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Permalink

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Journal

Hormone and Metabolic Research, 48(10)

ISSN

0018-5043

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Publication Date

2016-09-01

DOI

10.1055/s-0042-115644

Peer reviewed

Diurnal Plasma Cortisol Measurements Utility in Differentiating Various Etiologies of Endogenous Cushing Syndrome

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Abstract

Cortisol diurnal variation may be abnormal among patients with endogenous Cushing syndrome (CS). The study objective was to compare the plasma cortisol AM/PM ratios between different etiologies of CS. This is a retrospective cohort study, conducted at a clinical research center. Adult patients with CS that underwent adrenalectomy or trans-sphenoidal surgery (n = 105) were divided to those with a pathologically confirmed diagnosis of Cushing disease (n = 21) and those with primary adrenal CS, including unilateral adrenal adenoma (n = 28), adrenocortical hyperplasia (n = 45), and primary pigmented nodular adrenocortical disease (PPNAD, n = 11). Diurnal plasma cortisol measurements were obtained at 11:30 PM and midnight and at 7:30 and 8:00 AM. The ratios between the mean morning levels and mean late-night levels were calculated. Mean plasma cortisol AM/PM ratio was lower among CD patients compared to those with primary adrenal CS (1.4 ± 0.6 vs. 2.3 ± 1.5, p < 0.001, respectively). An AM/PM cortisol ratio 2.0 among patients with unsuppressed ACTH (> 15 pg/ml) excludes CD with a 85.0 % specificity and a negative predictive value (NPV) of 90.9 %. Among patients with primary adrenal CS, an AM/PM cortisol 1.2 had specificity and NPV of 100 % for ruling out a diagnosis of PPNAD. Plasma cortisol AM/PM ratios are lower among patients with CD compared with primary adrenal CS, and may aid in the differential diagnosis of endogenous hyper-cortisolemia.

Keywords

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Introduction

The diagnostic algorithm for Cushing syndrome (CS) is divided into 2 separate stages, starting with establishing the diagnosis of endogenous CS, followed by identifying its cause [1]. The hypercortisolemia might evolve from either ACTH dependent sources, namely a pituitary neoplasm or an ectopic ACTH or CRH secreting tumor, or from primary adrenal cortisol secretion [2]. Both steps present a clinical challenges; the diagnosis may be elusive due to the combination of nonspecific clinical presentation and the rare incidence of the syndrome [1, 3], while identification of the source of hypercortisolemia is complicated by the overlap between the biochemical profiles of the different CS etiologies [4]. Normal cortisol secretion follows a diurnal variation, with plasma levels peaking shortly after awakening in the morning, and decreasing thereafter reaching a nadir around midnight [5, 6]. This pattern is maintained by changes in ACTH secretion amplitude throughout the day [7]. Diminished diurnal cortisol variation among patients without CS has been associated with cardiovascular morbidity and mortality [8, 9]. The diminished diurnal cortisol variation found among patients with CS [5] has led to the use of late-night salivary cortisol in the screening for CS [10] and to its implementation in the CS diagnostic algorithm [1].

Single midnight plasma cortisol was suggested as a rule-out test for CS [11, 12], with limitations [13, 14]. However, the ratio between morning and late-night plasma cortisol levels (AM/PM plasma cortisol ratio), as a measure for diurnal variation have not been evaluated as a diagnostic tool. Moreover, the patterns of plasma cortisol diurnal variation in different etiologies of CS have not been extensively described. Hence, in the current analysis we assessed the plasma cortisol AM/PM ratios in various etiologies of endogenous CS, and evaluated the utility of these measurements for the diagnosis of endogenous CS.

Patients and Methods

We conducted a retrospective cohort study, including consecutive patients that were admitted to the National Institutes of Health for evaluation of suspected CS between 1995 and 2015. All patients were recruited through clinical protocols 97-CH-0076, 95-CH-0059, and 00-CH-0160 conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and gave written informed consent. All patients were hospitalized and underwent screening tests for CS, including serial 24-h urinary free cortisol (UFC) collections and diurnal plasma cortisol measurements. Diurnal plasma cortisol measurements were performed during an in-house evaluation, using an indwelling venous catheter with designated extension to preclude sleep disturbance during the night measurements. Blood samples were drawn at 7:30 and 8:00 AM for the morning measurements and at 11:30 PM and midnight for the late-night samples. Calculated mean values of the AM and PM samples, respectively, were used for the final analysis. Serum cortisol was measured by fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL, USA) with an intra- and interassay coefficients of variation (CV) of 2.1 and 4.1 %, respectively. The current analysis included adult patients with either elevated 24 h UFC, insufficient suppression of morning plasma cortisol after dexamethasone suppression (> 1.8 mcg/dl) or elevated midnight plasma cortisol (> 1.8 mcg/dl). Patients diagnosed with CS underwent further investigation in order to locate the source of hypercortisolemia.

Patients then went on to surgical intervention, either adrenalectomy or trans-sphenoidal surgery, as appropriate.

All pathological reports were reviewed, CD was defined as a pituitary adenoma positively stained to ACTH, and adrenal lesions were defined as previously described [15]. Patients with adrenal hyperplasia included 28 with macronodular hyperplasia (multiple nodules > 1 cm each) and 17 subjects with micronodular disease. Most of the patients with adrenocortical hyperplasia (31/41) had radiological evidence for bilateral disease. Patients with macro- and micronodular disease had comparable 24-h UFC collections, diurnal plasma cortisol values and AM/PM ratio, and were analyzed as a single group. Primary pigmented nodular adrenocortical disease (PPNAD) was defined as micronodular hyperplasia in the presence of pigment (lipofuscin) and consisted a separate group.

Statistical analysis

Statistical calculations were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± standard deviation (SD) unless otherwise indicated. For group comparisons, the independent Student's *t*-test or one-way analysis of variance (ANOVA) was used to analyze differences in numerical variables, the chi-square test was employed to analyze differences in categorical variables. The Pearson product was used for analysis of correlations between variables. The receiver operating characteristic (ROC) curve method was used to decide on the ideal AM/PM cortisol ratio cutoff for ruling out the diagnosis of CD among the study population and PPNAD among patients with primary adrenal CS. The p-value for significance was set at less than 0.05.

Results

The current analysis included 105 patients, aged 44.5 ± 13.4 years, with 72 females (68.6 %). Twenty one patients (20.0 %) had CD, 28 (26.7 %) had adrenal adenoma, 45 (42.9 %) had nodular adrenocortical hyperplasia, and 11 (10.5 %) had PPNAD.

For the full cohort, mean morning and midnight plasma cortisol measurements were 14.6 ± 6.4 and 10.4 ± 7.9 mcg/dl, respectively, and the calculated mean AM/PM cortisol ratio was 2.1 ± 1.4. Concordant morning and midnight mean ACTH levels were 23.6 ± 27.7 and 20.5 ± 41.4 pg/ml, respectively, with a mean AM/PM ACTH ratio of 1.8 ± 1.5. Mean 24-h UFC collections were 6.1 ± 20.9 times the upper limit of normal range (× ULN), with 22 patients (21.0 %) having UFC levels > 5 × ULN. Demographic and biochemical characteristics according to the patient's diagnoses are depicted in Table 1.

CD vs. primary adrenal CS

Patients with CD (n = 21) had higher morning (20.8 ± 7.8 vs. 13.1 ± 5.0 mcg/dl, p < 0.001) and midnight plasma cortisol levels (17.3 ± 8.5 vs. 8.7 ± 6.8 μg/dl, p < 0.001) compared with patients with primary adrenal CS of any etiology (n = 84), respectively. Both morning (60.3 ± 43.4 vs. 15.8 ± 14.4, p = 0.006) and midnight ACTH plasma levels (61.5 ± 41.0 vs. 8.3 ± 4.9, p = 0.002) were higher among patients with CD compared to those with an adrenal source for CS, respectively.

Mean plasma cortisol AM/PM ratio was lower among CD patients compared to those with corticotropin-independent adrenal CS (1.4 ± 0.6 vs. 2.3 ± 1.5, $p < 0.001$), and the calculated AM/PM ratio for plasma ACTH levels had similar trend (1.1 ± 0.5 vs. 2.0 ± 1.6, $p = 0.07$), respectively. Comparison of plasma cortisol AM/PM ratio according to patients' diagnoses is shown in Fig. 1.

We further evaluated the utility of AM/PM ratio in patients with plasma ACTH > 15 pg/ml. Among those patients, an AM/PM cortisol ratio 2.0 excludes CD with a 85 % specificity and 80 % sensitivity, yielding a negative predictive value (NPV) of 90.9 % (Fig. 2a).

Plasma cortisol AM/PM ratio according to 24-h UFC collections

Patients with CD had higher mean 24-h UFC collections compared with primary adrenal CS patients (12.8 ± 15.5 vs. $4.4 \pm 21.9 \times$ ULN, $p < 0.001$, respectively). Moreover, 14 patients with CD (66.7 %) had 24-h UFC collection measurements $> 5 \times$ ULN, compared with only 8 patients (9.5 %) from the primary adrenal CS group ($p < 0.001$). Mean AM/PM plasma cortisol ratios were higher among patients with 24-h UFC collections $< \times 5$ ULN both in the CD group (1.8 ± 0.9 vs. 1.2 ± 0.4, $p = 0.03$) and the primary adrenal CS group (2.4 ± 1.6 vs. 1.0 ± 0.1, $p = 0.002$, respectively; Fig. 3)

ACTH levels and AM/PM plasma cortisol ratio

Midnight ACTH levels negatively correlated ($r = -0.6$, $p = 0.03$) negatively with AM/PM cortisol ratio among patients with CD ($n = 12$), but positively ($r = 0.3$, $p = 0.03$) among those with primary adrenal CS ($n = 64$). Morning ACTH plasma levels correlated positively with AM/PM cortisol ratio among patients with primary adrenal CS in general ($r = 0.5$, $p < 0.001$), and specifically among subjects harboring adrenal adenoma ($r = 0.8$, $p < 0.001$) or adrenocortical hyperplasia ($r = 0.5$, $p = 0.02$). In contrast, no correlation was found between morning ACTH levels and AM/PM cortisol ratio among patients with CD.

Plasma cortisol AM/PM ratio among patients with PPNAD

Patients with PPNAD ($n = 11$) had a calculated AM/PM cortisol ratio ranging between 0.8 and 1.1 (Table 1). The mean AM/PM plasma cortisol ratio of patients with PPNAD was lower compared to either CD ($p = 0.005$), adrenal adenoma ($p < 0.001$), and nodular adrenocortical hyperplasia ($p < 0.001$; Table 1 and Fig. 1). Moreover, an AM/PM cortisol cutoff of 1.1 or 1.2 had specificity of 82 and 100 % for ruling-out PPNAD diagnosis among patients with primary adrenal CS, yielding a NPVs of 96.8 and 100 %, respectively (Fig. 2b).

ACTH levels among PPNAD patients were undetectable (< 5 pg/ml) in most measurements, both late-night (7/7) and morning (6/7) samples. These findings are even more striking considering the wide range of 24-h UFC measurements among PPNAD patients in our cohort (range, $1.7–13.7 \times$ ULN).

Discussion

In the current analysis, we evaluated the diurnal cortisol variation among patients with different etiologies of CS using the ratio between AM and PM plasma cortisol measurements. We found lower plasma cortisol AM/PM ratio among patients with CD compared with those with primary adrenal CS. This difference can be utilized in practice, as among patients with unsuppressed ACTH (> 15 pg/ml) a cortisol AM/PM ratio 2.0 excludes CD with a NPV of 90.9 %. ACTH negatively correlated with AM/PM cortisol ratio among CD patients, whereas among subjects with primary adrenal CS we found a positive correlation. This correlation was consistent among subjects with adrenal adenoma or adrenocortical hyperplasia but not among those with PPNAD. Moreover, patients with PPNAD had consistently low plasma cortisol AM/PM ratio that enables its use as a rule-out test (cutoff, 1.2) with a NPV of 100 % (Fig. 2b). Finally, we have shown that lower 24-h UFC collections were associated with higher AM/PM cortisol ratios both among patients with CD and primary adrenal CS (Fig. 3).

Plasma ACTH levels are essential for the distinction between ACTH-dependent and ACTH-independent causes of CS. However, a significant number of patients with autonomous cortisol secretion from the adrenals have nonsuppressed ACTH levels [2]. According to our analysis, patients with ACTH > 15 pg/ml whose source of hypercortisolemia is uncertain may be evaluated using diurnal plasma cortisol measurements: even a minimal preservation of AM/PM cortisol ratio effectively excludes the diagnosis of CD with high predictability (Fig. 2a).

On the other hand, patients with PPNAD in our analysis had minimal diurnal variation, and significantly lower mean plasma cortisol AM/PM ratio compared with other etiologies of ACTH-independent adrenal CS (Fig. 1). As a direct practical consequence, even mildly elevated AM/PM ratios (1.2) effectively exclude the diagnosis of PPNAD (NPV of 100 %, Fig. 2b). A possible mechanism for these differences is the lack of sufficient amount of unaffected adrenocortical tissue in within the PPNAD-affected cortex, a disease that is caused by germline mutations in the PRKAR1A gene [16]. This is in contrast to the development of other adrenal secreting neoplasms that may develop within (and are surrounded by) pre-existing normal tissue as the result of a somatic genetic event [17, 18] even where there are germline defects (different than in the *PRKAR1A* one) [19].

We found lower mean AM/PM cortisol ratio among patients with CD compared to those with primary adrenal CS (Fig. 1). This difference might be explained by the higher mean 24-h UFC collections among CD patients, with tighter suppression of the corticotroph function. This finding is also supported by the lower plasma cortisol AM/PM ratio among patients with higher 24-h UFC collections, both in the CD or primary adrenal CS groups. The diminished differences in AM/PM cortisol ratio between the etiologies after stratification by UFC levels might support this theory (Fig. 3), although the small sample size limits our ability to conclude. Other possible mechanisms for this difference include the presence of clock genes in the adrenal cortex [20].

ACTH levels among patients with adrenocortical adenoma or hyperplasia positively correlated with plasma cortisol AM/PM ratio. Among those patients ACTH levels probably indicated the functional level of the hypothalamic-pituitary-adrenal (HPA) axis. In contrast, the negative correlation between ACTH levels and plasma cortisol AM/PM ratio among CD patients stems from the additive effect of autonomous secretion from the pituitary adenoma that lacks diurnal variation [21], and the suppressive effect of high cortisol levels on the HPA axis activity.

Our study has several strengths. First, our data on diurnal cortisol levels are based on the mean of 2 samples at each time point (AM and PM), reducing a possible error. Second, the diagnoses of all the study subjects are supported by their pathological reports. Third, all diagnostic procedures have been performed uniformly under strict research protocols. Our main study limitation is the lack of patients with ectopic ACTH- secreting neoplasms.

In conclusion, plasma cortisol AM/PM ratios are lower among patients with CD compared with primary adrenal CS, and can assist in the diagnostic process of CS. Moreover, plasma cortisol AM/PM ratio is a valid rule-out clinical tool for CD among patients with unsuppressed (> 15 pg/ml) ACTH levels, and for PPNAD among patients with Corticotropin independent adrenal CS.

Acknowledgements

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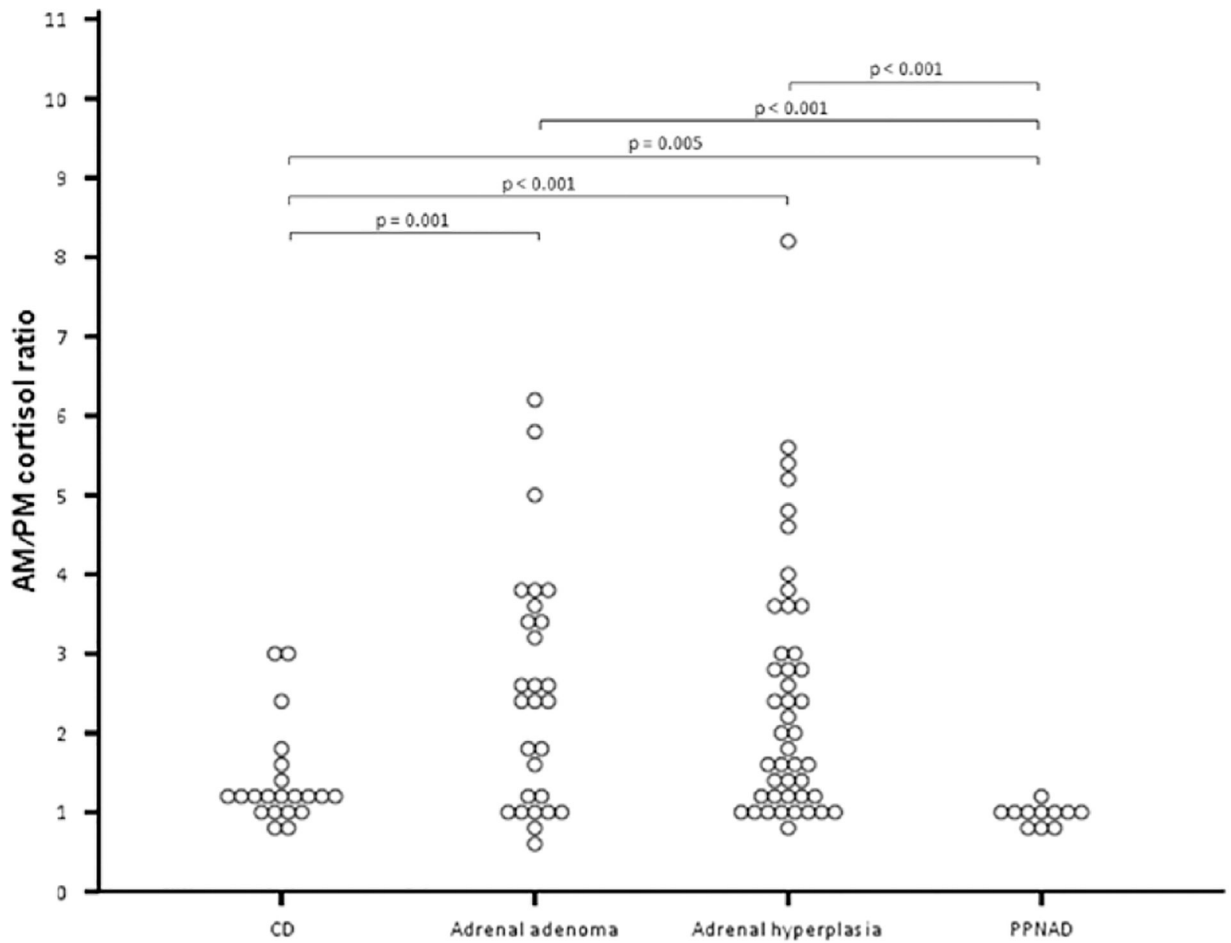
Fig. 1.
Comparison of plasma cortisol AM/PM ratio values according to patient's diagnoses. CD: Cushing's disease; PPNAD: Primary pigmented nodular adrenocortical disease.
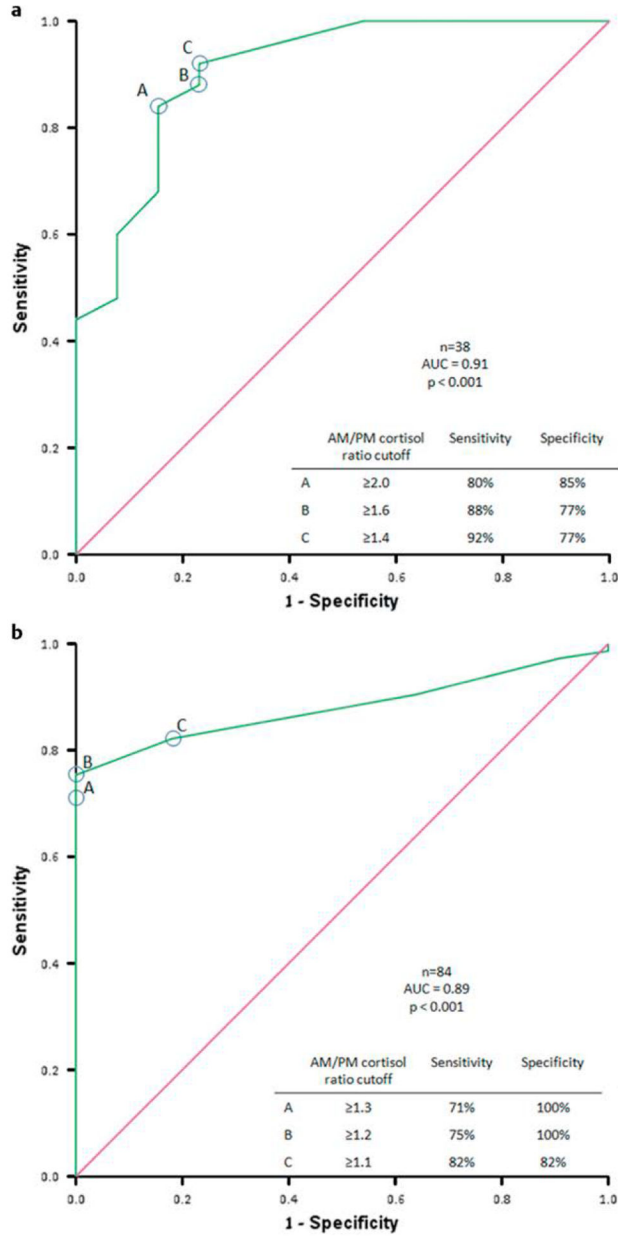
Fig. 2.
ROC curve analysis for the utility of AM/PM cortisol ratio in distinguishing primary adrenal CS from CD * **a** and PPNAD from other etiologies of primary adrenal CS **b**. * Included only patients with ACTH > 15 pg/ml. [+] Included only patients with elevated 24-h urinary free cortisol collections. ROC: receiver operator characteristic; CD: Cushing's disease; CS: Cushing's syndrome; PPNAD: Primary pigmented nodular adrenocortical disease; AUC. Area under the curve.
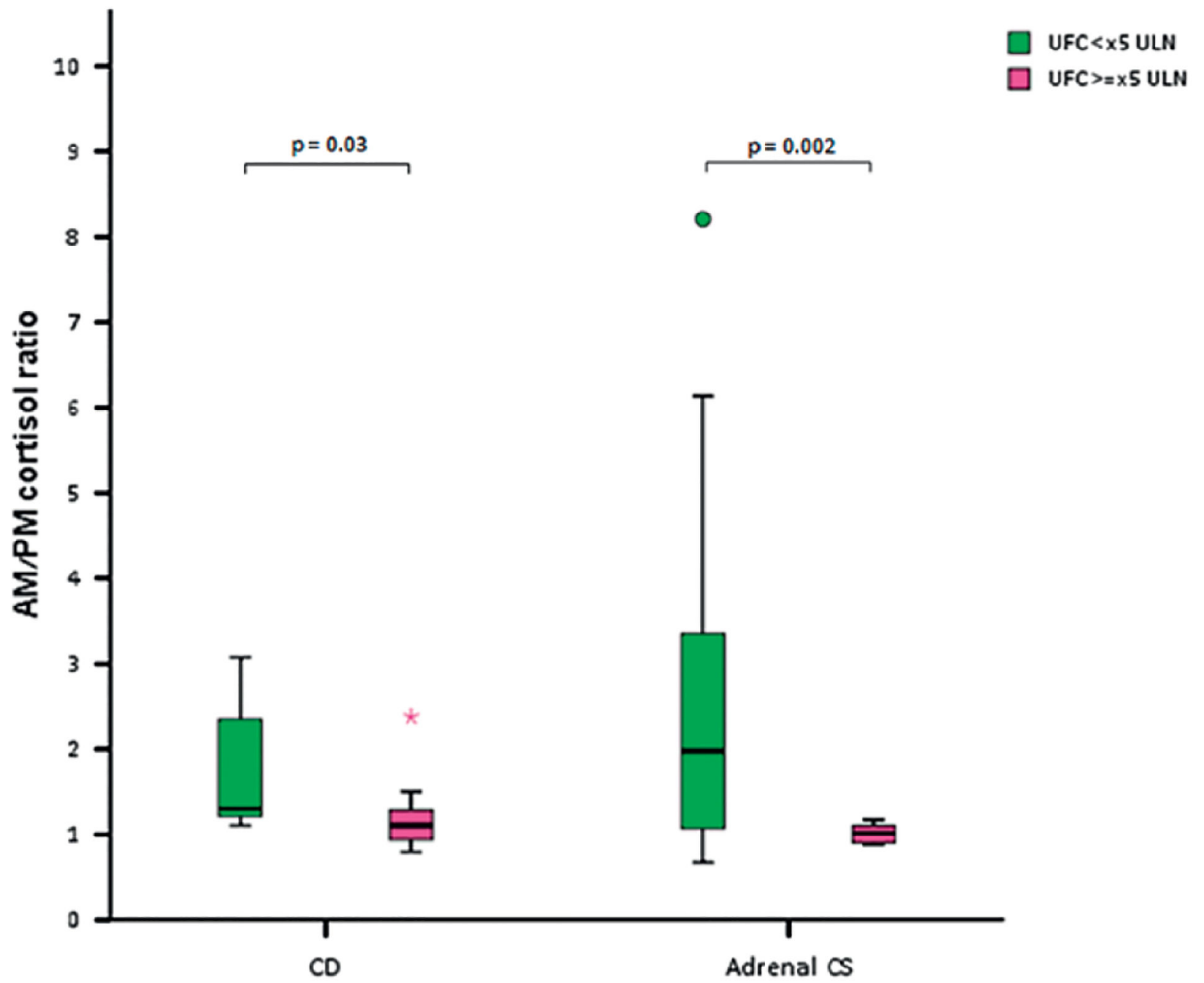
Fig. 3.
Comparison of plasma cortisol AM/PM ratio among patients with CD or primary adrenal CS, according to 24-h UFC collections. ACTH: Adrenocorticotropic hormone; CD: Cushing's disease; CS: Cushing's syndrome; UFC. 24-h urinary free cortisol; ULN-Upper limit of the reference range.

Table 1

Patients' demographic and baseline biochemical characteristics according to patients' diagnoses.

	CD	Adrenal adenoma	Adrenal nodular hyperplasia	PPNAD	p-Value
	n=21	n=28	n = 45	n = 11	
Female gender n (%)	15 (71.4)	21 (75.0)	30 (66.7)	6 (54.5)	NS
Age (years)	36.2 ± 14.9	46.6 ± 12.2	50.0 ± 10.5	32.9 ± 9.0	< 0.001
Ethnicity					
Caucasian	15 (71.4)	18 (64.3)	23 (51.1)	8 (72.7)	0.001
African-American	0	5 (17.9)	14 (31.1)	0	
Asian	2 (9.5)	2 (7.1)	2 (4.4)	0	
Other/unknown	4 (19.1)	3 (10.7)	6 (13.2)	3 (27.3)	
Cortisol					
AM plasma levels (µg/dl)	20.8 ± 7.8	12.9 ± 4.7	12.0 ± 4.1	17.9 ± 6.9	< 0.001
PM plasma levels (µg/dl)	17.3 ± 8.5	7.4 ± 5.7	7.1 ± 5.1	18.7 ± 7.3	< 0.001
AM/PM ratio	1.4 ± 0.6	2.5±1.5	2.4 ± 1.6	1.0 ± 0.1	< 0.001
ACTH					
AM plasma levels (µg/ml)	60.3 ± 43.4	18.3 ± 17.0	16.8 ± 13.5	5.8 ± 2.2	< 0.001
PM plasma levels (µg/ml)	80.2 ± 77.9	8.6 ± 5.6	8.8 ± 4.7	5.0 ± 0.0	< 0.001
AM/PM ratio	1.0 ± 0.6	2.1 ± 1.9	2.4 ± 1.6	1.2±0.4	NS
24 h UFC					
Mean level (× ULN)	12.8 ± 15.5	9.1 ± 37.7	1.6 ± 1.3	4.2 ± 3.4	NS
× 5 ULN n (%)	14 (66.7)	4 (14.3)	1 (2.2)	3 (27.3)	< 0.001

Data are presented as mean ± SD unless mentioned otherwise

CD: Cushing's disease; PPNAD: Primary pigmented nodular adrenocortical disease; ACTH: adrenocorticotropic hormone; UFC: Urinary free cortisol; ULN: Upper limit of the reference range; NS: Not significant

Comparisons were performed using ANOVA and chi square tests for continuous and categorical variables, respectively