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ORIGINAL ARTICLE

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Cardiometabolic profile of non-functioning and autonomous cortisol-secreting adrenal incidentalomas. Is the cardiometabolic risk similar or are there differences?

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Abstract

Objective To study the differences in the cardiometabolic profile between patients with non-functioning adrenal incidentalomas (NFAI) and incidentalomas with autonomous cortisol secretion (ACS).

Methods A total of 149 patients with adrenal incidentalomas were retrospectively evaluated and followed-up for a mean time of 34.6 months at Departments of Endocrinology and Metabolic Diseases Units of four tertiary Spanish hospitals. Patients were grouped as NFAI or ACS adenomas based on two cutoffs in the dexamethasone suppression test (DST): 3.0 µg/dl ($NFAI_{DST3}$ or ACS_{DST3}) and 1.8 µg/dl ($ACS_{DST1.8}$ and $NFAI_{DST1.8}$).

Results The mean age of both groups was 62.0 (10.31) and was similar in ACS and NFAI. The prevalence of diabetes, high blood pressure, cardiovascular, and cerebrovascular disease was higher in ACS than in NFAI, but differences only reached statistical significance for cerebrovascular disease using the 3.0 µg/dl cutoff (15.8% vs 2.3%, $p = 0.01$) and for diabetes using the 1.8 µg/dl cutoff (38.0% vs 22.0%, $p = 0.04$). No differences were found in the prevalence of dyslipidemia. The prevalence of obesity was lower in patients with ACS than in NFAI 26.3% vs 39.2%, $p = 0.18$ ($NFAI_{DST3}$ vs ACS_{DST3}) and 32.1% vs 40.6%, $p = 0.56$ ($ACS_{DST1.8}$ vs $NFAI_{DST1.8}$), but the differences did not reach statistical significance. Maximum adenoma diameter (R-squared = 0.15, $p < 0.001$) and cerebrovascular disease (OR = 1.59, $p = 0.04$) were the only parameters that could be predicted by the DST. The DST was an inadequate predictor of clinical (systolic and diastolic blood pressure, body mass index), hormonal (DHEAS, ACTH, UFC, and basal serum cortisol), biochemical (glucose, cholesterol, LDL, HDL, and triglycerides), and other radiological (laterality, lipid content) parameters. Throughout the follow-up, patients did not develop overt Cushing's Syndrome; three $NFAI_{DST3}$ developed ACS_{DST3}, eight $NFAI_{DST1.8}$ developed $ACS_{DST1.8}$, and one $NFAI_{DST1.8}$ progressed to ACS_{DST3}. In both groups (NFAI and ACS) the metabolic profile remained stable.

Conclusions Our data suggest higher prevalence of diabetes and cerebrovascular disease in ACS patients compared with NFAI. However, probably because of the small sample size, the differences only reached statistical significance using the cutoffs of 1.8 µg/dl for diabetes and 3.0 µg/dl for cerebrovascular disease. Patients with ACS and NFAI rarely progress to more aggressive forms of hypercortisolism, and the metabolic profile usually remains stable during the follow-up.

Keywords Autonomous cortisol secretion (ACS) · Non-functioning adrenal incidentaloma (NFAI) · Adrenal incidentaloma (AI) · Dexamethasone suppression test (DST)

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Introduction

Adrenal incidentalomas (AI) are an asymptomatic adrenal masses detected on imaging not performed for suspected adrenal disease [1]. Generally, they are non-functioning adrenocortical adenomas that do not require a specific treatment approach. However, a significant percentage may have hormonal hypersecretion, whose most prevalent form is autonomous cortisol secretion (ACS). Although ACS has been reported to be present in up to 50% of patients with AI [2], most reports describe an estimated prevalence between 2 and 20% [3–6].

There is growing evidence about the association between ACS and various metabolic disorders such as type 2 diabetes (T2DM), high blood pressure (HBP), obesity, and dyslipidemia, among others; and an increase in cardiovascular risk and mortality [7–10]. Data for adverse events and comorbidities in patients with ACS and non-functioning adrenal incidentalomas (NFAI) are obtained mainly from retrospective and cross-sectional studies; there is a lack of long-term follow-up studies focusing on cardiovascular events and mortality. Therefore, the natural history of these conditions from a long-term perspective is unknown. However, there is a tendency to deterioration over time of lipid metabolism, glycemia, and blood pressure control in patients with AI, which is greater in patients with ACS than in NFAI [11–13]. Di Dalmazi et al. reported that patients with increased cortisol secretion had higher prevalence of cardiovascular events (3.5% in patients with NFAI versus 15.9% in those with an intermediate phenotype and 31.6% in individuals with ACS [9]). Although supported by less evidence, cardiovascular events have also been found in patients with apparently NFAI [14, 15], possible because these cases NFAI might secrete glucocorticoids, which current traditional methods are inadequate to detect [11].

The aim of this study was to evaluate the metabolic abnormalities in patients with ACS and compare them with NFAI patients. We also aimed to identify possible associations between DST and clinical, radiological, biochemical, and hormonal parameters, with the aim to identify parameters able to predict ACS and the metabolic profile in patients with AI.

Methods

Study population

We retrospectively evaluated 149 consecutive patients with AI (85 women and 64 men, mean age of 62.0 years (SD = 10.31)) referred to Endocrinology and Metabolic Diseases Units of four Spanish tertiary hospitals (Princesa University Hospital (Madrid); La Paz University Hospital (Madrid); Salamanca University Hospital (Salamanca), and San Carlos Clinical University Hospital (Madrid)) from 2010 to 2019. We analyzed patients' data at baseline and at the last follow-up visit (mean follow-up = 34.6 (SD = 32.97) months).

Patients between 18 and 90 years with unilateral or bilateral AI/s (adrenal lesion detected in imaging tests not performed because of suspicion of adrenal pathology, in which at least one of the AI/s is bigger than 1 cm) were included in the study. Exclusion criteria were: (1) presence of hereditary syndromes associated with adrenal tumors, (2) patients on chronic treatment with glucocorticoids or with a history of glucocorticoid treatment for more than 3 months with a dose of 10 mg/day of prednisone (or equivalent) 3 months prior to the performance of the study, (3) patients under treatment with oral hormonal contraceptives (treatment should be suspended at least 6 weeks before performing the functionality study), (4) patients in whom the imaging test was performed in the context of the study of extension of an extra-adrenal primary cancer, and (5) patients with congenital adrenal hyperplasia, adrenal carcinoma, pheochromocytoma, overt Cushing's syndrome, and primary hyperaldosteronism.

ACS was considered an abnormal result in the suppression test with 1 mg of dexamethasone (DST) when cortisol was ≥3 μg/dl with no typical data of Cushing syndrome (CS) (ACS_{DST3}). This cutoff was selected based on several European studies which identified this cutoff point as the one offering the best sensitivity-specificity balance for morbidity and mortality [12, 13, 16–19], and also based on our current clinical practice. We also analyzed the cutoff of ≥1.8 μg/dl with the aim to evaluate whether it offered better sensitivity to detect comorbidities ($ACS_{DST1.8}$) [9]. ACS and NFAI (without subindex) referred in the text to both groups (ACS_{DST3}/$ACS_{DST1.8}$ and $NFAI_{DST3}$/$ACS_{DST1.8}$).

NFAI were considered when hormonal evaluation was normal (DST, aldosterone/renin ratio (when appropriate), and urinary metanephrines) and the radiological features were of adrenal adenoma or myelolipoma in the CT scan/MRI.

Clinical evaluation

Medical record review provided details on: pertinent demographic information (age, sex), prevalent medical diagnoses (HBP, T2DM, obesity, dyslipidemia, cerebrovascular, and cardiovascular disease), physical examination including evaluation of anthropometric characteristics (body mass index (BMI, kg/m^2)) and general physical examination, including the measurement of systolic (SBP) and diastolic blood pressure (DBP). These parameters were assessed at baseline and at the last follow-up visit.

HBP was diagnosed when SBP was ≥140 mmHg and/or DBP was ≥90 mmHg or when patients were receiving antihypertensive treatment. Diagnosis of T2DM and dyslipidemia was based on well-accepted criteria [20, 21]. Obesity was defined as a BMI >30 kg/m^2; cardiovascular disease was defined as ischemic heart disease or heart failure; and cerebrovascular disease as transient ischemic attack or acute stroke.

Hormone study

At baseline all AI patients underwent DST and urinary metanephrines determination (normetanephrine and metanephrine). The study was completed with baseline serum cortisol, adrenocorticotropic hormone (ACTH), dehydroepiandrosterone sulfate (DHEA-S), and 24-urinary free cortisol (UFC) measurement in some patients (according to the physician's judgment). Aldosterone/renin ratio was also evaluated in hypertensive patients. In the follow-up visit DST was evaluated again and the other parameters were determined according to the physician's judgment.

The normal ranges for hormonal parameters were: serum cortisol (3.7–19.4 µg/dl), ACTH (5–60 pg/ml), DHEA-S (33.6–249.0 µg/dl), UFC (4.3–176.0 µg/24 h), and DST (serum cortisol <3 µg/dl or <1.8 µg/dl (depending on the classification used) at 8.00 am after administration of 1 mg dexamethasone the previous night at 23.00 pm).

Biochemical study

All patients underwent routine biochemical evaluation after a 12-h overnight fast, at the initial evaluation and at the last follow-up visit. Glucose, total cholesterol, LDL, HDL, and triglyceride levels were evaluated. HbA1c was also measured in some patients.

Measurement methods

Serum cortisol was measured by competitive chemiluminescence in solid phase and electrochemiluminescence assays, intra-assay coefficient of variation (CV) was <5%; ACTH by electrochemiluminescence and sandwich type sequential immunoassay in solid phase, intra-assay CV was <10% and UFC by chemiluminescence assay of microparticles, chemiluminescence assay in immulite and in centaur with extraction in dichloromethane; intra-assay CV was <15%.

Radiological evaluation

Abdominal CT was performed in all patients, the maximum diameter of the adenoma/s (MAD), laterality, presence of necrosis, calcification, atypical characteristics, lipid content, and Hounsfield Units were evaluated. In 72 patients the study was completed with MRI (including the evaluation of MAD and laterality).

Statistical analysis

The statistical analysis was performed with STATA.15. Only patients with ACS and NFAI were included in the statistical analysis ($n = 149$). In the descriptive analysis, categorical variables were expressed as percentages; quantitative variables were expressed as mean ± standard deviation (SD).

Student's t test and lineal regression test were performed accordingly to compare differences in continuous parameters between two subgroups. Pearson's correlation coefficients (r) were used to evaluate linear correlations between continuous variables. The chi-squared-test and the logistic regression test were performed for the comparison of categorical variables between independent samples. Comparisons between paired-samples (baseline vs follow-up evaluation values) were assessed by paired t-test and McNemar test. In all cases, a two-tailed p value < 0.05 was considered as statistically significant. A nonparametric ROC curve was performed in order to determine which cutoff for DST had the best diagnostic accuracy in predicting comorbidities.

Results

Clinical characteristics by subgroups

Based on the 3.0 µg/dl cutoff for DST, 87.3 % ($n = 130$) of the patients had $NFAI_{DST3}$, and 12.8% ($n = 19$) ACS_{DST3}. According to the 1.8 µg/dl cutoff, 64.4% ($n = 96$) had $NFAI_{DST1.8}$, and 35.6% ($n = 53$) $ACS_{DST1.8}$. The mean age of the patients was 62.0 (10.31) years and 57.1% were women. Mean age was similar in ACS and in NFAI, and the female/male ratio was 1.1 and 1.4 in ACS and NFAI, respectively. No differences in sex or age were found between groups (Table 1).

The prevalence of HBP and cardiovascular disease was higher in ACS than in NFAI patients, but differences were not statistically significant. The prevalence of diabetes was significantly higher in $ACS_{DST1.8}$ than in $NFAI_{DST1.8}$ ($p = 0.04$), but the differences did not reach statistical significance with the 3 µg/dl cutoff. Cerebrovascular disease was significantly higher in ACS_{DST3} than in $NFAI_{DST3}$ ($p = 0.01$) but the differences did not reach statistical significance using the 1.8 µg/dl cutoff.

In the ROC curve, the cutoffs with the maximum global value were 1.54 µg/dl (Sensitivity (Se) = 63.4% and specificity (Sp) = 59.3%) for the diagnosis of diabetes; 1.61 µg/dl

Table 1 Clinical characteristics and biochemical and hormonal profile in the ACS and NFAI subgroups for the 3 µg/dl and 1.8 µg/dl cutoffs in the DST

Variable	ACS_{DST3} ($n = 19$)	$NFAI_{DST3}$ ($n = 130$)	*P* value	$ACS_{DST1.8}$ ($n = 53$)	$NFAI_{DST1.8}$ ($n = 96$)	*P* value
Age (years)	62.7 (4.86)	61.9 (1.62)	0.86	64.8 (2.74)	60.3 (1.76)	0.15
HTA (%)	57.9	50.0	0.52	54.7	49.0	0.50
T2DM (%)	42.1	25.4	0.13	37.7	21.9	**0.04**
Dyslipidemia (%)	47.4	50.0	0.83	52.8	47.9	0.57
Obesity (%)	26.3	39.2	0.18	32.1	40.6	0.56
CBD (%)	15.8	2.3	**0.01**	11.3	5.	0.17
CVD (%)	15.8	6.2	0.13	5.7	3.1	0.45
SBP/DBP (mmHg)	128.5 (3.89)/76.2 (2.05)	132.8 (1.63)/78.4 (1.02)	0.37/0.48	129.8 (2.35)/77.3 (1.66)	133.7 (1.94)/78.5 (1.14)	0.22/0.55
BMI (kg/m2)	25.2 (3.27)	30.01 (0.67)	**0.03**	27.4 (1.41)	30.6 (0.76)	**0.03**
Glucose (mg/dl)	106.8 (10.00)	106.9 (2.36)	0.98	105.1 (4.13)	108.0 (3.00)	0.57
HbA1c (%)	6.8 (0.71)	6.1 (0.12)	0.08	6.5 (0.30)	6.1 (0.14)	0.19
TC (mg/dl)	192.1 (11.07)	187.6 (3.91)	0.69	184.4 (6.34)	190.2 (4.53)	0.45
LDL (mg/dl)	123.1 (12.61)	114.1 (3.62)	0.40	115.9 (6.33)	114.9 (4.26)	0.89
HDL (mg/dl)	47.0 (3.37)	51.5 (1.84)	0.37	47.7 (2.53)	52.6 (2.14)	0.16
TG (mg/dl)	133.7 (18.05)	121.4 (6.14)	0.47	126.3 (9.90)	121.2 (7.24)	0.67
DHEAS (µg/dl)	45.5 (12.66)	47.7 (4.80)	0.88	39.9 (5.72)	51.5 (6.11)	0.22
Basal SeC (µg/dl)	17.0 (1.95)	16.0 (0.75)	0.65	16.7 (1.01)	15.9 (0.90)	0.60
ACTH (pg/ml)	14.9 (2.40)	20.76	0.22	15.4 (1.50)	22.7 (2.59)	0.04
UFC (µg/24 h)	66.0 (16.44)	45.8 (5.07)	0.12	62.2 (8.34)	36.3 (5.31)	**0.02**
DST (µg/dl)	4.6 (0.35)	1.5 (0.05)	**<0.001**	3.1 (0.20)	1.2 (0.03)	**<0.001**

Quantitative variables are expressed as mean values (standard deviation)

ACS_{DST3} autonomous cortisol secretion, *$NFAI_{DST3}$* non-functioning adrenal incidentalomas (cutoff of 3 µg/dl in dexamethasone suppression test (DST)), *$ACS_{DST1.8}$* autonomous cortisol secretion, *$NFAI_{DST1.8}$* non-functioning adrenal incidentalomas (cutoff of 1.8 µg/dl in DST), *T2DM* type two diabetes, *CBD* cerebrovascular disease, *CVD* cardiovascular disease, *SBP* systolic blood pressure, DBP diastolic blood pressure, *BMI* body mass index, *TC* total cholesterol, *TG* triglyceride, *DHEAS* dehydroepiandrosterone sulfate, *basal SeC* basal serum cortisol, *UFC* urinary free cortisol, *DST* dexamethasone suppression test

The bold values are statistically significant

(Se = 44.7% and Sp = 58.9%) for HBP and 1.54 µg/dl (Se = 64.7% and Sp = 58.3%) for diabetes + HBP. The cutoffs with the highest sensitivity (100%) were 0.74 µg/dl for diabetes; 0.64 µg/dl for HBP and 0.74 µg/dl for diabetes + HBP Fig 1.

Cardiovascular disease was significantly associated with HBP (13.2% in HBP patients vs 1.4% in non-HBP patients, $p = 0.01$), dyslipidemia (12.2% in dyslipidemia patients vs 2.7% in non-dyslipidemia patients, $p = 0.03$), and diabetes (14.6% in diabetics vs 4.6% in nondiabetic patients, $p = 0.04$), but not with obesity (7.1% in obese vs 6.6% in nonobese, $p = 0.07$).

No significant differences were found in the prevalence of dyslipidemia between ACS and NFAI. Dyslipidemia was significantly associated with HBP (63.2% in HBP patients vs 35.6% in non-HBP patients, $p = 0.001$), T2DM (73.2% in diabetic vs 40.7% in nondiabetic patients, $p < 0.001$). The prevalence of obesity was lower in patients with ACS than in patients with NFAI, although differences did not reach statistical significance.

No significant differences were found in mean SBP or DBP between ACS and NFAI. Mean BMI was greater than 25 kg/m^2 in both groups, and significantly lower in ACS patients. A negative correlation was found between BMI and DST (Pearson correlation (r) = −0.30, $p = 0.001$), but DST had a low-predictive value for BMI (R-squared = 0.09, $p = 0.001$).

Biochemical and hormonal evaluation (ACS vs NFAI)

No statistically significant differences were found in mean fasting plasma glucose (FPG), HbA1c, total cholesterol, LDL, HDL, and triglycerides between ACS and NFAI. Serum cortisol and DHEAS did not show differences either. Moreover, no differences in ACTH or DHEA were found between ACS_{DST3} and $NFAI_{DST3}$. However, ACTH levels were lower in $ACS_{DST1.8}$ patients than in $NFAI_{DST1.8}$ patients (15.4 vs 22.7 pg/ml, $p = 0.04$). There were not significant differences in DHEAS levels between bilateral and unilateral adenomas (40.8 (10.11) µg/dl vs 45.1 (4.95) µg/dl, $p = 0.74$). DHEAS did not show linear correlation with adenoma size ($r = -0.17$, $p = 0.20$) (Table 1).

As expected, DST was significantly higher in patients with ACS than in NFAI patients. UFC was higher in ACS than in NFAI, but only reached statistical significance for $ACS_{DST1.8}$ vs $NFAI_{DST1.8}$ ($p = 0.02$).

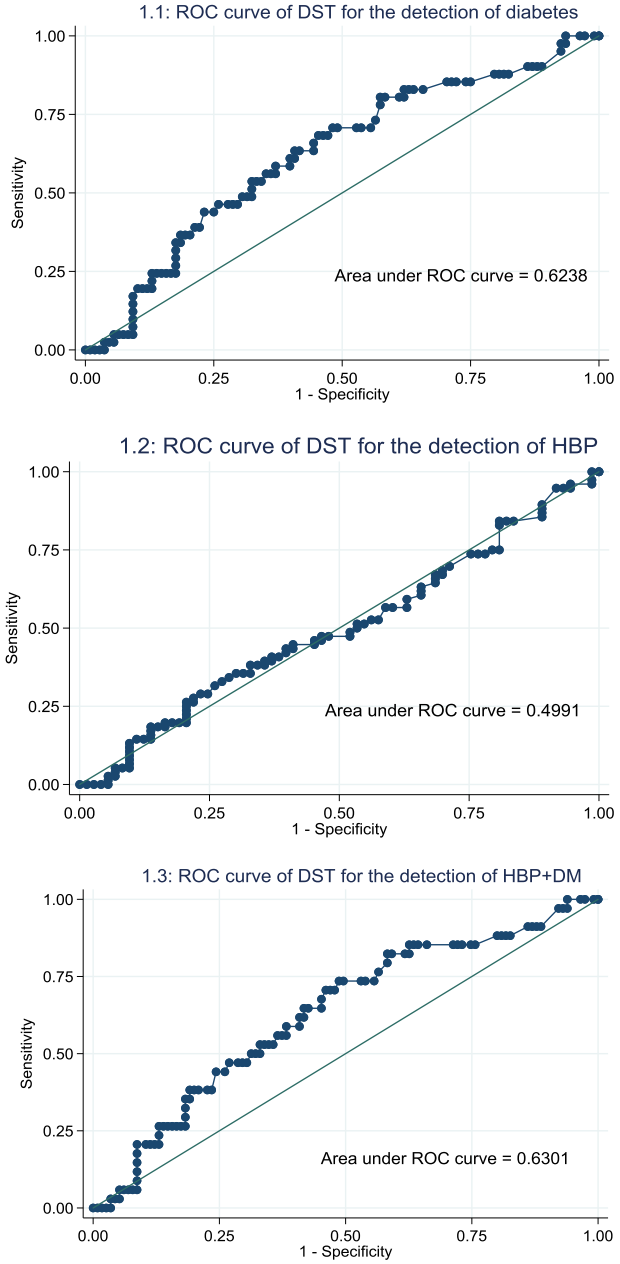

Fig. 1 ROC curve with the DST for the detection of diabetes, HBP and diabetes + HBP

Study of association of DST with cardiovascular risk factors and hormonal profile

No significant correlation was found between DST and FPG, HbA1c, total cholesterol, LDL, HDL, or triglycerides ($p > 0.05$ for r^2) (Table 2).

We found that the DST was inadequate to predict the levels of ACTH, basal serum cortisol, UFC, DHEAS, FPG, cholesterol, LDL, HDL, or triglycerides. Neither was it a good predictor of T2DM, dyslipidemia, HBP, obesity, and cardiovascular disease ($p > 0.05$ for LR-chi^2). However, it seems to be a good predictor of cerebrovascular disease (OR = 1.59, $p = 0.04$).

On the other hand, baseline serum cortisol, ACTH, UFC, and DHEAS were not good predictors of DST ($p > 0.05$ for R-squared). We only found a significant correlation of DST with MAD, although with a low-predictive power (Table 2).

We showed that there is a significant positive correlation between DST and MAD ($r = 0.38$, $p = 0.01$). Like DST, MAD was not a good predictor of FPG, HbA1c, total cholesterol, LDL, HDL, and triglycerides.

Radiological parameters predictive of ACS

The only radiological feature detected as a possible good predictor of ACS was MAD (OR = 1.1, $p < 0.001$). No association was found with bilaterality (OR = 2.1 and 1.9; $p = 0.32$ and 0.29 for the 3 µg/dl and 1.8 µg/dl cutoffs, respectively) or with the lipid content of adrenal lesions (OR = 0.94 and 0.98, $p = 0.44$ and 0.70 for the 3 µg/dl and 1.8 µg/dl cutoffs, respectively).

The prevalence of bilaterality was similar in ACS and NFAI 21.4% vs 11.3% ($p = 0.29$) (ACS_{DST3} vs $NFAI_{DST3}$) and 17.1% vs 10% ($p = 0.28$) ($ACS_{DST1.8}$ vs $NFAI_{DST1.8}$).

Follow-up study

There was not conversion of ACS or NFAI to overt CS in any case. The majority of patients with $NFAI_{DST3}$ remained stable throughout the follow-up, except three patients who developed ACS_{DST3} (average increase in DST in follow-up = 2.31 µg/dl). Five patients with ACS_{DST3} spontaneously reverted to $NFAI_{DST3}$ (average decrease in DST = 1.55 µg/dl) and only one to $NFAI_{DST1.8}$. In $NFAI_{DST1.8}$, eight patients developed $ACS_{DST1.8}$ (average increase in DST = 1.08 µg/dl) and only one ACS_{DST3} (Table 3).

During the follow-up, diabetes developed in 5.3% vs 4.6% (ACS_{DST3} vs $NFAI_{DST3}$) and 5.6% vs 4.2% ($ACS_{DST1.8}$ vs $NFAI_{DST1.8}$); HBP in 5.3% vs 4.6% (ACS_{DST3} vs $NFAI_{DST3}$) and 7.5% vs 3.1% ($ACS_{DST1.8}$ vs $NFAI_{DST1.8}$); and dyslipidemia in 15.8% vs 6.2% (ACS_{DST3} vs $NFAI_{DST1.8}$) and 7.5% vs 7.3% ($ACS_{DST1.8}$ vs $NFAI_{DST1.8}$). In both groups (ACS and NFAI) the metabolic profile remained stable throughout the follow-up (Table 3).

Discussion

The prevalence of ACS in AI in our study was 13% and 36%, using 3.0 µg/dl and 1.8 µg/dl cutoffs in the DST, respectively. This is in accordance with previous reports [1, 9] that showed widely variable prevalence of ACS ranging from 2 [22] to 18% [23] and from 5 to 20% in other

Table 2 Correlation between DST and cardiovascular risk factors and hormonal profile parameters

	Glucose	CT	LDL	HDL	TG	UFC	DHEAS	ACTH	MAD
r^2 (p value)	−0.06 (0.47)	−0.02 (0.11)	0.08 (0.47)	−0.11 (0.30)	0.06 (0.50)	0.26 (0.07)	−0.11 (0.33)	−0.14 (0.90)	**0.38 (<0.001)**
R-squared (p value)	0.00 (0.47)	0.00 (0.86)	0.01 (0.47)	0.01 (0.30)	0.00 (0.50)	0.07 (0.07)	0.01 (0.33)	0.02 (0.25)	**0.15 (<0.001)**

Correlation was analyzed by linear regression. r^2 = Pearson correlation coefficient

CT total cholesterol, *TG* triglycerides, *UFC* urinary free cortisol, *MAD* maximum adenoma diameter

The bold values are statistically significant

Table 3 Evolution of metabolic parameters during follow-up in ACS and NFAI for the cutoffs of 3 µg/dl and 1.8 µg/dl in the DST

Variable	ACS_{DST3}				$NFAI_{DST3}$			
	X0 (SD)	X1 (SD)	*d*	*P*	X0 (SD)	X1 (SD)	*d*	*p*
BMI (kg/m2)	26.4 (3.32)	25.8 (3.09)	−0.64 (0.57)	0.29	31.0 (0.84)	30.7 (0.99)	−0.30 (0.61)	0.62
SBP/ DBP (mmHg)	130.2 (5.02)/ 75.2 (2.82)	127.2 (5.38)/ 73.8 (2.37)	−3.0 (7.70)/ −1.44 (4.62)	0.71/ 0.76	131.1 (1.76)/ 77.5 (1.23)	130.4(1.69)/ 77.1 (1.28)	−0.61 (2.16)/ −0.36 (1.64)	0.78/ 0.83
Glucose (mg/dl)	106.8 (10.00)	114.4 (8.66)	7.67 (11.88)	0.53	106.8 (2.60)	109.8 (3.15)	2.93 (3.55)	0.41
LDL (mg/dl)	123.1 (12.6)	111.0 (10.54)	−12.08 (10.49)	0.27	108.7 (4.54)	108.5 (4.54)	−0.26 (3.58)	0.94
HDL (mg/dl)	47.0 (3.37)	50.0 (2.89)	3.03 (1.95)	0.15	52.1 (2.38)	53.5 (2.03)	1.34 (1.96)	0.50
Triglycerides (mg/dl)	134.9 (19.3)	120.3 (14.79)	−14.59 (18.26)	0.44	119.5 (7.28)	111.6 (7.97)	−7.87 (7.85)	0.32
BMI (kg/m2)	28.1 (1.37)	27.8 (1.25)	−0.28 (0.50)	0.59	32.1 (1.11)	31.6 (1.40)	−0.42 (0.85)	0.63
SBP/ DBP (mmHg)	130.1 (2.74)/ 77.0 (2.06)	129.1 (2.79)/ 76.8 (1.60)	−1.03 (3.42)/ −0.13 (2.32)	0.76/ 0.95	131.5 (2.08)/ 77.3 (1.32)	130.7 (1.96)/ 76.6 (1.63)	−0.80 (2.67)/ −0.71 (2.06)	0.77/ 0.72
Glucose (mg/dl)	105.2 (4.30)	109.2 (4.14)	3.98 (5.089)	0.44	107.9 (3.37)	111.3 (4.10)	3.41 (4.71)	0.47
LDL (mg/dl)	113.8 (7.29)	109.0 (6.49)	−4.79 (6.18)	0.45	109.5 (5.43)	108.9 (5.47)	−0.68 (4.07)	0.87
HDL (mg/dl)	47.8 (2.83)	53.2 (2.51)	5.43 (2.97)	0.08	53.7 (2.85)	52.5 (2.41)	−1.21 (1.69)	0.48
TG (mg/dl)	129.8 (11.13)	113.7 (9.65)	−16.16 (11.15)	0.16	116.5 (8.65)	112.6 (10.03)	−3.88 (9.41)	0.68

Variables are expressed as mean values (standard deviation)

ACS autonomous cortisol secretion, *NFAI* non-functioning adrenal incidentalomas, *X0* initial mean value, *X1* last mean value, *SD* standard deviation, *d* difference between means, *P p* value, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TG* triglyceride

[a]Patients with missing values in the variables in the last visit were excluded of the analysis

series [6]. This is mainly justified by the lack of universal consensus on the definition of ACS.

In our series, we found that AI were more common in middle-aged and elderly subjects. This finding is in agreement with other studies in which the prevalence of AI increases with age [24]. One hypothesis that tries to explain this finding is the tendency to hyperplasia of cortical cells in response to aging, related to ischemia, and atrophy [25]. We did not find significant differences in age between ACS and NFAI. However, the female/male ratio was higher in ACS than in NFAI patients. This is in agreement with most of the previous reports, which described a slightly higher prevalence of ACS in females [6, 26].

In the last decade several authors have addressed the question of whether AI could be associated with metabolic problems and poor cardiovascular outcome. Although in our study higher prevalence of HBP, diabetes, cerebrovascular, and cardiovascular diseases has been found in ACS patients compared with patients with NFAI, our data suggest that alterations in cortisol secretion in patients with AI is a continuum, rather than two clearly differentiated states (non-functioning adenomas and cortisol autonomous secretion).

The prevalence of HBP in our cohort was similar to that reported in other series of AI, such as Papanastasiou's [26] (74% in ACS and 50% in NFAI) and Morelli's [19] (66.7% in ACS and 53.9% in NFAI) and higher than the prevalence reported for the Spanish general population (around 35%) [27]. Glucocorticoid-induced hypertension could be explained by imbalance between vasodilators and vasoconstrictors and by hyperactivation of the mineralocorticoid receptor [28], or by higher pressor effects of catecholamines in the presence of cortisol. Nevertheless, as our study, most studies have failed to detect any relationship between HBP and markers of cortisol secretion [28]. HBP seems to be more related with the duration of the hypercortisolism [29].

We did not find significant differences in the prevalence of T2DM between ACS_{DST3} (42.1%) and $NFAI_{DST3}$ (25.4%), but statistical significance was reached with the 1.8 μg/dl DST cutoff. Moreover, the cutoff with the highest sensitivity for the detection of diabetes in the ROC curve was 1.61 μg/dl. However, other studies advocate for a cutoff point of 1.8 μg/dl, since it is considered more sensitive for the detection of comorbidities [9, 10]. The prevalence of diabetes in our study was 3 and 2-fold higher in ACS and NFAI, respectively, than that reported for the general Spanish population (12.7%) [27]. A cohort study also showed that NFAI had approximately a twofold higher risk for diabetes than controls with no adrenal tumors [30]. Moreover, Mazziotti et al. reported a prevalence of T2DM in ACS patients higher than in the general population but lower than in our series (22%) [31]. Hyperglycemia in ACS occurs as a consequence of an insulin-resistant state coupled with impaired insulin secretion, which are both induced by high-glucocorticoid levels [31]. Nevertheless, the relationship between obesity, insulin resistance, and AI is bidirectional [32]. Insulin can bind to IGF-1 receptor, leading to initiation of subsequent pathways such as PI3K and MAPK [33, 34], which lead to increased cellular glucose uptake [35], and to cell division and possibly adenoma formation [36]. Therefore, the higher prevalence of AI in obese patients might be explained, at least in part, as a result of AI growth in the context of hyperinsulinemia.

We found a significantly higher prevalence of cerebrovascular disease (seven times higher) in ACS_{DST3} than in $NFAI_{DST3}$, but the prevalence was only twice higher with the 1.8 μg/dl cutoff. Similar findings are described in Dalmazi's cohort [37]. These results are in favor of using the 3.0 μg/dl cutoff point for the screening of ACS, as defended by other authors [12, 19]. We also found that the risk of cardiovascular disease in patients with ACSDST3 was three times greater than in NFAIDST3 patients and twice greater than in the general population (10%) [27], although it did not reach statistical significance. Regarding the mechanism responsible of the increased cardiovascular and cerebrovascular risk, some authors defend that cortisol is an independent factor of vascular risk [10], whereas others support that this is mediated by cortisol association with HBP, T2DM, dyslipidemia, and other cardiovascular risk factors. In our study DST was a good predictor of cerebrovascular disease, but not of cardiovascular disease. On the other hand, we found a significant association between cardiovascular disease and HBP, diabetes, and dyslipidemia, but not between cerebrovascular disease and these cardiovascular risks factors. Therefore, there is probably a combined effect of both mechanisms.

No significant differences were found in the levels of FPG, HbA1c, triglyceride, cholesterol and fractions, SBP, and DBP. However, these findings are of poor value because most patients were under intensive treatment with antihypertensive, antidiabetic, and lipid-lowering drugs. We did not find differences in basal serum cortisol between ACS and NFAI. UFC was significantly higher, and ACTH lower in $ACS_{DST1.8}$ than in $NFAI_{DST1.8}$ patients but we did not find significant differences with the 3.0 μg/dl cutoff. This is in line with other studies and it points to the low diagnostic usefulness of low levels of ACTH (<10 pg/ml) and basal morning cortisol in the diagnosis of ACS when they are used as isolated parameters [38, 39]. The usefulness of UFC in the diagnosis of ACS has been assessed in some series, although its sensitivity to detect minimal elevations in cortisol secretion is limited (5–30%); therefore, it is not recommended as an initial-screening test for AI [40]. In contrast to Yener series, where the strongest predictor of ACS was low DHEAS, with an OR of 9.41 [41], we did not find significant differences in DHEAS between ACS and NFAI. Many series [17, 39, 42, 43] have found data similar to ours.

As described in other studies, we identified MAD as a good predictor of ACS. However, no association was found with other radiological characteristics or hormonal parameters. The high sensitivity of MAD > 4 cm in predicting malignancy is known [44]. But the diagnostic value of MAD is not limited to this circumstance. MAD is also considered an adequate marker of functionality. Yener et al. in their series with 317 AI found that a larger tumor size at diagnosis and a longer follow-up duration were associated with ACS development [45].

Regarding the hormonal and metabolic alterations during the follow-up, the present study showed that 2.3% of $NFAI_{DST3}$ and 1.9% of $NFAI_{DST1.8}$ developed ACS_{DST3} after a mean follow-up of 2.9 years. This risk is rather lower than in other series, such as the one reported by Papanastasiou et al. [26], which showed that 31% of $NFAI_{DST1.8}$ developed $ACS_{DST1.8.}$ However, the follow-up period was longer (5.5 years) and the design of the study was prospective. Other studies reported a risk of 6.6–15%, but also with follow-up periods longer than 3 years [24, 46, 47]. This risk is also higher in studies using DST > 3 μg/dl: for example, 11.1% in the study of Morelli et al. [46]. Furthermore, the reproducibility of the DST is not perfect, since known factors affecting dexamethasone metabolism may lead to false positive results. We cannot exclude that some patients classified as ACS were indeed false positives in the DST. The risk of evolution to overt CS was zero in our series, which is in line with the low risk described in other studies [48].

In our study, no significant deterioration of the lipid or glycemic parameters, BP or BMI was observed during the follow-up. The cardiometabolic profile in the last visit did not present significant differences between subgroups. In contrast with our results, in the study of Morelli et al. the

glycemic profile worsened in 25% of the patients, the lipid profile in 13%, weight in 26%, and BP in 34% [46], whereas they did not find differences between ACS and NFAI during the follow-up. These differences are probably related with different study populations, different study design (prospective vs retrospective), and the short follow-up in our patients.

The lack of association between DST and different components of metabolic syndrome and the lack of differences in the prevalence of different cardiovascular risk factors between groups could be explained by the small number of patients, a type 2 statistical error and the different individual sensitivity to glucocorticoids due to different polymorphism in the glucocorticoid receptor [49]. In addition, the classic statistic approach may be inadequate to study the association of metabolic syndrome and cortisol secretion. Morrelli et al. have demonstrated that the Artificial Neural Networks approach is probably more adequate than classic statistics for assessing the relationship between cortisol secretion and cardiovascular risk [50].

Conclusion

These data suggest that patients with ACS have higher risk of diabetes and cerebrovascular disease than NFAI patients. The risk of metabolic alterations (HBP, T2DM, and dyslipidemia) seems to be higher in both groups than in the general population. This suggests a continuum in the alterations of cortisol secretion. However, we found that, generally, the DST is not a reliable predictor of metabolic alterations in patients with AI.

Patients with ACS and NFAI rarely progress to more aggressive forms of hypercortisolism, and the metabolic profile usually remains stable during the follow-up. However, a longer monitoring-period would help to confirm this stability.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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