

ORIGINAL RESEARCH ARTICLE

The ACTH × DHEAS product as an ancillary biomarker of mild autonomous cortisol secretion in adrenal incidentalomas

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Abstract

Introduction: Mild autonomous cortisol secretion (MACS) is usually classified by the 1-mg overnight dexamethasone suppression test (DST). However, a single post-DST cortisol value may not fully capture the chronicity or biological relevance of cortisol autonomy. Although adrenocorticotrophic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEAS) reflect different but biologically linked components of hypothalamic–pituitary–adrenal (HPA) axis suppression, their combined assessment has not been sufficiently evaluated in adrenal incidentalomas.

Objective: To evaluate the association between the ACTH × DHEAS product and DST-defined cortisol autonomy and to explore its potential role as an ancillary biochemical marker in adrenal incidentalomas.

Methods: This retrospective study included 157 patients classified as having MACS or a nonfunctioning adrenal adenoma based on post-DST cortisol levels. Patients with overt endocrine syndromes, malignant or rare adrenal lesions, and *ARMC5*-positive disease were excluded. Correlation analyses, sex-adjusted multivariable logistic regression, exploratory receiver operating characteristic analyses, and a DeLong test were performed.

Results: MACS was present in 79 patients (50.3%). Patients with MACS had larger tumors, higher basal and post-DST cortisol, and lower ACTH, DHEAS, and $\ln(\text{ACTH} \times \text{DHEAS})$. $\ln(\text{ACTH} \times \text{DHEAS})$ was inversely correlated with post-DST cortisol and larger tumor size. After adjustment for age, sex, basal cortisol, and larger tumor size, lower $\ln(\text{ACTH} \times \text{DHEAS})$ remained independently associated with MACS (odds ratio = 0.473, 95% CI: 0.304–0.737, $p = 0.001$). The corrected $\ln(\text{ACTH} \times \text{DHEAS})$ showed moderate discrimination (area under the curve = 0.693) but did not significantly outperform –DHEAS alone.

Conclusion: $\ln(\text{ACTH} \times \text{DHEAS})$ may serve as a practical ancillary marker of chronic HPA-axis suppression, particularly in borderline or discordant cases. It should not replace DST or DHEAS alone, and prospective outcome-based validation is required before clinical implementation.

Keywords: Adrenal incidentaloma; Mild autonomous cortisol secretion; Dexamethasone suppression test; Adrenocorticotrophic hormone; Dehydroepiandrosterone sulfate; Hypothalamic–pituitary–adrenal axis

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1. Introduction

Adrenal incidentalomas are adrenal masses of at least 1 cm detected on imaging performed for reasons unrelated to suspected adrenal disease. Their recognition has increased with the widespread use of cross-sectional imaging and population aging, with a prevalence approaching 10% in older adults.¹ Initial evaluation is directed toward two key questions: whether the lesion is malignant and whether it is hormonally functional.² This distinction is particularly important because the pre-test probability of malignancy differs substantially between lesions detected incidentally and those discovered during oncological staging. On non-contrast computed tomography, a homogeneous adrenal lesion with attenuation of 10 Hounsfield units or less is highly suggestive of a benign lipid-rich adenoma, which accounts for most adrenal incidentalomas.³ In such radiologically benign lesions, the major clinical challenge often shifts from imaging surveillance to interpreting subtle hormonal activity.

All patients with adrenal incidentalomas should undergo hormonal assessment, including a 1-mg overnight dexamethasone suppression test (DST), because mild cortisol autonomy is associated with cardiometabolic and skeletal morbidity.² Overt hormone-producing tumors, such as classic Cushing syndrome, primary aldosteronism, and pheochromocytoma, are usually identified by characteristic clinical and biochemical findings. By contrast, mild autonomous cortisol secretion (MACS) is defined by biochemical evidence of cortisol autonomy in the absence of overt clinical features of Cushing syndrome, most commonly using a post-DST cortisol threshold greater than 1.8 µg/dL.⁴ Current ESE/ENSAT guidelines also recommend confirmation of adrenocorticotrophic hormone (ACTH) independence to support adrenal-origin cortisol secretion.⁵

Despite its central role, the DST has important limitations when used as a single categorical test for MACS. Post-DST cortisol reflects acute feedback dynamics rather than the duration or cumulative burden of cortisol exposure.⁶ In addition, DST-based classification is affected by assay-related factors, medications, comorbidities, and insufficient dexamethasone exposure, and there is no universally accepted gold standard for clinically meaningful cortisol autonomy.⁷ Because adrenal incidentalomas are usually detected unexpectedly, it is unclear how long the adenoma has been present before diagnosis. Therefore, a single post-DST cortisol value may not fully capture the chronicity or biological relevance of cortisol autonomy. In the absence of additional indicators of sustained hypothalamic–pituitary–adrenal (HPA) axis suppression, the distinction between truly nonfunctional adrenal disease

and biologically relevant low-grade hypercortisolism may remain uncertain.^{4,8,9}

Ancillary markers reflecting chronic suppression of the HPA axis may therefore improve the biological interpretation of DST results. Sustained adrenal cortisol secretion suppresses pituitary ACTH through glucocorticoid-mediated negative feedback, including inhibition of ACTH release and proopiomelanocortin (POMC) transcription.¹⁰ Chronic ACTH suppression subsequently reduces adrenal androgen production, particularly dehydroepiandrosterone sulfate (DHEAS).^{10,11} ACTH and DHEAS are both lower in many patients with MACS than in those with nonfunctioning adrenal adenomas, but each marker has limitations as a standalone diagnostic test because of pulsatility, assay variability, age and sex effects, and overlap between groups.^{12–14}

Although ACTH and DHEAS have previously been evaluated as individual supportive markers of cortisol autonomy, their combined utility as an integrated index of pituitary feedback suppression and downstream adrenal androgen suppression has not been systematically evaluated in a cohort of this size.⁸ This is an important knowledge gap because MACS is not simply a disorder of incomplete cortisol suppression after dexamethasone; it may represent a continuum of chronic alterations in HPA-axis feedback with variable clinical relevance. A composite index may therefore be useful not because it is expected to replace the DST, but because it can provide a second biological dimension when interpreting DST-defined cortisol autonomy.

From a methodological perspective, the present index was not designed to create a new diagnostic category but to address a narrower, clinically relevant uncertainty: whether a DST-defined biochemical phenotype is accompanied by basal evidence of chronic ACTH-dependent adrenal androgen suppression. This distinction is important because patients with adrenal incidentalomas may have similar post-DST cortisol values but different degrees of HPA-axis adaptation.¹² A single post-DST cortisol measurement reflects the response to an acute pharmacological challenge, whereas basal ACTH and DHEAS reflect different time scales of feedback physiology.⁴ Therefore, evaluating these signals together may help separate isolated borderline DST abnormality from a more coherent biochemical pattern suggestive of sustained cortisol autonomy. This conceptual role is complementary to current guideline-based evaluation and does not imply replacement of the DST or of clinical assessment of cortisol-related comorbidities.⁵

We therefore hypothesized that an integrated index combining pituitary feedback suppression and downstream

adrenal androgen suppression may better capture sustained cortisol autonomy than either component alone. The present study evaluates the association of the ACTH × DHEAS product with post-DST cortisol and assesses its potential role as an ancillary biomarker in patients with adrenal incidentalomas. In response to the biological dependence of DHEAS on age and sex, the revised analysis also adjusted for sex in addition to age, basal cortisol, and larger tumor size.

2. Materials and methods

2.1. Study design and population

This retrospective observational study included patients evaluated for adrenal incidentalomas at a single tertiary endocrine center. The study included patients evaluated between 2013 and 2024. An adrenal incidentaloma was defined as an adrenal mass measuring at least 1 cm detected on imaging performed for an unrelated clinical indication. Patients were eligible if their clinical records included adrenal imaging data and the biochemical variables required to assess cortisol autonomy, including basal cortisol, ACTH, DHEAS, and post-DST cortisol.

The study was approved by the local ethics committee (approval number 0277, approved May 23, 2024). The study was conducted in accordance with the principles of the Declaration of Helsinki and used data obtained from routine clinical records.

Patients with missing DHEAS and/or ACTH values, which were required for calculation of the primary ACTH × DHEAS index, were excluded. To obtain a homogeneous cohort of benign adrenal adenomas and to avoid confounding from overt endocrine or malignant adrenal disease, patients were excluded if they had overt Cushing syndrome, pheochromocytoma, primary aldosteronism (Conn syndrome), angiomyolipoma, malignant adrenal lesions including adrenocortical carcinoma and adrenal metastases, adrenal cysts, other rare adrenal masses, or *ARMC5*-positive disease. *ARMC5*-positive disease was defined as adrenal disease associated with a known pathogenic or likely pathogenic variant in *ARMC5*, usually associated with primary bilateral macronodular adrenal hyperplasia (PBMAH) and a distinct genetic mechanism of cortisol autonomy. In PBMAH, cortisol secretion may involve intra-adrenal/paracrine ACTH production and aberrant G protein-coupled hormone receptor signaling, which could confound the interpretation of circulating ACTH and DHEAS, and their product as markers of HPA-axis suppression. Therefore, these patients were excluded to maintain a more homogeneous study population.

2.2. Clinical and biochemical assessment

Demographic data, adrenal laterality, tumor size, basal cortisol, basal ACTH, DHEAS, and 1-mg DST cortisol values were obtained from hospital records. In patients with unilateral lesions, tumor size was recorded as the largest diameter reported on adrenal imaging. In patients with bilateral adrenal lesions, larger tumor size was defined as the largest diameter of the dominant/larger adrenal lesion and was used in the revised regression and correlation analyses. MACS was defined as post-DST cortisol greater than 1.8 µg/dL in the absence of overt clinical features of Cushing syndrome, whereas patients with post-DST cortisol of 1.8 µg/dL or less were classified as having nonfunctioning adrenal adenomas. The term “DST-defined cortisol autonomy” was used throughout this study to emphasize that the classification is biochemical and not a validated clinical outcome measure.

Serum DHEAS concentrations were analyzed using measured values obtained from the hospital laboratory database. Because DHEAS concentrations physiologically declined with age and differ by sex, laboratory-reported age-stratified reference intervals were used for clinical interpretation. Formal external age- and sex-standardized DHEAS z-scores could not be calculated retrospectively because patient-specific lower and upper reference limits were unavailable in the dataset. Therefore, no additional mathematical age-adjusted transformation was performed; instead, age and sex were included as covariates in the revised multivariable model to account for their potential effects on DHEAS concentrations.

2.3. Composite adrenocorticotrophic hormone–dehydroepiandrosterone sulfate index

The ACTH × DHEAS product was calculated by multiplying basal ACTH by DHEAS. Because the product distribution was right-skewed and endocrine variables often showed non-normal distributions, the product was natural-log transformed and analyzed as $\ln(\text{ACTH} \times \text{DHEAS})$. Lower values of this index were expected to reflect greater HPA-axis suppression, consistent with simultaneous pituitary ACTH suppression and reduced ACTH-dependent adrenal androgen production.

The multiplicative product was selected because ACTH and DHEAS were expected to change in the same biological direction during chronic ACTH-independent cortisol secretion. In this context, both lower ACTH and lower DHEAS were considered to support the same physiological interpretation: suppression of the HPA axis. A ratio was not preferred because ACTH and DHEAS did not represent opposing signals in MACS, and a ratio

could remain unchanged when both variables were suppressed in parallel. A summed z -score approach could be useful in prospective studies with standardized external reference data; however, such reference distributions were not uniformly available in this retrospective cohort. The product therefore provided a physiologically interpretable index of concordant suppression at two linked levels of the adrenal axis.

2.4. Statistical analysis

Continuous variables were assessed for distributional characteristics and were presented as mean $\pm$ standard deviation or median with interquartile range, as appropriate. Categorical variables were presented as number and percentage. Comparisons between MACS and nonfunctional adenoma groups were performed using the independent-samples t -test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were summarized descriptively.

Receiver operating characteristic (ROC) analysis was performed to compare the discriminatory ability of basal cortisol, ACTH, DHEAS, and $\ln(\text{ACTH} \times \text{DHEAS})$ for predicting DST-defined cortisol autonomy. Because ACTH, DHEAS, and the $\text{ACTH} \times \text{DHEAS}$ product were expected to decrease as cortisol autonomy increases, these variables were multiplied by -1 for ROC plotting and area under the curve (AUC) estimation so that higher transformed values corresponded to a higher probability of MACS and the curves were displayed above the reference line. The corrected $-\ln(\text{ACTH} \times \text{DHEAS})$ curve therefore represented the sign-corrected natural-log-transformed $\text{ACTH} \times \text{DHEAS}$ product. The ROC analysis was interpreted as exploratory and ancillary rather than as a test to replace the DST. The optimal threshold was determined using the Youden index, and the log-derived threshold was back-transformed to the original $\text{ACTH} \times \text{DHEAS}$ scale for clinical interpretation. A DeLong comparison was performed to evaluate whether the AUC of corrected $-\ln(\text{ACTH} \times \text{DHEAS})$ differed significantly from the AUC of $-\text{DHEAS}$ alone. All tests were two-tailed, and $p < 0.05$ was considered statistically significant.

Correlation analyses were performed to examine the relationship between $\ln(\text{ACTH} \times \text{DHEAS})$ and clinical or biochemical parameters. Spearman rank correlation coefficients were used for non-normally distributed variables, whereas Pearson correlation was used for $\ln(\text{DST cortisol})$.

Binary logistic regression analysis using the Enter method was performed to identify independent predictors of DST-defined cortisol autonomy. The model included

age, sex, $\ln(\text{ACTH} \times \text{DHEAS})$, basal cortisol, and tumor size. Gender was entered as male versus female, with female sex as the reference category. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported.

3. Results

3.1. Study population

The study included 157 patients with adrenal incidentalomas after application of the eligibility and exclusion criteria. Of these, 120 patients (76.4%) were female, and 37 (23.6%) were male. According to DST-defined cortisol status, 79 patients (50.3%) were classified as having MACS and 78 (49.7%) as having nonfunctioning adrenal adenomas. Bilateral MACS was present in 40 patients (25.5%), unilateral MACS in 39 (24.8%), bilateral nonfunctional adenomas in 28 (17.8%), and unilateral nonfunctional adenomas in 50 (31.8%) (Table 1).

3.2. Clinical and biochemical differences according to dexamethasone suppression test category

Group comparisons were first performed to determine whether patients classified as MACS differed from those with nonfunctional adenomas in variables expected to reflect cortisol autonomy and HPA-axis suppression. Age was similar between groups. Tumor size was larger in the MACS group, whereas basal ACTH, DHEAS, and $\ln(\text{ACTH} \times \text{DHEAS})$ were lower. Basal cortisol, post-DST cortisol, and $\ln(\text{DST cortisol})$ were higher in patients with MACS (Table 2).

3.3. Correlation analysis

Correlation analysis was then performed to evaluate whether $\ln(\text{ACTH} \times \text{DHEAS})$ behaved as an integrated marker of pituitary and adrenal androgen suppression. $\ln(\text{ACTH} \times \text{DHEAS})$ was strongly and positively correlated with its two components, basal ACTH and DHEAS. By contrast, it showed inverse relationships with 1-mg DST cortisol, $\ln(\text{DST cortisol})$, and tumor size, indicating that lower $\ln(\text{ACTH} \times \text{DHEAS})$ values were associated with higher post-DST cortisol levels and larger adrenal lesions. Basal cortisol was not significantly correlated with $\ln(\text{ACTH} \times \text{DHEAS})$ (Table 3).

3.4. Independent predictors of dexamethasone suppression test-defined cortisol autonomy

Group comparisons and correlation analyses suggested associations among tumor size, basal cortisol, DHEAS, and $\ln(\text{ACTH} \times \text{DHEAS})$; therefore, multivariable logistic regression was performed to determine whether the composite index remained independently associated with post-DST cortisol autonomy after adjustment for

Table 1. Distribution of the study population by sex, cortisol status, and adrenal laterality

Variable	Category	<i>n</i>	%
Gender	Male	37	23.6
	Female	120	76.4
Mild autonomous cortisol secretion	Bilateral	40	25.5
	Unilateral	39	24.8
Nonfunctional adenoma	Bilateral	28	17.8
	Unilateral	50	31.8

Table 2. Descriptive statistics and group comparisons according to DST-defined cortisol status (*n* = 157)

Variable	Overall	Nonfunctional (<i>n</i> = 78)	MACS (<i>n</i> = 79)	<i>p</i> -value
Age (years)	59.57 ± 9.33	59.50 (16.00)	59.0 (13.00)	0.518
Tumor size (mm)	30.00 (20.50)	25.00 (18.25)	37.0 (25.00)	<0.001
Basal cortisol (µg/dL)	12.80 (6.55)	11.80 (6.52)	13.20 (6.30)	0.013
Basal ACTH (pg/mL)	11.00 (7.00)	12.00 (8.25)	9.93 (7.00)	0.033
DHEAS (µg/dL)	62.00 (58.00)	73.50 (62.97)	46.00 (51.70)	<0.001
1-mg DST cortisol (µg/dL)	1.82 (1.48)	1.10 (0.61)	2.58 (1.30)	<0.001
ln(ACTH × DHEAS)	6.43 ± 0.91	6.75 ± 0.88	6.12 ± 0.90	<0.001
ln(DST cortisol)	0.56 ± 0.55	0.06 ± 0.35	1.06 ± 0.40	<0.001

Notes: Values are presented as mean ± SD for normally distributed variables and median (IQR) for non-normally distributed variables. IQR is calculated as the difference between the 75th and 25th percentiles. Group comparisons were performed using the Mann–Whitney *U* test or independent-samples *t*-test, as appropriate. The overall column presents age as mean ± SD, whereas the subgroup columns present age as median (IQR), according to the distributional format used for subgroup comparison. *p*-values are two-tailed.

Abbreviations: ACTH: Adrenocorticotrophic hormone; DHEAS: Dehydroepiandrosterone sulfate; DST: Dexamethasone suppression test; IQR: Interquartile range; MACS: Mild autonomous cortisol secretion; SD: Standard deviation.

Table 3. Correlation of $\ln(\text{ACTH} \times \text{DHEAS})$ with clinical and biochemical parameters ($n = 157$)

Variable	Correlation coefficient	<i>p</i> -value
Basal cortisol ($\mu\text{g/dL}$)	0.061	0.448
Basal ACTH (pg/mL)	0.662	<0.001
DHEAS ($\mu\text{g/dL}$)	0.827	<0.001
1-mg DST cortisol ($\mu\text{g/dL}$)	−0.347	<0.001
Tumor size (mm)	−0.244	0.002
$\ln(\text{DST cortisol})^a$	−0.358	<0.001

Notes. Spearman rank correlation coefficients are presented unless otherwise indicated. ^aPearson correlation coefficient is shown for $\ln(\text{DST cortisol})$.

Negative correlation coefficients indicate inverse relationships. All *p*-values are two-tailed.

Abbreviations: ACTH: Adrenocorticotrophic hormone; DHEAS: Dehydroepiandrosterone sulfate; DST: Dexamethasone suppression test.

these factors. In this model, lower $\ln(\text{ACTH} \times \text{DHEAS})$ remained independently associated with DST-defined cortisol autonomy after adjustment for age, sex, basal cortisol, and larger tumor size. Specifically, each one-unit increase in $\ln(\text{ACTH} \times \text{DHEAS})$ was associated with lower odds of MACS/high cortisol status (OR = 0.473, 95% CI: 0.304–0.737, $p = 0.001$). Larger tumor size and higher basal cortisol were also independently associated with MACS, whereas age and sex were not significant predictors (Table 4).

3.5. Receiver operating characteristic analysis

The ROC analysis was reframed as a secondary and exploratory analysis supporting the potential ancillary role of the $\text{ACTH} \times \text{DHEAS}$ product, rather than as a primary test of diagnostic discrimination or as evidence that the index could replace the DST. Because lower ACTH, lower DHEAS, and lower $\text{ACTH} \times \text{DHEAS}$ values indicate greater HPA-axis suppression, ACTH, DHEAS, and $\ln(\text{ACTH} \times \text{DHEAS})$ were sign-corrected by multiplying them by −1 before ROC analysis. This transformation did not change the biological interpretation of the original variables; rather, it aligned the direction of prediction so that higher transformed values indicated a higher probability of MACS.

The corrected $-\ln(\text{ACTH} \times \text{DHEAS})$ curve showed moderate discrimination (AUC = 0.693, 95% CI: 0.611–0.775, $p < 0.001$), followed closely by −DHEAS (AUC = 0.688, 95% CI: 0.605–0.770, $p < 0.001$). Basal cortisol showed

modest discrimination, whereas −ACTH had the lowest AUC among the evaluated parameters (Table 5 and Figure 1). Importantly, the DeLong comparison showed that the AUC difference between corrected $-\ln(\text{ACTH} \times \text{DHEAS})$ and −DHEAS alone was not statistically significant (AUC difference = 0.005; $p = 0.845$). Therefore, the composite index should not be interpreted as diagnostically superior to DHEAS alone.

The optimal threshold for the corrected $-\ln(\text{ACTH} \times \text{DHEAS})$ variable, determined by the Youden index, was approximately −6.24, corresponding to 55.7% sensitivity and 78.2% specificity. Back-transformation of this log-derived threshold corresponded to an approximate $\text{ACTH} \times \text{DHEAS}$ product of 511 on the original scale; values below this raw-scale product were consistent with greater HPA-axis suppression. Because sensitivity was limited, this threshold should be viewed as supportive rather than exclusionary.

4. Discussion

In this study, we evaluated the $\text{ACTH} \times \text{DHEAS}$ product, expressed as $\ln(\text{ACTH} \times \text{DHEAS})$, as a composite marker of HPA-axis suppression in patients with adrenal incidentalomas. The principal finding was that $\ln(\text{ACTH} \times \text{DHEAS})$ was significantly lower in patients with DST-defined MACS and was inversely associated with post-DST cortisol. In multivariable analysis, lower $\ln(\text{ACTH} \times \text{DHEAS})$ remained independently associated with post-DST cortisol $> 1.8 \mu\text{g/dL}$ after adjustment for age,

Table 4. Binary logistic regression analysis for predictors of DST-defined cortisol autonomy (*n* = 157)

Variable	B	Adjusted OR	95% CI	<i>p</i> -value
Age	0.026	1.026	0.983–1.071	0.235
Male sex	0.445	1.561	0.632–3.854	0.334
ln(ACTH × DHEAS)	−0.748	0.473	0.304–0.737	0.001
Basal cortisol (µg/dL)	0.113	1.120	1.027–1.222	0.011
Tumor size (mm)	0.084	1.087	1.041–1.136	<0.001

Notes: Binary logistic regression analysis was performed using the Enter method. Outcome variable: DST-defined cortisol status, coded as normal = 0 and high cortisol/MACS = 1. Female sex was used as the reference category. All *p*-values are two-tailed.

Abbreviations: ACTH: Adrenocorticotrophic hormone; B: Regression coefficient; CI: Confidence interval; DHEAS: Dehydroepiandrosterone sulfate; DST: Dexamethasone suppression test; MACS: Mild autonomous cortisol secretion; OR: Odds ratio.

Table 5. Comparative ROC analysis of hormonal parameters for predicting DST-defined cortisol autonomy (*n* = 157)

Variable	AUC	95% CI	<i>p</i> -value
Corrected −ln(ACTH × DHEAS)	0.693	0.611–0.775	<0.001
−DHEAS (µg/dL)	0.688	0.605–0.770	<0.001
Basal cortisol (µg/dL)	0.615	0.527–0.703	0.013
−ACTH (pg/mL)	0.598	0.510–0.687	0.034

Notes: ROC analysis was performed to evaluate the discriminatory ability of hormonal parameters for predicting DST-defined cortisol autonomy.

ACTH, DHEAS, and ln(ACTH × DHEAS) were multiplied by −1 before ROC analysis because lower original values indicate greater cortisol autonomy. AUC values with 95% CIs are presented. All *p*-values are two-tailed.

Abbreviations: ACTH: Adrenocorticotrophic hormone; AUC: Area under the curve; CI: Confidence interval; DHEAS: Dehydroepiandrosterone sulfate; DST: Dexamethasone suppression test; ROC: Receiver operating characteristic.

basal cortisol, and tumor size. These findings suggest that combined assessment of ACTH and DHEAS may provide additional biological information beyond a single post-DST cortisol value.

The diagnosis of MACS remains challenging because it relies primarily on the 1-mg DST despite the absence of a validated gold standard for clinically relevant cortisol autonomy.⁶ Current guidelines define MACS using a post-DST cortisol threshold greater than 1.8 µg/dL and recommend confirmation of ACTH independence to

establish adrenal-origin disease.⁵ However, DST results should be interpreted within a broader biological context because the test is highly sensitive but has limited specificity, and false-positive results may occur when dexamethasone exposure is inadequate or when cortisol dynamics are affected by medications, comorbidities, or assay-related factors.^{1,2,7} Ancillary markers reflecting ACTH-independent cortisol secretion may therefore be valuable in patients with borderline or intermediate DST results.

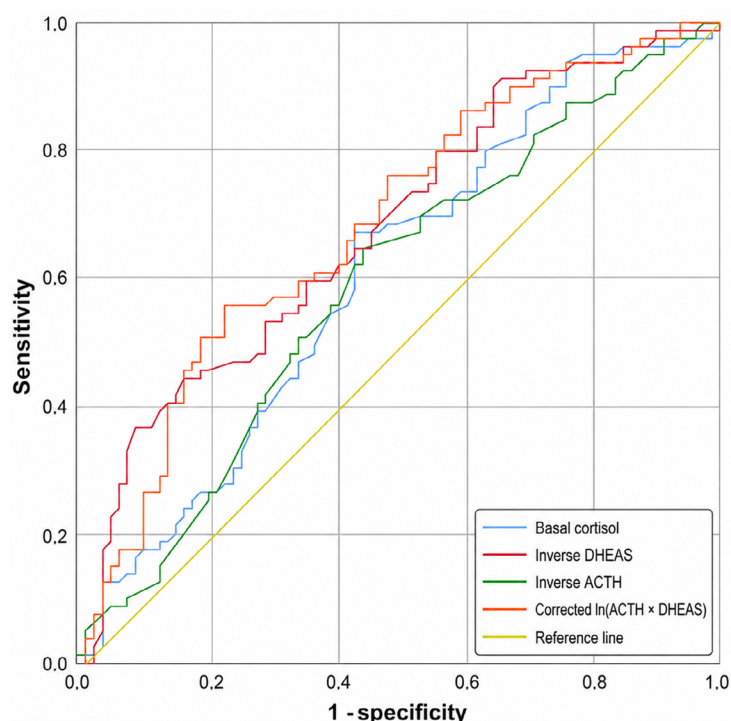


Figure 1. Comparative ROC curves for hormonal predictors of DST-defined cortisol autonomy.

Note: Inverse DHEAS and inverse ACTH were used because lower DHEAS and ACTH values reflect greater HPA suppression in cortisol autonomy. Corrected $\ln(\text{ACTH} \times \text{DHEAS})$ refers to the direction-adjusted natural-log-transformed ACTH $\times$ DHEAS product. Lower raw ACTH $\times$ DHEAS values indicate greater HPA-axis suppression; therefore, direction correction was applied for ROC visualization so that the curve was displayed in the diagnostically relevant direction. The yellow diagonal line represents the reference line. Blue line: basal cortisol. Red line: inverse DHEAS. Green line: inverse ACTH. Orange line: corrected $\ln(\text{ACTH} \times \text{DHEAS})$.

Abbreviations: ACTH: Adrenocorticotrophic hormone; CI: Confidence interval; DHEAS: Dehydroepiandrosterone sulfate; DST: Dexamethasone suppression test; HPA: Hypothalamic–pituitary–adrenal axis; ROC: Receiver operating characteristic.

Our results are consistent with the physiological concept that adrenal cortisol autonomy should suppress pituitary ACTH secretion and, over time, reduce ACTH-dependent adrenal androgen production. Cortisol exerts negative feedback at the hypothalamic–pituitary level through rapid and delayed mechanisms, including inhibition of ACTH release and POMC transcription. Accordingly, sustained adrenal cortisol secretion would be expected to lower ACTH and subsequently reduce DHEAS production.^{10,11} In the present cohort, ACTH and DHEAS were lower in patients with MACS, and $\ln(\text{ACTH} \times \text{DHEAS})$ showed stronger discrimination than ACTH alone. This supports the rationale that a composite marker may better reflect chronic HPA-axis suppression than ACTH alone, although the ROC findings did not demonstrate superior discriminative performance compared with DHEAS alone.

Previous studies have evaluated ACTH or DHEAS as supportive markers of cortisol autonomy, but their diagnostic utility as standalone tests remains limited.

ACTH is central to confirming ACTH-independent cortisol secretion, yet it is influenced by pulsatility, preanalytical variability, assay differences, and overlap between MACS and nonfunctioning adrenal adenomas.¹⁵ DHEAS may better reflect chronic ACTH suppression and has shown useful negative predictive value in selected populations, particularly for excluding MACS, but it is also influenced by age, sex, and adrenal reserve.^{13,16} In line with these observations, ACTH alone showed the lowest ROC performance in our analysis, whereas DHEAS performed better and corrected $-\ln(\text{ACTH} \times \text{DHEAS})$ showed the highest, although still moderate, discriminative ability.

The multiplicative ACTH $\times$ DHEAS product should be interpreted mechanistically. Both ACTH and DHEAS were expected to decline when ACTH-independent cortisol secretion suppressed the HPA axis. Therefore, a low product reflects concordant suppression at the pituitary and adrenal androgen levels. This is different from a ratio, which is most useful when two variables move in opposite

directions or when one variable is used to normalize another. In MACS, however, both components move in the same direction. A summed z-score approach could be attractive in future studies, particularly if harmonized age- and sex-specific DHEAS reference distributions are available, but it was not feasible as the primary approach in this retrospective clinical dataset.

The moderate ROC performance of corrected $-\ln(\text{ACTH} \times \text{DHEAS})$ should be interpreted cautiously. The AUC of 0.693 was only slightly higher than that of $-\text{DHEAS}$ alone, and the DeLong comparison did not show a statistically significant difference. Therefore, the present study does not support the use of $\text{ACTH} \times \text{DHEAS}$ as a superior diagnostic marker compared with DHEAS alone. Rather, the index may serve as a physiologically integrated ancillary marker that captures two linked consequences of chronic cortisol autonomy. This distinction is important because the proposed index is intended to refine biological interpretation, not to replace existing guideline-based testing. Therefore, the ROC findings should be interpreted as exploratory and hypothesis-generating. They support the potential ancillary value of the $\text{ACTH} \times \text{DHEAS}$ product as a biologically integrated marker of HPA-axis suppression, but do not establish it as a standalone diagnostic test.

The proposed threshold also requires caution. The corrected $\ln(\text{ACTH} \times \text{DHEAS})$ cutoff of approximately 6.24, corresponding to an $\text{ACTH} \times \text{DHEAS}$ product of about 511 on the raw scale, showed higher specificity than sensitivity. This means that a low value may support the presence of biologically relevant HPA-axis suppression, but a higher value cannot exclude MACS. The threshold is therefore cohort-specific, exploratory, and unsuitable for clinical adoption without external validation. This limitation is particularly important because MACS exists on a continuum and because threshold-based approaches may oversimplify the biological heterogeneity of adrenal cortisol autonomy.

A further issue is that DST-defined categories do not necessarily capture the full biological spectrum of cortisol autonomy. The 1-mg DST remains the cornerstone of biochemical assessment, but it reflects an acute pharmacological feedback response and may be influenced by dexamethasone exposure, metabolism, assay-related factors, and comorbid conditions. Therefore, a mildly elevated post-DST cortisol value does not always prove sustained or clinically meaningful cortisol autonomy, whereas a value within the nonfunctional range may not completely exclude subtle or intermittent low-grade cortisol secretion over time.¹² This distinction is particularly relevant because cardiometabolic comorbidities are common in patients with adrenal incidentalomas, including those

classified as nonfunctional, and their presence alone does not establish cortisol causality. In this context, basal ACTH and DHEAS may provide complementary information because they are not post-dexamethasone measurements and may reflect more sustained HPA-axis feedback suppression.

From a practical clinical perspective, the 1-mg DST should remain the first-line biochemical test for evaluating cortisol autonomy in adrenal incidentalomas. The $\text{ACTH} \times \text{DHEAS}$ product should not be used as a screening test or to exclude MACS because of its limited sensitivity. Instead, it may be most useful in patients with borderline or intermediate post-DST cortisol values, or in those with discordant biochemical and clinical findings. In such cases, a low $\text{ACTH} \times \text{DHEAS}$ product may provide supportive evidence of sustained HPA-axis suppression and may justify closer metabolic and skeletal follow-up.

Future studies should therefore evaluate whether $\text{ACTH} \times \text{DHEAS}$, DHEAS alone, or integrated multi-marker models are more closely associated with hypertension, diabetes, osteoporosis, cardiovascular outcomes, postoperative adrenal insufficiency, and clinical benefit after adrenalectomy than DST-based categories alone.

The issue of dexamethasone exposure is also clinically relevant. Insufficient dexamethasone absorption or accelerated dexamethasone metabolism can cause false-positive DST results, and serum dexamethasone concentrations were not available in this retrospective study.¹⁷ However, basal ACTH and DHEAS were not post-dexamethasone measurements; they were routine basal markers obtained independently of the acute dexamethasone challenge. Therefore, concordantly low basal ACTH and DHEAS are less likely to be explained solely by poor dexamethasone exposure during the DST. This provides indirect physiological support for chronic HPA-axis suppression, although it cannot replace prospective confirmation using measured dexamethasone levels or independent measures of cortisol dynamics.

A central clinical problem in MACS is determining whether DST-defined cortisol autonomy is biologically and clinically meaningful in an individual patient. Cardiometabolic and skeletal comorbidities such as hypertension, type 2 diabetes mellitus, obesity, cardiovascular disease, and osteoporosis are common both in MACS and in the older population in whom adrenal incidentalomas are frequently detected.^{1,2,4,9,18} Therefore, their coexistence with an adrenal adenoma does not necessarily establish cortisol-related morbidity. This uncertainty is reinforced by the absence of a validated gold standard for clinically relevant cortisol autonomy and by the interpretation of DST values as a continuum rather

than a strict dichotomy.^{4,6} Although current guidelines define MACS using a post-DST cortisol threshold > 1.8 µg/dL and recommend confirmation of ACTH independence, there is no universally accepted upper boundary that determines when cortisol autonomy becomes clinically actionable.^{2,5}

Previous research supports the use of additional markers such as late-night salivary cortisol, ACTH, and DHEAS to improve the identification of patients with subtle cortisol autonomy and cardiometabolic risk.^{12,19} In surgical studies, adrenalectomy decisions have incorporated additional evidence of HPA-axis dysregulation, including suppressed ACTH, abnormal midnight cortisol, elevated late-night salivary cortisol, or mildly increased urinary free cortisol.²⁰ The postoperative improvement in cortisol-related comorbidities, together with the frequent occurrence of postoperative adrenal insufficiency in such patients, provides indirect evidence of sustained and biologically relevant cortisol autonomy.²¹

In this context, lower ACTH × DHEAS may provide mechanistic support for chronic HPA-axis suppression and may help identify patients in whom comorbidities are more plausibly related to sustained cortisol autonomy rather than coincidental age-related disease. Therefore, an important hypothesis for future studies is whether patients with lower ACTH × DHEAS values have a higher burden or progression of hypertension, type 2 diabetes mellitus, cardiovascular disease, or osteoporosis, and whether this marker predicts postoperative adrenal insufficiency or clinical benefit after adrenalectomy. Such studies may help move the field beyond reliance on a single DST threshold toward a biologically informed model that better distinguishes biochemical MACS from clinically meaningful cortisol excess.²²

The tumor-size variable also warrants consideration. Tumor size was greater in patients with MACS and remained independently associated with DST-defined cortisol autonomy. This finding is compatible with the concept that larger adenomas may have greater steroidogenic capacity or may be more likely to produce MACS. Nevertheless, tumor size is not a specific marker of cortisol biology, and it should not be interpreted in isolation. The inverse correlation between $\ln(\text{ACTH} \times \text{DHEAS})$ and larger tumor size suggests that larger lesions may be associated with stronger HPA-axis suppression in some patients, but this association remains indirect and should be tested in prospective cohorts with standardized imaging, steroid profiling, and longitudinal outcomes.

The age and sex dependence of DHEAS is another important consideration.²³ In the present cohort, women

accounted for 76.4% of the sample, reflecting the sex distribution commonly observed in adrenal incidentaloma cohorts but may limit generalizability. We therefore added sex to the revised regression model. The persistence of the association between $\ln(\text{ACTH} \times \text{DHEAS})$ and MACS after adjustment for age and sex supported the robustness of the signal within this cohort. However, statistical adjustment is not the same as reference-based standardization. Future studies should ideally collect the lower and upper limits of age- and sex-specific DHEAS reference intervals for each patient, calculate standardized DHEAS scores, and compare raw DHEAS, reference-adjusted DHEAS, ACTH, and composite indices within a single analytic framework.

Another reason to avoid overinterpreting the ROC analysis is that the outcome was itself defined as MACS based on DST results. A biomarker may show good agreement with DST-defined classification without necessarily predicting clinical morbidity or treatment benefit. Conversely, a marker with only moderate discrimination against DST may still provide biologically relevant information if it identifies a subgroup with sustained HPA-axis suppression or postoperative adrenal insufficiency.²⁰ For this reason, the current findings should be regarded as hypothesis-generating. The most clinically meaningful validation would not be a higher AUC against the DST alone, but demonstration that the marker improves prediction of cortisol-related comorbidities, progression over time, or postoperative outcomes beyond established clinical and biochemical variables.

The need for outcome-based validation is central to the interpretation of this study. Because the dependent variable in the present analysis was DST-defined cortisol autonomy, the results demonstrated biochemical concordance rather than proof of clinical benefit. A marker that is mechanistically plausible and independently associated with DST status may still fail to identify the subgroup of patients who develop progressive hypertension, worsening glycemic control, fractures, cardiovascular events, postoperative adrenal insufficiency, or measurable benefit after adrenalectomy.²¹ Future prospective studies should therefore evaluate whether lower ACTH × DHEAS values predict these clinically meaningful endpoints independently of post-DST cortisol and DHEAS alone. Such studies should ideally include serum dexamethasone concentrations, repeated hormonal testing, age- and sex-standardized DHEAS measures, medication review, and longitudinal assessment of cortisol-related comorbidities. This will allow the ACTH × DHEAS product to be tested not only as a biochemical correlate of MACS, but also as a candidate component of a probabilistic model for clinically meaningful cortisol autonomy.

A future multi-marker approach can incorporate post-DST cortisol as a continuous variable, basal ACTH, DHEAS or age- and sex-standardized DHEAS, late-night salivary cortisol, urinary free cortisol when appropriate, dexamethasone concentration, tumor size and imaging phenotype, and patient-level comorbidity burden. Such a model will better reflect the probabilistic nature of MACS than a single dichotomous cutoff. Within this type of framework, ACTH × DHEAS can function as a mechanistic marker of chronic feedback suppression rather than as an independent diagnostic endpoint. This conceptual role is consistent with the clinical reality that decisions about follow-up or adrenalectomy are individualized and depend on biochemical severity, comorbidities, surgical risk, patient preference, and expected reversibility of cortisol-related complications.

This study has several limitations. Its retrospective and cross-sectional design prevents assessment of whether lower $\ln(\text{ACTH} \times \text{DHEAS})$ predicts incident cardiometabolic outcomes, postoperative adrenal insufficiency, or long-term morbidity. DST-defined cortisol status was used as the outcome, so the analysis evaluated concordance with current biochemical classification rather than performance against an independent clinical gold standard. Dexamethasone levels, cortisol awakening response, detailed medication effects, and standardized prospective comorbidity outcomes were not available. ACTH and DHEAS may be influenced by analytical and biological factors, and formal external age- and sex-specific DHEAS z-scores could not be calculated from the available retrospective data. Although sex was added to the revised regression model, this adjustment did not replace the need for validation using standardized reference-based DHEAS metrics. Finally, the proposed threshold (approximately 6.24 for corrected $\ln[\text{ACTH} \times \text{DHEAS}]$ and approximately 511 for ACTH × DHEAS) was derived from a single cohort and should not be applied clinically without external validation.

5. Conclusion

Adrenal incidentalomas are increasingly encountered in clinical practice, particularly with wider use of cross-sectional imaging and population aging, and MACS represents the most common form of adrenal hormonal hyperfunction in this setting. However, deciding which patients require closer follow-up or active treatment remains challenging, because hypertension, type 2 diabetes mellitus, cardiovascular disease, and osteoporosis are frequent in the same older population and cannot always be attributed to cortisol autonomy on the basis of post-DST cortisol alone. In this study, $\ln(\text{ACTH} \times \text{DHEAS})$ was

lower in patients with DST-defined cortisol autonomy and remained independently associated with elevated post-DST cortisol after adjustment for age, sex, basal cortisol, and larger tumor size, supporting its biological relevance as a marker of altered HPA-axis feedback.

By integrating pituitary ACTH suppression and downstream DHEAS reduction, this composite index may provide a simple mechanistic adjunct to DST interpretation and help refine the phenotyping of cortisol autonomy in adrenal incidentalomas. However, its moderate AUC, lack of significant improvement over DHEAS alone, and limited sensitivity indicated that it should not be used as a standalone diagnostic test or as a rule-out marker. Future research should move toward multi-marker, probabilistic models of clinically meaningful MACS, in which ACTH × DHEAS may serve as one component of integrated HPA-axis phenotyping, alongside DST results, ACTH independence, clinical comorbidities, dexamethasone exposure, and longitudinal outcomes. Prospective outcome-based studies are needed to determine whether lower ACTH × DHEAS identifies patients with clinically meaningful cortisol-related morbidity or benefit from adrenalectomy.

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Conflict of interest

The authors declare they have no competing interests.

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Conceptualization: All authors

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Katip Celebi University in Izmir (approval number: 0277, approval date: May 23, 2024). Because of the retrospective design, the ethics committee waived the requirement for informed consent. All data were extracted from hospital records and anonymized before analysis.

Consent for publication

Not applicable.

Availability of data

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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