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Clinical Research Article

Performance of Dehydroepiandrosterone Sulfate and Baseline Cortisol in Assessing Adrenal Insufficiency

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Abstract

Context: Diagnosing adrenal insufficiency (AI) often requires complex testing, which can be time-consuming and expensive. Dehydroepiandrosterone sulfate (DHEAS) is a promising marker of hypothalamic-pituitary-adrenal axis function; however, its diagnostic performance has not yet been evaluated in a large-scale study.

Objective: Evaluate the performance of DHEAS and baseline cortisol in assessing AI.

Design: Single-center retrospective cohort study.

Setting: Referral center.

Patients: Adults who underwent cosyntropin stimulation testing (CST) between 2005 and 2023 and had DHEAS measured within 3 months prior to CST.

Main Outcome Measures: Area under receiver operating characteristic curve (AUROC) for DHEAS and baseline cortisol. Prevalence of AI based on various DHEAS and baseline cortisol concentrations.

Results: Among 1135 patients, 195 (17.2%) had AI. Both baseline cortisol and DHEAS independently had good diagnostic performance with AUROC 0.81 [95% confidence interval (CI) 0.77-0.84 and 0.81 (95% CI 0.78-0.85)], respectively. Time of CST performance had no significant effect on diagnostic accuracy of baseline cortisol while recent glucocorticoid use decreased diagnostic performance of DHEAS (AUROC 0.72 vs 0.83). Only 1.2% of patients with baseline cortisol ≥10 mcg/dL had AI based on CST. Among patients with baseline cortisol between 5 and 9.9 mcg/dL, only 1.3% had AI if DHEAS was ≥60 mcg/dL. Conversely, the majority (72.2%) of patients with both baseline cortisol <5 mcg/dL and DHEAS <25 mcg/dL were found to have AI.

Conclusion: DHEAS has good diagnostic performance in assessing AI. Measuring both baseline cortisol and DHEAS concentrations may eliminate the need for further dynamic testing in many patients.

Key Words: DHEAS, cortisol, diagnosis, CST, cosyntropin, synacthen, stimulation test

Adrenal insufficiency (AI) is characterized by insufficient cortisol production due to adrenal gland hypofunction. Primary AI results from direct damage to the adrenal glands while central AI is caused by hypothalamic-pituitary-adrenal (HPA) axis dysfunction often in the setting of hypothalamic/pituitary tumors or chronic exogenous glucocorticoid (GC) use (1). Diagnosing AI remains a challenge for clinicians due to vague symptomatology, insidious onset, and complex multistep testing. However, untreated AI can lead to impaired quality of life as well as life-threatening consequences such as adrenal crisis (2-4).

The HPA axis is a highly dynamic endocrine system with diurnal fluctuations in cortisol concentrations throughout the day, which limits current testing modalities for AI. Measurement of a morning cortisol is often the initial test performed. While this is a simple screening test, samples are time-sensitive and median cortisol concentrations have been shown to drop by 30 nmol/L (1.1 mcg/dL) per hour between 7 AM and noon (5). Various cutoffs for baseline cortisol have been proposed, and in general, a cortisol concentration of <3 mcg/dL is considered to be suggestive of AI while cortisol >15 mcg/dL is indicative of an intact HPA axis (6, 7). However, for many patients with baseline cortisol between 3 and 15 mcg/dL, the test is indeterminate and additional testing may be needed.

The most common dynamic test performed is the cosyntropin stimulation test (CST), which requires measurement of cortisol concentrations at baseline, 30, and 60 minutes after

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injection of synthetic ACTH. Either low-dose (1 mcg) or high-dose (250 mcg) CST can be performed. Although low-dose CST may have higher sensitivity for detection of secondary AI, subsequent studies and meta-analyses have suggested similar diagnostic performance of low- and high-dose CST in assessment of secondary AI (8). Other dynamic tests include the insulin tolerance test (ITT), glucagon stimulation test, overnight metyrapone test, and corticotrophin-releasing hormone test (9-11). While more accurate than baseline morning cortisol testing, these tests tend to be time-consuming and resource-intensive and vary in availability across different institutions.

Dehydroepiandrosterone sulfate (DHEAS) is almost exclusively secreted from the adrenal cortex under the control of ACTH with small contributions from the ovaries and testes; it is one of the most abundant steroid hormones in the circulation (12). Several studies have suggested that baseline DHEAS is an accurate marker of HPA axis integrity as patients with AI often have low age- and sex-matched DHEAS concentrations (13-15). Additionally, DHEAS has a long half-life, lacks diurnal variation, and can be measured with widely available assays, which makes it an attractive potential diagnostic test (9). As HPA axis impairment generally affects androgen secretion before cortisol secretion, DHEAS may also be more sensitive in detecting early-stage AI (16). While there have been a few studies evaluating the diagnostic value of DHEAS in assessing AI (13, 15, 17, 18), these studies have been limited by smaller sample sizes, and no consensus exists yet regarding optimal cutoff concentrations of DHEAS to diagnose AI. Consequently, we aimed to (1) evaluate the accuracy of DHEAS in diagnosing AI based on CST and assess the impact of GC use, (2) evaluate the accuracy of baseline cortisol in diagnosing AI based on CST and assess the impact of CST time (<10 AM vs ≥10 AM), and (3) determine prevalence of CST-based AI diagnosis based on various cutoff concentrations of DHEAS and baseline cortisol.

Methods

Design

We conducted a single-center retrospective cohort study after approval by the Institutional Review Board with a waiver of consent. All patients provided research authorization for their medical record use.

Patients

Adult patients who underwent CST in the outpatient setting between 2005 and 2023 and had a DHEAS measurement within 3 months prior to or 1 week after CST were included in this study. Patients were excluded if they (1) died within 6 months of CST, (2) were hospitalized within 30 days of CST, (3) were taking oral estrogen at the time of CST, or (4) had congenital adrenal hyperplasia (CAH) defined as a 17-OH progesterone >400 ng/dL at any point or DHEAS greater than upper limit of normal. In our study, we defined primary AI as a baseline ACTH concentration >60 pg/mL. Postmenopausal status was defined as women 50 years or older. The Elixhauser Comorbidity Index was used to assess the burden of comorbidities based on a list of 30 International Classification of Diseases diagnosis codes.

CST and Assays

CST was performed using 250 mcg of Cosyntropin (Mylan Institutional, Amsterdam, Netherlands). Total cortisol levels were measured at baseline, 30, and 60 minutes after cosyntropin injection. Baseline cortisol was defined as cortisol concentration at 0 minutes after CST while peak cortisol was defined as cortisol at 60 minutes after CST. AI was defined as cortisol concentration <18 mcg/dL at 60 minutes after cosyntropin stimulation. The DHEAS level obtained closest to the time of CST was utilized in our analysis. Total cortisol (mcg/dL) was quantified using a competitive binding immune-enzymatic assay that utilizes monoclonal antibiotics (Beckman Coulter, Brea, CA). DHEAS (mcg/dL) was measured using a chemiluminescent immunoassay (Siemens Immulite 2000, Tarrytown, NY) between 2005 and 2018 and using an immunoenzymatic assay from 2018 to 2023 (Beckman Coulter DXI-800). Comparability between the 2 methods was established by in-house method comparison studies using 50 serum samples that spanned the analytical measuring range of the 2 assays; the average percentage difference between results was found to be 3.7%.

Statistical Analysis

Continuous data were summarized using median and interquartile range (25-75%) and categorical data reported using n (percent). Wilcoxon rank sum tests were used to compare continuous variables, and chi-square tests were used to compare categorical variables. Spearman correlations were used to summarize the relationship between age and concentration of cortisol and DHEAS. Exact binomial confidence intervals (CIs) were reported for sensitivity and specificity measurements. Area under the receiver operating characteristic curve (AUROC) was calculated for DHEAS and baseline cortisol; the optimal cutoff concentration to maximize both sensitivity and specificity was calculated using the approach of minimizing absolute (sensitivity-specificity). For baseline cortisol and DHEAS, sensitivity and specificity were calculated for various cutoff points. Subgroup analyses were performed to evaluate (1) the effect of CST time on baseline cortisol concentration (CST <10 AM vs ≥10 AM) and (2) the effect of GC use on DHEAS concentration (GC use within 2 months of CST vs no recent GC use). DHEAS was also standardized by dividing the DHEAS value by the value of the lower limit of normal range based on the patient's age and sex. Statistical significance was defined as $P < .05$. Statistical analyses were performed with SAS (Version 9.4) and R (version 4.3.2).

Results

Baseline Characteristics

In our cohort of 1135 patients (78.6% women), the median age was 42.8 years (interquartile range 32.6-54.8 years), and AI was diagnosed in 195 (17.2%) of patients. Patients with AI were older (median age of 49.9 years vs 41.5 years, $P < .001$) and had a lower proportion of women (70.3% vs 80.3%, $P = .002$). Median body mass index was 27.5 kg/m^2 and did not differ between groups (Table 1). Patients with AI had a higher Elixhauser Comorbidity Index than patients without AI (score ≥ 5 in 16.4% vs 9.9%, $P = .028$). Patients with AI had a lower median baseline cortisol concentration

Table 1. Baseline characteristics of patients with and without adrenal insufficiency

Variable	n available	All patients (n = 1135)	Peak cortisol ≥18 µg/dL (n = 940)	Peak cortisol <18 µg/dL (n = 195)	*P*-value
Age, years, median (IQR)	1135	42.8 (32.6-54.8)	41.5 (31.8-53.3)	49.9 (36.9-62.1)	**<.001**
Women, n (%)	1135	892 (78.6)	755 (80.3)	137 (70.3)	**.002**
Ethnic background, n (%)	1090				**<.001**
Black		26 (2.4)	20 (2.2)	6 (3.2)	
Asian		18 (1.7)	14 (1.6)	4 (2.1)	
White		1008 (92.5)	840 (93.2)	168 (88.9)	
American Indian/Alaskan Native		4 (0.4)	0 (0)	4 (2.1)	
Other		34 (3.1)	27 (3.0)	7 (3.7)	
Body mass index, kg/m^2, median (IQR)	1114	27.5 (23.0-33.2)	27.2 (22.9-33.3)	27.9 (24.0-32.6)	.322
Glucocorticoid use within 2 months of CST, n (%)	1135	223 (19.6)	135 (14.4)	88 (45.1)	**<.001**
CST performed prior to 10 AM, n (%)	1135	497 (43.8)	408 (43.4)	89 (45.6)	.567
Elixhauser Comorbidity Index, median (IQR)	1135	1 (0-3)	1 (0-3)	2 (0-3)	.105
Elixhauser Comorbidity Index score ≥5 n (%)		125 (11.0)	93 (9.9)	32 (16.4)	**.028**
eGFR, mL/min, median (IQR)	863	90.6 (76.1-105.2)	91.6 (77.7-106.3)	82.5 (67.4-97.3)	**<.001**
Sodium, mmol/L, median (IQR)	885	140.0 (139.0-142.0)	140 (139.0-142.0)	141 (138.0-142.0)	.718
Hyponatremia (Na <135 mmol/L), n (%)		27 (3.1)	20 (2.7)	7 (4.5)	.258
Potassium, mmol/L, median (IQR)	884	4.3 (4.1-4.5)	4.3 (4.1-4.6)	4.3 (4.1-4.5)	.178
Primary adrenal insufficiency, n (%)	883	47 (5.3)	26 (3.6)	21 (12.7)	**<.001**
ACTH, pg/mL, median (IQR)	883	17.0 (11.0-27.0)	17.0 (11.0-25.0)	16.0 (7.8-35.0)	.939
DHEAS, µg/dL, median (IQR)	1135	72.0 (33.0-137.0)	83.0 (46.0-150.5)	20.0 (14.9-46.0)	**<.001**
$DHEAS_{standard}$, median (IQR)	1135	2.5 (1.3-4.4)	2.9 (1.7-4.7)	1.0 (0.6-1.8)	**<.001**
Baseline Cortisol, µg /dL, median (IQR)	1129	7.2 (5.0-10.0)	7.9 (5.7-11.0)	4.2 (2.2-6.2)	**<.001**
Peak Cortisol, µg /dL, median (IQR)	1135	23.0 (19.0-26.0)	24.0 (21.0-27.0)	14.0 (9.0-16.0)	**<.001**

Bolded text indicates $P < .05$.
Abbreviations: CST, cosyntropin stimulation test; DHEAS, dehydroepiandrosterone sulfate; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

compared to patients without AI (4.2 vs 7.9 mcg/dL, $P < .001$), similar ACTH (median of 16.0 vs 17.0 pg/mL, $P = .939$), lower DHEAS concentration (median of 20.0 vs 83.0 mg/dL, $P < .001$), and lower standardized DHEAS (median 1.0 vs 2.9, $P < .001$) (Table 1). Peak cortisol concentration did not correlate with age [unadjusted Spearman R = −0.04 ($P = .136$)]. Baseline cortisol demonstrated a slight negative association with age in women [unadjusted Spearman R = [unadjusted Spearman R = −0.11 ($P = .001$)] but not men [unadjusted Spearman R = 0.04 ($P = .574$)]. DHEAS decreased with age in both men [unadjusted Spearman R = −0.67 ($P < .001$)] and women [unadjusted Spearman R = −0.55 ($P < .001$)] [Supplementary Fig. S1 (19)]. In our cohort, 497 (43.8%) of patients underwent CST prior to 10 AM. We also found that 223 (19.6%) of our cohort had an active prescription for GCs within 2 months prior to CST. Notably, among patients with abnormal CST, 88 (45.1%) were found to have recent GC use.

Table 2. Diagnostic accuracy of baseline cortisol and DHEAS

Variable	n	AUROC (95% CI)
Baseline cortisol		
Total cohort	1129	0.81 (0.77-0.84)
CST performed before 10 AM	495	0.83 (0.79-0.88)
CST performed at 10 AM or later	634	0.80 (0.75-0.85)
DHEAS		
Total cohort	1135	0.81 (0.78-0.85)
No glucocorticoid use within 2 months	912	0.83 (0.79-0.87)
Glucocorticoid use within 2 months	223	0.72 (0.64-0.79)
Standardized DHEAS[a]		
Total cohort	1135	0.80 (0.77-0.84)

[a]Specify equation for standardization.
Abbreviations: AUROC, area under the receiver operating characteristics curve; CI, confidence interval; CST, cosyntropin stimulation test; DHEAS, dehydroepiandrosterone sulfate.

Baseline Cortisol

Across the whole cohort, baseline cortisol demonstrated an AUROC of 0.81 (95% CI 0.77-0.84) when both sensitivity and specificity were maximized [Table 2, Supplementary Fig. S2 (19)]. Based on subgroup analysis, the diagnostic accuracy of baseline cortisol was slightly higher in patients who underwent CST before 10 AM compared to those who underwent CST at or after 10 AM with AUROC 0.83 (95% CI 0.79-0.88) vs 0.80 (95% 0.75-0.85) [Table 2, Supplementary Fig. S2 (19)]. Among patients with normal CST results, median baseline cortisol concentrations were higher before 10 AM compared to ≥10 AM (9.0 vs 6.9 mcg/dL, $P < .001$) [Supplementary Table S1 (19)]. Among patients with abnormal CST results, baseline cortisol levels did not differ based on time of CST [Supplementary Table S1 (19)]. Separate subgroup analysis demonstrated that GC use within

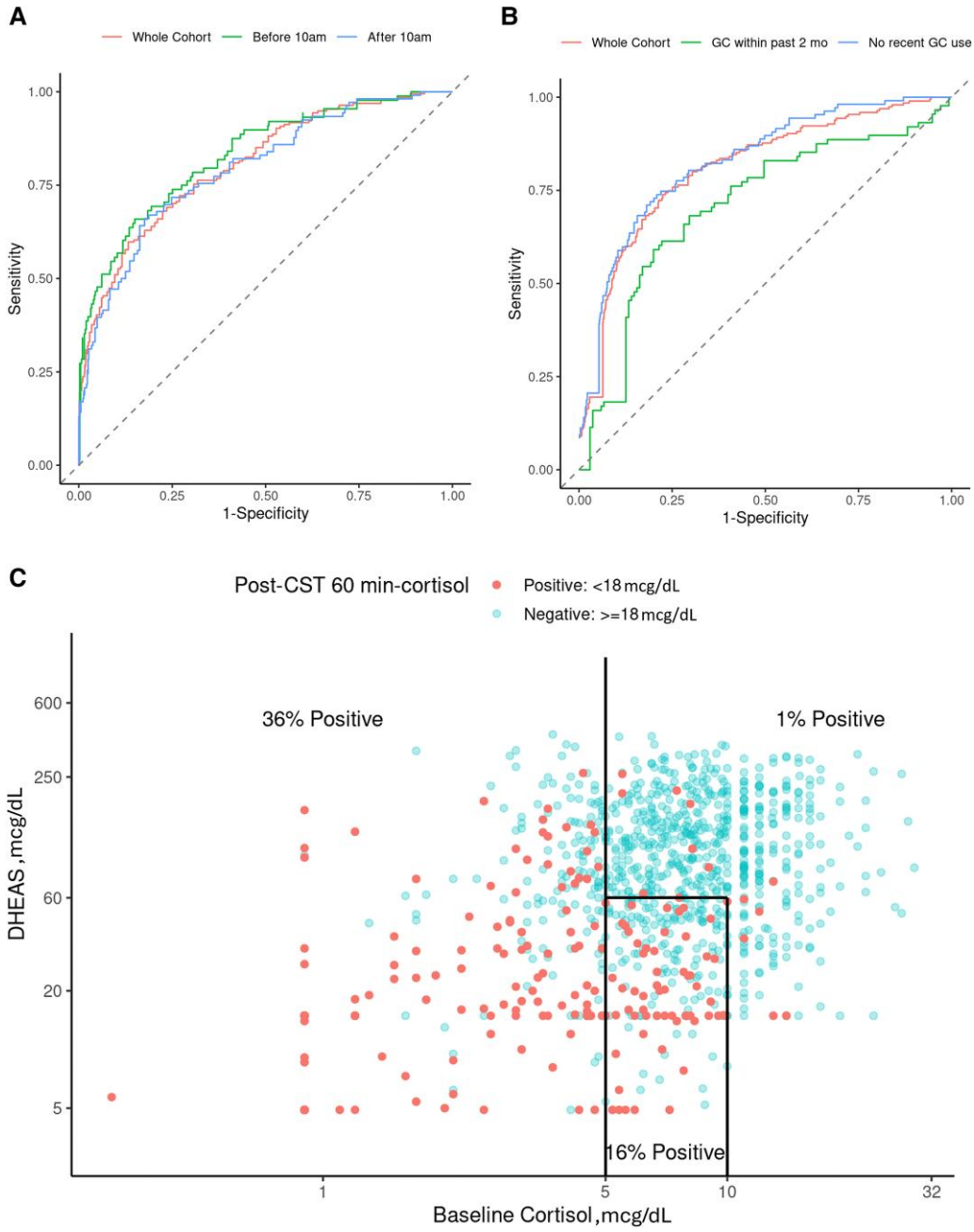

Figure 1. (A) Comparison of AUROC for baseline cortisol based on time of CST. (B) Comparison of AUROC curves for DHEAS based on glucocorticoid use. (C) CST outcome based on baseline cortisol and DHEAS concentration after excluding patients with recent glucocorticoid use. The scales for the x- and y-axis in (C) are nonlinear.

Abbreviations: CST, cosyntropin stimulation test; DHEAS, dehydroepiandrosterone sulfate; GC, glucocorticoid.

the past 2 months had no significant effect on baseline cortisol concentration regardless of whether CST was normal or abnormal [Supplementary Table S2 (19)].

DHEAS

Among all patients, DHEAS had good diagnostic accuracy with AUROC 0.81 (95% CI 0.78-0.85) when sensitivity and specificity were maximized [Table 2, Supplementary Fig. S3 (19)]. DHEAS demonstrated better diagnostic accuracy in patients without GC use within the past 2 months compared to patients with recent GC use with AUROC 0.83 (95% CI 0.79-0.87) vs 0.72 (95% CI 0.64-0.79), respectively [Fig. 1B, Supplementary Fig. S3 (19)]. Standardized DHEAS demonstrated similar performance as nonstandardized DHEAS with AUROC 0.80 (95% CI 0.77-0.84). Among patients with normal CST results, median DHEAS concentration was significantly lower in patients with GC use within the past 2 months compared to those without recent GC use (54.0 vs 90.9 mcg/dL, $P < .001$) [Supplementary Table S2 (19)]. Among patients with abnormal CST, median DHEAS was low but did not differ between those with and without recent GC use (20.2 vs 20.0 mcg/dL, $P = .877$) [Supplementary Table S2 (19)].

Table 3. Sensitivity and specificity of various baseline cortisol and DHEAS cutoff concentrations

Subgroups	Hormone cutoff	Overall cohort		Cohort with applied exclusions[a,b]	
		Sensitivity rate (exact binomial CI)	Specificity rate (exact binomial CI)	Sensitivity rate (exact binomial CI)	Specificity rate (exact binomial CI)
All patients (women, men, all ages)	**Cortisol, µg/dL**		**n = 935**		**n = 407**
	Baseline cortisol < 3	33 (26-40)	97 (96-98)	33 (23-44)	99 (98-100)
	Baseline cortisol < 5	61 (54-68)	84 (82-86)	51 (40-62)	93 (90-95)
	Baseline cortisol < 10	96 (93-99)	30 (27-33)	94 (87-98)	40 (35-45)
	Baseline cortisol < 12	98 (95-99)	19 (16-22)	98 (92-100)	25 (21-30)
	Baseline cortisol < 15	100 (98-100)	7 (6-9)	100 (96-100)	11 (8-15)
	DHEAS, µg/dL		**n = 940**		**n = 805**
	DHEAS < 25	57 (50-64)	89 (86-91)	55 (45-65)	90 (88-92)
	DHEAS < 40	70 (63-77)	79 (76-82)	69 (59-78)	82 (79-85)
	DHEAS < 60	82 (76-87)	65 (62-68)	80 (72-87)	69 (66-72)
	DHEAS < 100	90 (85-94)	43 (40-46)	92 (85-96)	46 (43-50)
	DHEAS < 120	92 (88-96)	35 (32-38)	94 (88-98)	38 (35-42)
Women ≥ 50 years	**DHEAS, µg/dL**		**n = 196**		**n = 159**
	DHEAS < 25	77 (64-87)	66 (59-72)	72 (53-86)	69 (61-76)
	DHEAS < 40	89 (78-96)	48 (41-56)	88 (71-96)	51 (43-59)
	DHEAS < 60	93 (83-98)	33 (27-40)	91 (75-98)	35 (28-43)
	DHEAS < 100	98 (91-100)	12 (8-17)	100 (89-100)	13 (8-19)
	DHEAS < 120	98 (91-100)	5 (2-9)	100 (89-100)	6 (3-11)

Bold text indicate categories and sample size.
[a]Exclusions for cortisol analysis: 528 patients excluded for CST performed at or after 10 AM.
[b]Exclusions for DHEAS analysis: 135 patients excluded for glucocorticoid use within 2 months.
Abbreviations: CI, confidence interval; DHEAS, dehydroepiandrosterone sulfate; M + F, males and females.

Predefined Baseline Cortisol and DHEAS Cutoff Levels

We investigated baseline cortisol concentrations cutoffs of 3, 5, 10, 12, and 15 mcg/dL, which demonstrated that baseline cortisol <10 mcg/dL had a high sensitivity of 96% (95% CI 93-99%) for the diagnosis of AI; however, it had a suboptimal specificity of 30% (95% CI 27-33%) (Table 3). After excluding patients with CST performed at or after 10 AM, specificity slightly increased to 40% (95% CI 35-45). Similarly, we investigated DHEAS cutoffs of 25, 40, 60, 100, and 120 mcg/dL across all patients as well as specifically in postmenopausal women. Among all patients, a DHEAS measurement <100 mcg/dL demonstrated a sensitivity of 90% (95% CI 85-94%) for diagnosing AI based on CST with specificity of 43% (95% CI 40-46%) (Table 3). After the exclusion of patients with recent GC use, sensitivity and specificity slightly improved to 92% and 46%, respectively. In postmenopausal women, we found that a DHEAS measurement <100 mcg/dL had a high sensitivity of 98% (95% CI 91-100%) (Table 3).

Prevalence of AI Based on Baseline Cortisol and DHEAS Concentration

After excluding patients with recent GC use, only 1.2% of patients with baseline cortisol concentration ≥10 mcg/dL demonstrated post-CST cortisol <18 mcg/dL compared to 36.3% of patients with baseline cortisol <5 mcg/dL and 6.3% of patients with baseline cortisol 5-9.9 mcg/dL (Figs. 1C and 2). CST-based diagnosis of AI was documented in 72.2% patients who had both low baseline cortisol (<5 mcg/dL) and DHEAS <25 mcg/dL (Fig. 2). When DHEAS was ≥25 mcg/dL, CST-based diagnosis of AI in patients with baseline cortisol <5 mcg/dL ranged from 16.8% to 50.0%, depending on DHEAS concentrations (Fig. 2). Among patients with baseline cortisol between 5 and 9.9 mcg/dL and DHEAS ≥60 mcg/dL, CST-based diagnosis of AI was 1.3% (Fig. 2).

Discussion

In our large cohort of patients, we found that DHEAS and baseline cortisol independently had good diagnostic performance and in combination were able to accurately identify patients with AI based on CST. While the diagnostic accuracy of DHEAS was reduced with recent GC use, the diagnostic performance of baseline cortisol was not significantly impacted by the timing of CST. Standardizing DHEAS by age- and sex-specific normal limits did not improve diagnostic performance. Based on our data, we propose no further testing for AI in patients with baseline cortisol ≥10 mcg/dL as AI is very unlikely. DHEAS is especially valuable in patients with indeterminate baseline cortisol (5-9.9 mcg/dL); if DHEAS is ≥60 mcg/dL, AI remains unlikely, and we also propose no further testing in these patients. With this approach, further testing would only be indicated in patients with baseline cortisol measurement between 5 and 9.9 mcg/dL and DHEAS <60 mcg/dL, or baseline cortisol measurement <5 mcg/dL with DHEAS ≥25 mcg/dL, which is a small subset of our cohort.

We found that only 17.2% patients who underwent CST had AI based on CST. Prevalence of AI was lower in our cohort than in prior studies which ranged from 33% to 57% (11, 14, 15, 17, 20), potentially reflecting that CST at our institution is available to any physician without the need for consulting endocrinology. Other studies had notably smaller sample sizes and primarily included patients with high clinical suspicion for HPA dysfunction based on comorbidities such as pituitary macroadenoma or prolonged history of GC use. We also found that while baseline and peak cortisol concentration did not demonstrate a robust association with age, DHEAS was negatively correlated with age among both men and women, which is consistent with prior findings that DHEAS declines with increasing age regardless of gonadal function (13, 21). Fischli et al also demonstrated that the diagnostic accuracy of DHEAS decreases with advancing age (20).

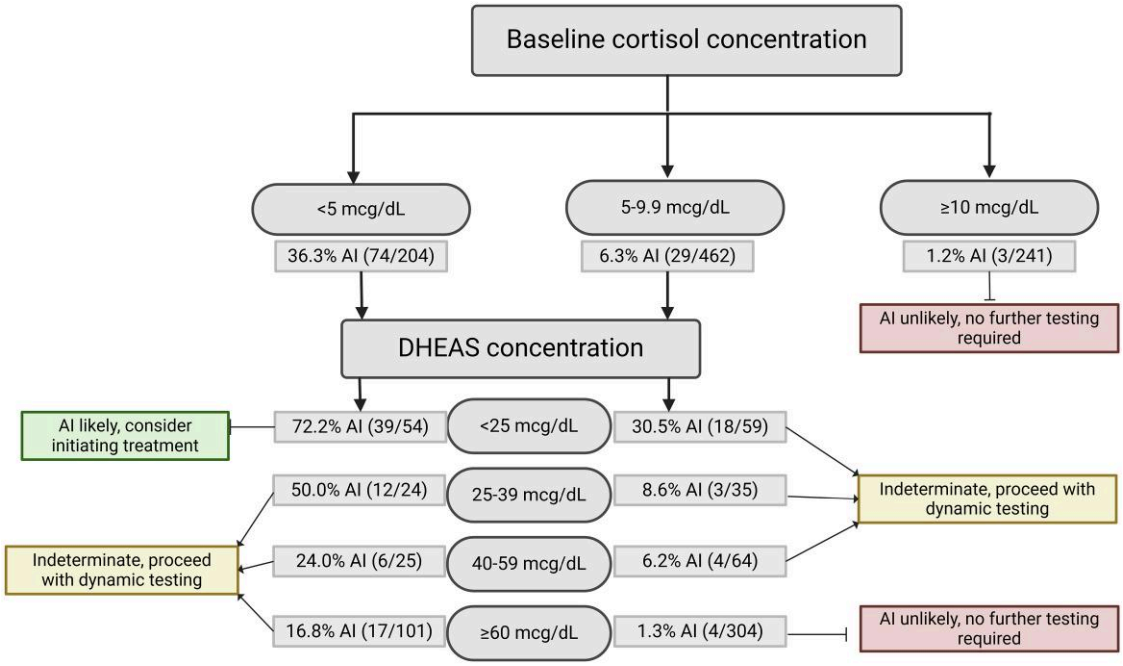

Figure 2. Flowchart displaying the prevalence of adrenal insufficiency based on various baseline cortisol and dehydroepiandrosterone sulfate concentrations.

Interestingly, standardizing DHEAS according to age- and sex-specific limits of normal did not improve diagnostic accuracy in our study. Consequently, to identify cutoff concentrations that would be easy to incorporate into clinical practice, we utilized nonstandardized DHEAS concentrations in our subsequent analyses.

As expected, we found that median DHEAS concentration in patients with AI was lower than in patients without AI (20.0 vs 83.0 mcg/dL), which is similar to values reported in Charoensri et al (30.4 vs 87.6 mcg/dL) (15). In our study, DHEAS demonstrated an AUROC of 0.81 while standardized DHEAS demonstrated an AUROC of 0.80 in diagnosing AI based on CST. In other smaller studies where DHEAS measurements were standardized, log-transformed, or adjusted for age and sex, AUROC ranged from 0.84 to 0.98 (13, 15, 17). Noted differences in the diagnostic accuracy of DHEAS between our study and previous reports are likely due to smaller sample sizes and different patient populations tested for AI. We further found that the diagnostic performance of DHEAS was better in patients without a history of recent GC use than in patients with GC use within the past 2 months (AUROC 0.83 vs 0.72). No other studies examined the impact of exogenous GC use on the accuracy of DHEAS in diagnosing AI. Our data suggest that lower DHEAS in patients with GC-induced adrenal insufficiency may not be reflective of AI, and we recommend cautious interpretation of results in this setting.

Median baseline cortisol concentration was also lower in patients with AI. Among patients with normal CST results, median baseline cortisol was 2.1 mcg/dL lower when CST was performed at 10 AM or later compared to when CST was performed before 10 AM. This is consistent with trends in prior studies that demonstrated that median baseline cortisol decreases by approximately 1.1 mcg/dL per hour between 7 AM and noon (5). Among patients with abnormal CST, neither median baseline cortisol nor median peak cortisol differed between groups, suggesting that patients with AI have low cortisol levels without significant diurnal variation. Similarly, ROC curve analysis demonstrated that the diagnostic accuracy of baseline cortisol is not significantly affected by CST time. While standard practice is to perform CST before 10 AM, our findings suggest that although there is an increased risk of false positives when CST is performed after 10 AM, diagnosis of AI is unlikely to be missed.

We demonstrated that baseline cortisol <10 mcg/dL was the lowest cutoff value with very high sensitivity for AI diagnosis. However, as specificity was suboptimal, we proposed a 2-step process to incorporate DHEAS measurements and reduce the number of false positives. We found that patients with indeterminate baseline cortisol (5-9.9 mcg/dL) but DHEAS ≥60 mcg/dL were unlikely to have AI and also do not require further testing unless very high clinical suspicion is present. Conversely, the majority of patients with baseline cortisol measurement <5 mcg/dL and DHEAS <25 mcg/dL were found to have AI, and empiric treatment could be considered without further testing. Specifically, among postmenopausal women, specificity of DHEAS was shown to be reduced, and caution should be taken when interpreting results in this patient population.

To our knowledge, this is the first large-scale study to evaluate the predictive value of DHEAS in conjunction with baseline cortisol concentrations for the assessment of AI. Strengths of this study include a robust sample size, a uniform testing/biochemical workup that was performed at a single center, and subgroup analyses that assess the impact of CST timing and history of exogenous GC use. We additionally excluded critically ill patients and patients with CAH. As CAH was excluded based on an International Classification of Diseases diagnosis and elevated 17OH-progesterone concentrations, some patients may have been missed. Although

CST is known to lack some sensitivity in diagnosing secondary AI and ITT is considered the gold standard (10, 13, 14), CST is the most frequently performed dynamic test and using it as the reference standard for this study is practical and generalizable. CST interpretation depends on several factors, including pretest probability (clinical suspicion), timing of assessment (30 or 60 minutes post-CST cortisol measurement), a cutoff definition of "abnormal," and the cortisol assay used. At our institution, an abnormal result is defined as post-CST cortisol at 60 minutes of <18 mcg/dL. Thus, our classification of patients as disease positive or negative may not be identical to cohorts of patients with different CST-based AI definitions. A lower 60-minute post-CST cortisol cutoff was recently proposed in a study that compared 48 samples measured by the Beckman Access cortisol assay to the Elecsys 1 measurements (22). Notably, another study that used a newer Roche Elecsys cortisol II assay reported the lowest post-CST 60 minutes cortisol of 17.9 mcg/dL (range 17.9-35.8 mcg/dL) (23). This suggests that the cutoff of 18 mcg/dL is reasonable even with newer assays, especially considering that comparative studies of CST with ITT demonstrated concerns with under- and not overdiagnosis of AI.

In summary, our study demonstrates that DHEAS measurement is a valuable diagnostic test that can eliminate the need for dynamic testing in a large subset of patients when assessed in conjunction with baseline cortisol. Recent GC use was shown to reduce the diagnostic accuracy of DHEAS. Although performing CST at or after 10 AM reduced median cortisol concentrations among patients with normal CST results, the diagnostic accuracy of baseline cortisol was not significantly affected by CST time. Baseline cortisol ≥10 mcg/dL is highly reassuring against AI. If baseline cortisol is between 5 and 9.9 mcg/dL but DHEAS is ≥60 mcg/dL, CST is likely to be normal and dynamic testing is not needed. Conversely, for patients with baseline cortisol <5 mcg/dL and DHEAS <25 mcg/dL, given the high likelihood of AI, empiric treatment should be considered if consistent with the patient's symptoms and medical history. DHEAS should be interpreted more cautiously in patients with recent GC use, postmenopausal women, and older patients. The small subset of patients with indeterminate baseline cortisol and DHEAS concentrations should proceed to further dynamic testing. As DHEAS is a simple lab test that is widely available and does not have significant diurnal variation, its routine use alongside baseline cortisol may simplify evaluation for AI.

Disclosures

I.B. reports advisory board participation, data safety monitoring board participating or consulting (fees to institution) with Corcept Therapeutics, Sparrow Pharmaceutics, Xeris, Recordati, Camurus, Crinetics, Diurnal, Spruce, NovoNordisk, AstraZeneca, Adrenas Pharmaceutics, and HRA Pharma with no relation to the submitted work. I.B. reports research support from Recordati and HRA Pharma, not related to this work. The remaining authors have no conflicts of interest to declare.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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