



Cardiovascular and metabolic risk factors in patients with subclinical Cushing

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Abstract

Purpose Adrenal incidentalomas (AI) are discovered after work-up unrelated to adrenal gland diseases; up to 30% of AI show subclinical endogenous cortisol excess (SH), frequently associated to hypertension, obesity, metabolic disorders and increased incidence of cardiovascular events (CVEs).

Methods We analysed 628 AI patients divided into two groups: 471 non-functional adrenal adenoma (NFA) and 157 SH. All patients underwent complete examinations, 24-h ambulatory blood pressure monitoring, biohumoral parameters and vascular damage markers, such as c-IMT and ankle brachial index. After long-term follow-up, we registered newly onset of CVEs such as myocardial infarction (MI), percutaneous stenting and surgical bypass (PTA/CABG), stroke, overall/cardiovascular mortality. Moreover, SH patients underwent to surgical (SSH) or pharmacological treatment (MSH).

Results SH patients showed higher prevalence of metabolic syndrome, diabetes mellitus, and previous CVEs respect NFA at baseline. After follow-up MSH group showed higher recurrence of major CV events compared with NFA and SSH (RR 2.27 MSH vs NFA for MI; RR 2.30 MSH vs NFA for PTA/CABG; RR 2.41 MSH vs NFA for stroke). In SSH there was a significant reduction of the number of antihypertensive medications needed to reach target blood pressure levels (2.3 ± 1.0 to 1.5 ± 0.4 drugs). None differences were found in SH patients, distinguished in relation to cortisol plasma levels after dexamethasone suppression test ($1.8\text{--}5 \mu\text{g/dL}$, above $5 \mu\text{g/dL}$).

Conclusions SH is linked to relevant cardiovascular and metabolic alterations, leading to worsen clinical outcomes. In eligible patients, adrenalectomy is valid and safe option to treat SH, reducing cardiometabolic abnormalities.

Keywords Subclinical Cushing · Cardiovascular risk · Glucose metabolism · Metabolic syndrome · Atherosclerosis

Introduction

The incidentally discovered adrenal mass during exams not strictly related to adrenal disorders is defined “adrenal incidentaloma” (AI) [1, 2]. High availability and routinely use of radiological imaging leads to discover AI in more than 10% of adults, up to 30% in subjects older than

80 years [2, 3]. Standardized and widespread hormonal evaluation allowed to find in more than 30% of AI an autonomous overproduction of cortisol from AI, not related to ACTH levels, without clinical signs or symptoms of Cushing’s disease, condition called “subclinical Cushing’s syndrome” or “subclinical hypercortisolism” (SH) [4]. In the past decades, many international Guidelines have suggested different hormonal assessment in order to identify SH, but recently the use of 1-mg overnight dexamethasone suppression test (DST) is the pivotal test in detection of SH [5].

As well as it is well established that overt hypercortisolism is associated to several comorbidities, also SH has been associated with several metabolic and cardiovascular (CV) comorbidities [6], as hypertension, impairment of glucose metabolism (until type 2 diabetes), inducing high overall CV events and CV mortality [7]. In literature, despite heterogeneity of methodological approaches (retrospective or

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prospective models of study, different criteria for SH diagnosis, duration of follow-up, data recorded), it is obvious that SH is related to worsen CV and metabolic profile, with higher prevalence of CV events (almost 3-times) and CV mortality (more than 10-times) in SH patients respect non-functional AI (NFAI) [8]. Previously in SH it was found the significant improvement of 24-h blood pressure profile and reduction of metabolic syndrome (MS) prevalence after follow-up of 12 months from surgical treatment (unilateral adrenalectomy) [9]. Moreover, Di Dalmazi et al. showed that subjects with worsened secreting patterns of endogenous cortisol secretion after DST are characterized by higher rate of CV events and lower survival rates for all-cause mortality respect NFAI and patients with stable cortisol secretion [3].

The aim of this study was to evaluate, through a prospective study, the natural history of NFAI and SH subjects in a long-term follow-up setting. Moreover, we intend to evaluate different ongoinings of SH patients treated by surgical removal of cortisol-secreting adrenal adenoma (adrenalectomy) compared with patients pharmacologically treated.

Material and Methods

From January 2000 to June 2018, we consecutively enrolled 765 patients referred to Tertiary Centre of Secondary Arterial Hypertension Unit for AI. The diagnosis of AI was based on imaging procedures [abdominal computed tomography (CT) or magnetic nuclear resonance] performed during evaluations not related to adrenal diseases. The radiological features used to define a benign adrenal mass were precontrast Hounsfield units (≤ 10 HU), 15-min wash-out after contrast infusion ($\geq 50\%$), and regular margins [7, 10]. We excluded patients with known or suspected malignancies, renal disease, or cysts. We performed complete clinical and hormonal assessment in order to exclude pheochromocytoma, primary hyperaldosteronism, overt clinical signs of hypercortisolism or hyperandrogenism. We also excluded patients with an history of extended steroid intake or those patients without complete follow-up. Therefore, 137 patients were excluded from the study for the above reasons (18%).

Serum cortisol levels post dexamethasone (≤ 50 nmol/L or ≤ 1.8 $\mu\text{g/dL}$) was used as a diagnostic criterion for the exclusion of autonomous cortisol secretion. SH was defined with serum cortisol levels after 1 mg of DST greater than 1.8 mg/dL (or > 50 nmol/L) plus one abnormal hormonal tests of hypothalamic-pituitary-adrenal axis: (a) urinary free cortisol (UFC) level > 100 mcg/24 h; (b) morning (8:00 h) plasma ACTH levels < 10 pg/ml [1]. Moreover, we have distinguished SH patients in two groups in relation to

cortisol levels post dexamethasone test: between 1.8 and 5 $\mu\text{g/dL}$ and above 5 $\mu\text{g/dL}$.

ACTH was measured with radioimmunoassay method (RIA), normal limit 10–90 pg/ml, inter/intra-assay CV 8.3/6.2; plasma cortisol was measured with RIA, normal limit 9.6–26 lg/dl, inter/intra-assay CV 5.5/4.5; UFC was measured with RIA, normal limit 1.37–7.53 lg/24 h, inter/intra-assay CV 5.5/4.5. All hormone assays were performed with commercially available kits.

Each patient received detailed description of the study protocol and all subjects signed the informed consent form and gave written approval to be included in this study population, according to the latest version of the World Medical Declaration of Helsinki (2013).

Anthropometric and laboratory data were retrieved in all patients at baseline and during follow-up. The MS was defined by ATPIII-NCEP criteria [11]. The presence of diabetes was evaluated by serum glucose behaviors, HbA1c or oral glucose tolerance test. Ambulatory blood pressure monitoring (ABPM) was performed using the oscillometric technique, which involves a portable lightweight, noninvasive monitor with self-insufflating cuff (Spacelabs Medical, 90207, Issequah, WA, USA). ABPM readings were obtained at 15-min intervals from 6 AM to midnight and at 30-min intervals from midnight to 6 AM. The definitions of “dipper” and “non-dipper” were established where night-time SBP and DBP decrease was > 10 and $< 10\%$, respectively. Ambulatory hypertension was defined as 24-h BP [125/80 mmHg] [12]. Subjects without a complete 24-h BP measurement (14 diurnal and 7 nocturnal measurements) were excluded from the study.

In all patients we performed by the same operator (AC) eco-color Doppler evaluation of carotid arteries, using Hewlett-Packard Sonor 5500 Ultrasound system (Hewlett Packard, Andover, Massachusetts, USA), equipped with a 3.11 MHz realtime B-mode scanner was used for the evaluation. Imaging of the right common carotid artery (CCA) was performed with the individuals turning their head 45° to the left. The high-resolution images were analysed to calculate cIMT, defined as thickness of the vascular intima-media complex obtained in five consecutive regions of the wall of the CCA, every 4–5 mm beginning close to the bifurcation. The value attributed to each individual was the average value among the cIMT measurement, five from the left and five from the right carotid artery.

In patients with biochemical evidence of SH, clinical indication to surgical treatment [related to presence at least of two relevant comorbidities potentially related to autonomous cortisol secretion (as arterial hypertension, glucose intolerance or type 2 diabetes mellitus, MS, obesity, dyslipidaemia), at least one of these poorly controlled by medical measures], and expressed consent to its procedure, we performed an adrenocortical scintigraphy specific for the

adrenal cortex using radiopharmaceuticals (^{131}I -6b-iodio-methyl-nor-cholesterol) or ^{18}F -fluorodeoxyglucose (FDG) PET/CT total body (in relation to availability); unilateral uptake on the side of the adrenal mass (concordant up-take) confirmed unappropriated cortisol secretion due to single adrenal adenoma [13, 14]. We avoided to perform scintigraphy or FDG PET/CT in patients with large adrenal mass with absolute indication to surgical treatment (i.e., >6 cm).

In 39 SH patients we performed unilateral adrenalectomy (SSH group) with biochemical remission of cortisol excess evaluated during follow-up (thought normalization of serum cortisol after DST and 24-h urinary cortisol excretion); of these, 29 patients completed the entire period of follow-up of 36 months (at baseline, 78% with serum cortisol levels after DST between 1.8–5 $\mu\text{g}/\text{dL}$ or 50–138 nmol/L; 22% over 5 $\mu\text{g}/\text{dL}$ or 138 nmol/L). On the other hand, 118 SH patients did not undergo to adrenalectomy despite clinical indication because of different reasons (lack of unilateral and concordant uptake on scintigraphy or FDG PET/TC, refusal of surgical treatment, personal preference), and then were pharmacologically treated with optimized therapy in order to reduce metabolic and CV altered parameters (higher doses of drug tolerated by patients or fulfill dosed to get control) (MSH group) (at baseline, 70% with serum cortisol levels after DST between 1.8 and 5 $\mu\text{g}/\text{dL}$ or 50 and 138 nmol/L; 30% over 5 $\mu\text{g}/\text{dL}$ or 138 nmol/L).

Evaluation during follow-up

The same specialized physicians (CL, LP, GDT, FO) assessed patients every 12–15 months for the first 3 years after diagnosis through clinic evaluation (by hormonal and instrumental tests), and subsequently at 24–36 months through interview until a follow-up of 15 years. We recalled by telephone the participants who missed a scheduled follow-up visit. If a patient had died, we obtained date and cause of death by telephone contact with family or the patient's family physician.

During follow-up we evaluated the state of hypertension, dyslipidaemia, type 2 diabetes, and newly onset of major cardiovascular events (CVEs), and concomitant treatments.

We used long-term follow-up (15 years) to define Kaplan–Meier curves regarding newly CV events and CV mortality.

Statistical analysis

All data are expressed as mean standard deviation. Differences between means were assessed by the Student's *t* test or the Mann–Whitney U test in non-normally distributed data for two-sample comparison, or by one-way analysis of variance applying the Fisher least significant difference post

hoc test for multiple comparisons. χ^2 statistics were used to assess differences between categorical variables.

Univariate, multiple linear regression analysis and Cox proportional-hazards were made with Backward Stepwise Regression method to evaluate models for CV events and CV mortality.

We have evaluated several hormonal parameters (plasma cortisol post dexamethasone test, UFC, ACTH plasma levels), and plotted then on the receiver operating characteristic (ROC) curve for evaluate CV and metabolic outcomes in patients surgically or conservatively treated. The most appropriate cutoff values were established as the ones with higher result of the sum of sensitivity and specificity.

P values less than 0.05 were taken as statistically significant. Statistical analysis was performed using dedicated statistical software SPSS (Statistical Package for Social Sciences, software, version 24; SPSS Inc, Chicago, Illinois, USA) and e GraphPad (version 5.0; GraphPad Software, Inc, La Jolla, California, USA).

Results

We enrolled a total of 628 patients with AI (321 M, 253 F; mean age 60.2 ± 12.2 yrs), distinguished in 471 NFA (282 M, 189 F; mean age 59.6 ± 12.5), and 157 SH (93 M; 64 F; mean age 62.9 ± 11.1); bilateral AIs were found in 83 (13.2%) patients. The mean dimension of adrenal lesions was 22.8 ± 12.5 mm, without significant differences on mean diameter between NFA and SH patients (22.4 ± 12.7 mm vs 24.5 ± 13.6 mm, respectively).

Table 1 shows parameters of overall patients enrolled at baseline. SH patients show higher office systolic and diastolic blood pressure (SBP and DBP) compared with NFA patients (139 ± 20.9 mmHg vs 145 ± 2.4 mmHg, $p < 0.01$; 82.9 ± 10.9 mmHg vs 85.7 ± 12.5 mmHg, $p < 0.02$; respectively). SH patients showed higher levels of uric acid respect NFA patients (5.2 ± 1.5 mg/dl vs 4.8 ± 1.6 mg/dl; $p < 0.05$). Moreover, at baseline we found significantly higher prevalence of MS (classified by ATP III criteria) (42.8%) and diabetes mellitus (19%) in SH patients respect NFA group (12.7 and 7%, $p < 0.05$; respectively). None differences of number of medications used were observed (lipid-lowering, antihypertensive drugs and oral antidiabetic drugs) in all subjects, albeit we found an increasing trend to use more antihypertensive drugs in SH patients respect NFA group (2.4 ± 1.2 vs 2.0 ± 1.1).

At baseline, in overall subjects enrolled the anamnestic evaluation of previously diagnosed major CVEs leads to find a significant higher prevalence for all main categories in SH patients compared with NFA patients: myocardial infarction (MI) (6.4 vs 1.5%, $p < 0.05$), percutaneous transluminal angioplasty/coronary artery bypass graft

Table 1 Demographic, anthropometric and biochemical parameters, prevalence of ATP criteria, number of medications and major comorbidities in patients with non-functioning adenomas (NFA) and subclinical hypercortisolism (SH) at baseline

	NFA n: 471 (282 M; 189 F)	SH n: 157 (93 M; 64 F)	<i>p</i>
Age (yrs)	59.6 ± 12.5	62.9 ± 11	ns
BMI (kg/m ²)	28.6 ± 8.5	27.8 ± 5	ns
WC (cm)	99 ± 13.4	99 ± 12.4	ns
NC (cm)	37.6 ± 4.3	38.4 ± 4.2	ns
O-SBP (mmHg)	139 ± 20.9	145 ± 2.4	<0.01
O-DBP (mmHg)	82.9 ± 10.9	85.7 ± 12.5	<0.02
Glyc (mg/dL)	99.2 ± 22.3	98.9 ± 26.3	ns
Creat (mg/dL)	1.0 ± 0.6	0.9 ± 0.4	ns
TC (mg/dL)	202.0 ± 42.1	195.7 ± 38.8	ns
LDL-C (mg/dL)	121.8 ± 34.8	115.5 ± 34.0	ns
HDL-C (mg/dL)	52.1 ± 14.5	55.4 ± 15.5	ns
TGL (mg/dL)	121.5 ± 50.4	116.7 ± 51.7	ns
UA (mg/dl)	4.8 ± 1.6	5.2 ± 1.5	<0.05
WC (%)	43.4	38.2	ns
HDL-C (%)	27.4	28.7	ns
Hypert (%)	81.7	83.4	ns
HyperTGL (%)	19.3	19.1	ns
MS (%)	12.7	42.8	<0.05
Diabetes (%)	7	19	<0.05
OAD (Number)	1.3 ± 0.7	1.3 ± 0.5	ns
Statines or fibrates (Number)	1.0 ± 0.2	1.1 ± 0.3	ns
Antihypert drugs (Number)	2.0 ± 1.1	2.4 ± 1.2	ns
MI (%)	1.5	6.4	<0.05
PTA/CABG (%)	0.4	4.5	<0.05
Stroke (%)	1.5	5.1	<0.05

NFA non-functioning adenomas, SH subclinical hypercortisolism, BMI body mass index, WC waist circumference, NC neck circumference, O-SBP office systolic blood pressure, O-DBP office diastolic blood pressure, HR heart rate, Glyc glycaemia, Creat serum creatinine, Na serum sodium, K serum potassium, Ca serum calcium, TC total cholesterol, LDL-C LDL-cholesterol, HDL-C HDL-cholesterol, TGL triglycerides, UA serum uric acid, WC waist circumference more than 92 cm in men and 88 cm in women, expressed as percentage of incidence, HDL-C HDL-cholesterol less than 40 mg/dL in men, less than 50 mg/dL in women, expressed as percentage of incidence, HyperTGL tryglicerides more than 150 mg/dL, expressed as percentage of incidence, MS metabolic syndrome defined as any of three of five criteria in NCEP ATP III definition, expressed as percentage of incidence, OAD oral antidiabetic drugs, MI myocardial infarction, PTA/CABG percutaneous transluminal angioplasty/coronary artery bypass graft

(4.5 vs 0.4%, $p < 0.05$), and stroke (5.1 vs 1.5%, $p < 0.05$) (Table 1).

In total, 29 subjects (10 M, 19 F; mean age 61.7 ± 11.6 yrs) underwent unilateral adrenalectomy with

complete restoration of cortisol excess, completing the entire period of follow-up of 36 months; whereas 118 patients (M 50, 68 F; mean age 62.2 ± 11.2 yrs) were pharmacologically treated with optimized therapy in order to reduce metabolic and CV altered parameters. None of patients surgically-treated had peri- and post- procedural major complications.

Table 2 shows demographic, hemodynamic and biochemical parameters in overall patients distinguished in three groups: NFA, MSH, and SSH. Interestingly, at the end of ambulatory follow-up SSH showed significant decrease of waist circumference (100.5 ± 10.8 cm vs 94.1 ± 6.6 cm; $p < 0.05$) and neck circumference (38.1 ± 3.6 cm vs 34.2 ± 5.1 cm; $p < 0.05$), whereas MSH group showed increased neck circumference during follow-up (38.2 ± 3.5 cm vs 39.1 ± 3.8 cm; $p < 0.05$). Both SSH and MSH groups showed significant reduction of office-SBP (131.6 ± 17.4 mmHg vs 123.2 ± 8.45 mmHg, 145.6 ± 21.6 mmHg vs 134.9 ± 16.2 mmHg, respectively; $p < 0.05$) and office-DBP (80.2 ± 10.7 mmHg vs 73.8 ± 5.3 mmHg, 85.6 ± 12.4 mmHg vs 80.4 ± 9.1 mmHg, respectively; $p < 0.05$). Regarding biochemical parameters, after ambulatory follow-up in SSH patients we found significant reduction of total cholesterol (195.2 ± 34 mg/dl vs 189.5 ± 30.2 mg/dl; $p < 0.05$) and LDL-cholesterol (121.5 ± 32.2 mg/dl vs 112 ± 27.3 mg/dl; $p < 0.05$); whereas we observed significant increase of uric acid levels in MSH (5.1 ± 1.5 mg/dl vs 5.5 ± 1.3 mg/dl; $p < 0.05$) and NFA patients (4.8 ± 1.6 mg/dl vs 5.1 ± 1.6 mg/dl; $p < 0.05$).

Interestingly, in SSH group reduction of blood pressure behaviors was observed during at 24-h ABPM evaluation (Table 3), associated to significant reduction of mean number of anti-hypertensive medications used (2.3 ± 1.0 vs 1.1 ± 0.4; $p < 0.05$); whereas in MSH and NFA group significant reduction of blood pressure behaviors was not found as well as mean number of anti-hypertensive medications. Furthermore, SSH patients had also an improvement of circadian pressure profile, with significant reduction of “non-dipping pattern” after follow-up (from 48.3 to 27.5%; $p < 0.05$).

Moreover, after ambulatory follow-up in SSH patients we have described the significant reduction of MS prevalence (42.6 vs 18%; $p < 0.05$) as well as of diabetes mellitus (20.7 vs 7.4%; $p < 0.05$). Regarding ATPIII criteria, SSH patients showed a drastic reduction of incidence in hypertension (89.2 to 40.2%; $p < 0.05$), hypertriglyceridemia (27.8 to 10.8%, $p < 0.05$) and waist circumference altered (55 vs 35%, $p < 0.05$). On the other hand, in MSH group we observed significant increase of waist circumference altered (>102 cm for men and >88 cm for women) (46.7 vs 48.9%, $p < 0.05$). In MSH patients we found a significant thickening of c-IMT (from 0.91 ± 0.5 to 1.0 ± 0.2; $p < 0.01$) and an increased incidence of

Table 2 Demographic, anthropometric and biochemical parameters in patients with non-functioning adenomas (NFA), subclinical hypercortisolism with medical management (MSH) and subclinical hypercortisolism with surgical management (SSH) after follow up of 3 years

	NFA n. 471 (282 M; 189 F)	follow-up	<i>p</i>	MSH n. 118 (50 M; 68 F)	follow-up	<i>p</i>	SSH n. 29 (10 M; 19 F)	follow-up	<i>p</i>
BMI (kg/m ²)	28.6 ± 8.5	27.4 ± 4.4	ns	27.6 ± 5.3	28.1 ± 4.5	ns	29.1 ± 4.4	28.1 ± 4.1	ns
WC (cm)	99.1 ± 13.4	98.7 ± 14.6	ns	99.3 ± 11.5	101.2 ± 11.9	ns	100.5 ± 10.8	94.1 ± 6.6	<0.05
NC (cm)	37.6 ± 4.3	38.7 ± 7.1	ns	38.2 ± 3.5	39.1 ± 3.8	<0.05	38.1 ± 3.6	34.2 ± 5.1	<0.05
O-SBP (mmHg)	139.1 ± 21	135.2 ± 20.3	ns	145.6 ± 21.6	134.9 ± 16.2	<0.05	131.6 ± 17	123 ± 8.4	<0.05
O-DBP (mmHg)	82.9 ± 10.9	80.8 ± 10.3	ns	85.6 ± 12.4	80.4 ± 9.1	<0.05	80.2 ± 10.7	73.8 ± 5.3	<0.05
HR (sec)	66.8 ± 9.5	65.1 ± 9.4	ns	67.5 ± 10	65.2 ± 10.5	ns	67.7 ± 11	66.5 ± 8.8	ns
Glyc (mg/dL)	99.2 ± 22.3	96.8 ± 21.4	ns	97.3 ± 20.1	96.9 ± 20.1	ns	95.1 ± 16.8	92 ± 14.8	ns
Creat (mg/dL)	1.0 ± 0.6	1.0 ± 0.6	ns	0.9 ± 0.3	0.9 ± 0.5	ns	0.85 ± 0.22	0.87 ± 0.2	ns
TC (mg/dL)	202.0 ± 42.1	196.8 ± 34.9	ns	194.7 ± 37.5	192.9 ± 38.8	ns	195.2 ± 34.0	189.5 ± 30.2	<0.05
LDL-C (mg/dL)	121.8 ± 34.8	113.2 ± 32.8	ns	115.1 ± 35.3	114.5 ± 33.7	ns	121.5 ± 32.2	112.0 ± 27.3	<0.05
HDL-C (mg/dL)	52.1 ± 14.5	55.6 ± 13.4	ns	56.2 ± 14.7	53.9 ± 15.2	ns	54.3 ± 14.4	53.9 ± 14.3	ns
TGL (mg/dL)	121.5 ± 50.4	122.3 ± 66.6	ns	124.8 ± 51.4	120.5 ± 51.8	ns	122.6 ± 47.8	121.9 ± 45.3	ns
UA (mg/dl)	4.8 ± 1.6	5.1 ± 1.6	<0.05	5.1 ± 1.3	5.5 ± 1.3	<0.05	4.8 ± 1.1	4.9 ± 1.1	ns

NFA non functioning adenomas, MSH subclinical hypercortisolism with medical management, SSH subclinical hypercortisolism with surgical management, BMI body mass index, WC waist circumference, NC neck circumference, O-SBP office systolic blood pressure, O-DBP office diastolic blood pressure, HR heart rate, Glyc glycaemia, Creat creatinemia, Na serum sodium, K serum potassium, Ca serum calcium, TC total cholesterol, LDL-C LDL- Cholesterol, HDL-C HDL-Cholesterol, TGL triglycerides, UA uric acid, HyperTGL hypertriglyceridemia

Table 3 Ambulatory Blood Pressure Monitoring (ABPM) and number of antihypertensive medications in patients with non-functioning adenomas (NFA), subclinical hypercortisolism with medical management (MSH) and subclinical hypercortisolism with surgical management (SSH) after follow up of 3 years

	NFA n. 471 (282 M; 189 F)	follow-up	<i>p</i>	MSH n. 118 (50 M; 68 F)	follow-up	<i>p</i>	SSH n. 29 (10 M; 19 F)	follow-up	<i>p</i>
G-SBP (mmHg)	130.4 ± 15.2	124.4 ± 13.6	ns	131.2 ± 14.1	127.4 ± 14.8	ns	128.3 ± 15.8	120.9 ± 7.5	<0.05
G-DBP (mmHg)	79.6 ± 37.5	72.3 ± 9.1	ns	76.9 ± 10.6	77.2 ± 10	ns	79.1 ± 11.4	74.1 ± 8.0	<0.05
G-HR (bpm)	74.0 ± 9.8	73.3 ± 8.9	ns	71.5 ± 8.2	73.6 ± 8.2	ns	74.2 ± 9.7	72.5 ± 9.1	ns
D-SBP (mmHg)	133.1 ± 15.4	127.0 ± 8.9	ns	129.8 ± 17.2	130.9 ± 17.2	ns	131.3 ± 15.2	129.0 ± 12.7	<0.05
D-DBP (mmHg)	80.1 ± 10.7	75.8 ± 9.2	ns	79.7 ± 11.2	78.6 ± 10.2	ns	81.2 ± 10.8	77.4 ± 8.5	<0.05
D-HR (bpm)	76.1 ± 10.1	74.5 ± 9.2	ns	73.1 ± 9.5	76.2 ± 9	ns	76.3 ± 10.5	75.1 ± 8.75	ns
N-SBP (mmHg)	122.3 ± 16.4	116.4 ± 16.8	ns	121.6 ± 16.5	118.1 ± 15.6	ns	117.8 ± 19.6	116.5 ± 11.1	<0.05
N-DBP (mmHg)	70.1 ± 10.3	66.5 ± 8.9	ns	68.8 ± 10.8	68.8 ± 10.8	ns	69.6 ± 12.9	67.7 ± 8.2	<0.05
N-HR (bpm)	67.5 ± 10.3	67.7 ± 8.5	ns	65.9 ± 9.3	67.8 ± 8.9	ns	66.8 ± 9.0	68.0 ± 7.9	ns
“Non-Dipper” (%)	47.3%	53.8%	ns	44.2%	53.1%	ns	48.3%	27.5%	<0.05
Antihypert Drug (num.)	2.0 ± 1.1	2.1 ± 1.1	ns	2.45 ± 1.1	2.6 ± 1.4	ns	2.3 ± 1.	1.1 ± 0.4	<0.05

NFA non-functioning adenomas, MSH subclinical hypercortisolism with medical management, SSH subclinical hypercortisolism with surgery, G-SBP global average systolic blood pressure, G-DBP global average diastolic blood pressure, G-HR global average heart rate, D-SBP daytime average systolic blood pressure, D-DBP daytime average diastolic blood pressure, D-HR daytime average heart rate, N-SBP night-time average systolic blood pressure, N-DBP night-time average diastolic blood pressure, N-HR night-time average heart rate, NON-DIPPER non-dipper pattern

plaques (from 20 to 32%, $p < 0.01$) (Table 4). In brief in Table 5 are reported the main hormonal and clinical characteristics of each patient who underwent surgery should be added.

Figure 1a represents Kaplan–Meier curves showing incidence of newly CVE in overall patients during long-term follow-up (15 years). MSH patients have higher CV

events than patients with NFA and SSH, with Unadjusted risk ratio (RR) for CV events in MSH respect NFA and SSH of 2.56 and 2.10, respectively ($p < 0.001$). Figure 1b represents Kaplan–Meier curves for CV mortality. Compared with patients with NFA and SSH, unadjusted CV mortality at the end of the follow-up gets worse in patients with MSH, with cardio-vascular cumulative survival

Table 4 Prevalence of ATP criteria, number of medications, carotid intima-media thickness (c-IMT) and Ankle-Brachial Index (ABI) in patients with non-functioning adenomas (NFA), subclinical hypercortisolism with medical management (MSH) and subclinical hypercortisolism with surgical management (SSH) after follow up of 3 years

	NFA n. 471 (282 M; 189 F)	follow-up	<i>p</i>	MSH n. 118 (50 M; 68 F)	follow-up	<i>p</i>	SSH n. 29 (10 M; 19 F)	follow-up	<i>p</i>
WC (%)	43.4	35.9	ns	47.3	49.5	ns	55.0	35.0	< 0.05
HDL-C (%)	27.4	31.2	ns	28.2	31.6	ns	38.0	45.7	ns
Altered arterial hypertension (%)	81.7	81.0	ns	88.1	85.5	ns	89.2	40.2	< 0.05
HyperTGL (%)	19.3	19.7	ns	19.6	18.2	ns	27.8	10.8	< 0.05
Metabolic Syndrome (%)	12.7	7.0	ns	51.3	47.9	ns	42.6	18.0	< 0.05
Diabetes (%)	7.0	3.5	ns	26.7	23.5	ns	20.7	7.4	< 0.05
Oral Antidiabetic Drugs (num.)	1.3 ± 0.7	1.0 ± 1.0	ns	1.15 ± 0.4	1.5 ± 0.3	ns	1.0 ± 0.5	0.7 ± 0.5	ns
Statines or Fibrates (num.)	1.0 ± 0.2	1.1 ± 0.3	ns	1.1 ± 0.3	1.6 ± 0.2	ns	1.0 ± 0.8	0.8 ± 0.5	ns
c-IMT (mm)	0.95 ± 0.2	0.96 ± 0.2	ns	0.91 ± 0.5	1.0 ± 0.2	< 0.01	0.96 ± 0.2	0.96 ± 0.2	ns
Plaque (%)	15.2	20.4	ns	20	32	< 0.01	30	20	ns
ABI (mm)	1.1 ± 0.1	1.1 ± 0.1	ns	1.15 ± 0.3	1.2 ± 0.2	ns	1.1 ± 0.1	1.1 ± 0.9	ns
ABI < 0.9% (%)	0.4	0.2	ns	2	0.7	ns	0	0	ns

NFA non-functioning adenomas, MSH subclinical hypercortisolism with medical management, SSH subclinical hypercortisolism with surgical management, WC waist circumference more than 92 cm in men and 88 cm in women, expressed as percentage of incidence, HDL-C HDL-cholesterol less than 40 mg/dL in men, less than 50 mg/dL in women, expressed as percentage of incidence; Altered arterial hypertension: blood pressure behaviors more than 130/85 mmHg, HyperTGL Tryglicerides more than 150 mg/dL, expressed as percentage of incidence; metabolic syndrome defined as any of three of five criteria in NCEP ATP III definition, expressed as percentage of incidence, c-IMT carotid intima media thickness; plaque: percentage of incidence of plaques, ABI ankle-brachial index

significantly reduced (RR 2.64 respect NFA and 1.72 respect SSH; $p < 0.001$). Regarding incidence of newly CVE or CV mortality observed during follow-up, none differences were found in SH patients, either conservatively or surgically treated, distinguished in relation to cortisol plasma levels after DST (in SSH group 78% had values between 1.8 and 5 $\mu\text{g/dL}$, and 22% above 5 $\mu\text{g/dL}$; in MSH group 70% had values between 1.8 and 5 $\mu\text{g/dL}$, and 30% above 5 $\mu\text{g/dL}$).

Table 6 shows the results of univariate and Cox proportional-hazards models for CVE; CVE prevalence was independently associated positively with mean value of 24-h systolic blood pressure ($p < 0.0001$), with an independent contribution of plasma levels of LDL-cholesterol and plasma levels of serum glucose. Whereas, regarding univariate and Cox proportional-hazards models for CV mortality (Table 7), CV mortality was independently associated with plasma levels of serum glucose ($p < 0.001$). In Fig. 2 the ROC curve analysis shows the association between urinary serum cortisol (UFC) at baseline and (A) the reduction of office systolic blood pressure after surgery [best cutoffs for UFC was set at 109.65 nmol/24 h (AUC 0.720 ± 0.106, $p < 0.05$; sensibility 76.2%, specificity 37.5%)] and (B) and reduction of waist circumference after surgery [best cutoffs for UFC was set at 132 nmol/24 h (AUC 0.815 ± 0.153, $p < 0.05$; sensibility 63%, specificity 50%)].

Discussion

Several evidences show that the autonomous secretion of cortisol from AI, defined as SH, despite absence of overt phenotypical features of Cushing's Syndrome, is associated to metabolic and CV complications inducing reduction of either quality of life and survival, due to higher incidence of CV events and CV mortality [7, 8, 15–19]. In this respect, Di Dalmazi et al. [7] have evaluated in large registry of AI patients, newly onset of CVE and overall and CV-specific mortality, distinguishing the enrolled subjects in relation to response to DST during follow-up. In particular, these Authors revealed that SH patients showed higher incidence of CVE compared with NFAI patients. Moreover, subgroup of SH patients with worsened secreting patterns after DST during follow-up, showed highest incidence of CVE respect to patients with stable cortisol secretion. In this study, beyond age, the main factor related to worsen CV global risk factor was the mean concentrations of cortisol after DST, suggesting in SH patients the pivotal role of cortisol excess in the development of metabolic and CV abnormalities.

Recently, in a systematic review Park et al. [8] have compared all studies performed on AI and SH [7, 15–19]. Despite differences about methodological analysis (retrospective models, different diagnostic criteria for SH, extreme heterogeneity of metabolic and CV outcomes evaluated, short period of evaluation), the Authors have

Table 5 Anthropometric, biochemical parameters, prevalence of ATP criteria, number of medications in patients with subclinical hypercortisolism with surgical management (SSH) at baseline and after follow-up of 3 years

	BMI (Kg/m ²)		WC (cm)		NC (cm)		O-SBP (mmHg)		O-DBP (mmHg)		GLYC (mg/dl)		LDL (Mg/dl)		PC:DXM (mmol/L)		UFC (mmol/L)		WC alt (Y/N)		AH alt (Y/N)		TGL alt (Y/N)		MS (Y/N)		DM (Y/N)		OAD (Y/N)		AHD (num)		
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
1	25	25	97	92	43	37	110	120	70	65	92	88	128	110	75	37	210	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	30	28	112	101	36	31	145	125	85	75	104	95	169	125	215	45	154	179	1	0	1	0	1	0	1	0	0	0	2	1	3	0	
3	32	32	116	102	40	34	135	120	85	90	100	97	120	125	125	43	235	200	1	0	1	1	1	1	1	0	0	0	2	2	3	2	
4	32	30	87	86	35	33	140	130	90	75	94	98	136	110	198	25	175	145	0	0	1	1	0	0	0	0	0	1	1	3	3		
5	32	29	101	89	36	31	120	120	85	70	85	87	83	115	110	17	217	156	0	0	1	0	0	0	0	0	0	0	0	0	2	0	
6	33	33	90	85	37	34	130	120	80	70	95	85	125	115	115	47	243	198	0	0	1	0	0	0	0	0	0	1	0	2	0		
7	32	31	106	108	42	36	160	135	90	75	76	80	113	100	135	43	210	145	1	1	1	1	1	1	0	0	0	0	0	4	3		
8	30.4	30	100	102	40	39	135	120	85	85	95	90	115	105	125	25	229	109	1	1	1	1	1	0	0	1	1	0	1	1	3	3	
9	27.3	27.3	85	84	39	33	100	110	60	70	90	92	155	115	107	17	235	134	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	40	36	115	115	35	36	160	130	100	75	89	85	110	100	235	26	235	98	1	1	1	1	1	1	0	0	0	0	0	4	3		
11	23	23	100	92	34	32	160	130	70	75	92	88	69	85	265	31	258	127	0	0	1	1	1	1	0	0	0	0	1	1	3	2	
12	29.1	29.4	101	97	43	37	140	120	70	70	77	91	89	100	197	29	227	135	0	0	1	1	1	0	0	0	0	0	0	0	3	3	
13	29.2	23.1	111	89	35	31	135	130	80	75	85	88	110	100	124	24	210	85	1	0	1	0	1	0	1	0	0	0	0	0	3	0	
14	23.1	23.7	85	81	36	34	110	110	65	70	76	80	85	105	85	31	219	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	30.1	25.8	104	90	44	39	130	120	85	70	90	90	139	110	90	29	215	110	1	0	1	0	1	0	0	0	0	0	0	0	0	2	0
16	28.1	29	102	104	32	35	130	125	85	75	94	92	125	105	92	37	227	75	1	1	1	1	1	0	1	0	0	0	1	1	0	2	0
17	33	33	105	98	35	34	110	130	85	75	114	98	128	110	65	45	235	78	1	1	1	1	1	1	0	1	1	0	3	2	2	2	
18	22	22	85	81	43	39	130	125	85	75	152	125	77	100	94	27	216	89	0	0	1	0	0	0	0	0	0	1	1	3	3	2	0
19	32	32	110	102	45	36	130	125	90	80	94	93	137	125	91	39	254	117	1	1	1	1	1	0	0	0	0	0	1	1	3	0	
20	25.7	25.7	102	91	37	34	130	120	85	80	79	82	136	120	87	31	235	135	1	1	1	1	1	0	0	0	0	0	0	0	0	2	0
21	27	27	105	92	31	32	140	115	90	75	83	85	203	150	165	27	145	147	1	0	1	0	1	1	1	0	0	0	0	0	3	0	
22	33	33.8	102	95	36	33	115	120	70	70	79	80	116	120	89	45	215	98	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
23	22.9	23	85	81	38	32	145	130	80	75	123	115	95	110	176	47	198	125	0	0	1	1	1	0	0	0	0	1	0	3	2	3	2
24	25.5	24	92	81	35	34	110	115	90	80	85	90	97	105	65	23	223	107	1	1	1	1	1	0	0	0	0	0	0	0	0	2	0
25	29	29	99	99	34	33	135	125	70	70	105	101	125	120	97	26	216	115	1	1	1	1	1	0	0	1	1	0	0	0	2	0	0
26	27	27	98	89	38	34	135	130	80	75	115	101	81	100	125	19	210	78	0	0	1	1	1	0	0	0	0	1	1	3	2	3	3
27	32	29	103	103	41	34	132	125	80	85	102	95	148	120	130	49	231	98	0	0	1	1	1	0	0	0	0	1	0	3	2	2	3
28	30	29	115	99	44	35	130	120	70	65	95	88	169	135	127	39	254	74	1	1	1	1	1	1	0	0	0	0	1	1	3	0	
29	28.5	27.5	106	92	43	35	135	130	85	75	93	89	131	110	115	45	212	85	1	0	1	1	1	0	0	1	0	0	0	1	0	3	0
Meanor %	29.1 ± 3.9	28.1 ± 3.6	100.6 ± 9.4	94 ± 9.3	38.1 ± 3.9	34.3 ± 2.3	131.7 ± 15	123 ± 6.1	80.6 ± 9.2	73.8 ± 4.1	95 ± 16	92 ± 9.8	121.2 ± 30.5	112 ± 12.7	128 ± 51	33.4 ± 10	217 ± 26	120 ± 35	58% ± 2.6	35% ± 1.0	89% ± 3.5	40% ± 1.0	28% ± 0.4	11% ± 0.5	43% ± 0.5	18% ± 0.5	21% ± 0.5	7.4% ± 0.5	0.7 ± 0.5	2.3 ± 0.5	1.1 ± 0.4		
p	ns	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	ns	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	ns	<0.05	<0.05	<0.05		

BMI body mass index, WC waist circumference, NC neck circumference, O-SBP office systolic blood pressure, O-DBP office diastolic blood pressure, O-DBP office diastolic blood pressure, G_{lyc} glycaemia, LDL-C LDL-cholesterol, WC alt: waist circumference more than 92 cm in men and 88 cm in women, expressed as Y (presence) or N (absence), AH alt: altered arterial hypertension: blood pressure behaviors more than 130/85 mmHg, expressed as Y (presence) or N (absence), TGL alt: triglycerides more than 150 mg/dL, expressed as Y (presence) or N (absence), MS metabolic syndrome defined as any of three of five criteria in NCEP ATP III definition, expressed as Y (presence) or N (absence); DM diabetes mellitus presence, expressed as Y (presence) or N (absence), OAD oral antidiabetic drugs, expressed as number of drugs taken, AHD antihypertensive drugs, expressed as number of drugs taken

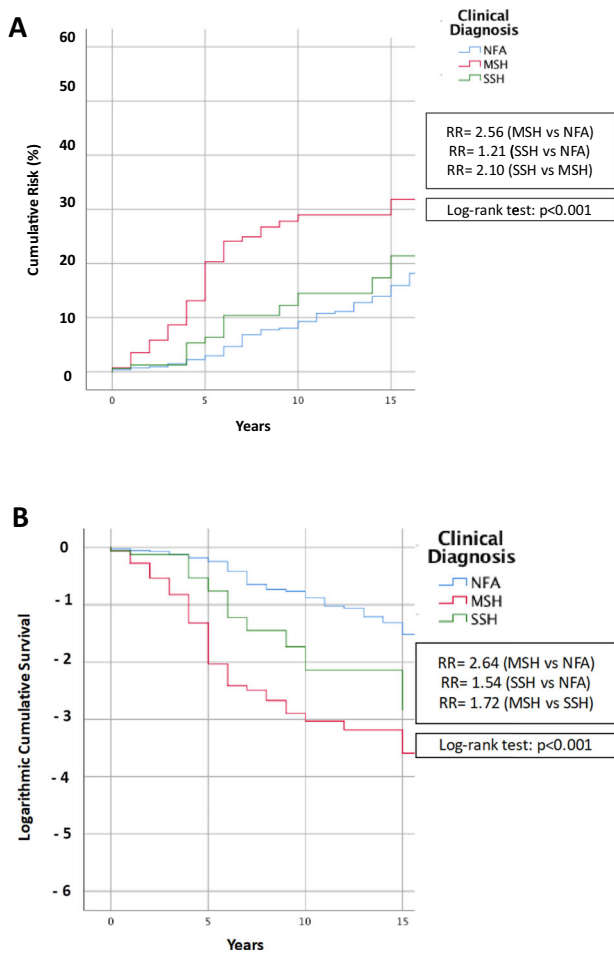


Fig. 1 Kaplan–Meier Curve showing global cardio-vascular events (a) (nonfatal acute myocardial infarction, percutaneous transluminal coronary angioplasty and surgical bypass for ischemic heart disease, or ischemic stroke) and logarithmic cumulative cardio-vascular survival (b) in patients with NFA, MSH and SSH during follow-up (15-years)

confirmed that SH is associated to higher incidence of CVEs and CV mortality, as well as to higher prevalence of diabetes mellitus and hypertension.

Our study agrees with previous evidences and reinforces thesis that chronic cortisol autonomous secretion by AI is related to progressive worsening of cardio-metabolic phenotype [20, 21].

In particular, we adopted clinical practice published guidelines, that are concordant with use of 1 mg DST for the screening of endogenous hypercortisolism, with cortisol threshold $<1.8 \mu\text{g/dl}$ to define adequate suppression [1], associated to other biochemical abnormalities: low ACTH levels ($<10 \text{ pg/ml}$), higher 24-h UFC excretion (over the upper limit of reference values) or missing circadian rhythm of cortisol [1, 21]. Moreover, other strengths of our study are the long-term prospective observation (follow-up more than 15-year), and the specific evaluation of major CV outcomes.

Interestingly, at the time of enrolment we found higher prevalence of MS and diabetes mellitus in SH patients compared with NFAI patients (42.8% vs 12.7% and 19% vs 7%, respectively), as well as higher prevalence of previous major CVE, as MI (6.4 vs 1.5%), stroke (5.1 vs 1.5%) and revascularization procedures (CABG or BPAC) (4.5 vs 0.4%), confirming in SH patients the higher CVE events and the role of metabolic abnormalities, as MS and altered glucose metabolism. Recently, Araujo-Castro M. et al have found in 149 AI patients, followed-up for a mean time of 34.6 months, higher prevalence of diabetes in subjects with plasma cortisol values $>1.8 \mu\text{g/dl}$ after DXM test respect to NFAI (38.0 vs 22.0%, $p < 0.04$) [22].

Chronic exposure to hypercortisolism can impair glucose homeostasis by modifying the complex network of signaling in adipose tissue biology [23], exerting pleiotropic effects on different fat depots [24]. In case of endogenous or exogenous glucocorticoid excess, dysregulated adipose physiology brings to abdominal obesity and high circulating levels of free fatty acids and triglycerides [25, 26]. Lipid overload is the consequence of altered insulin signaling in adipocytes, increased lipolysis, aberrant adipokine secretion and low-grade inflammation, inducing peripheral insulin resistance, up to overt diabetes mellitus [27, 28]. As consequence, some Authors had proposed screening, by overnight 1 mg DST, on type 2 diabetes population, especially if associated to poor glycemic control and advanced microvascular damage. Recently, Cansu et al. have found significant prevalence of SH (2%) in diabetic patients with high levels of HbA1c ($>8\%$) and macrovascular complications, as nephropathy, retinopathy and polyneuropathy [29].

We previously described impairment of adipokines levels and adipokines expression in intra-abdominal adipose tissue in SH patients; in fact, we found higher tissue expression of Leptin mRNA from fat surrounding adrenal gland in cortisol-producing adrenal adenomas than in NFAs [30]; on the other hand, in cortisol-secreting group, Adiponectin mRNA expression was reduced in fat surrounding cortisol-secreting adenoma of adrenal gland. As regard, in patients with overt Cushing Syndrome, Weise and Masuzaki found positive correlation between Leptin concentrations and BMI [31, 32]. Actually, there are several biochemical evidences that in SH patients adipocytokines as well several pro-inflammatory cytokines (i.e., IL-6) secretion from intra-abdominal fatty tissue (i.e., perirenal, perivisceral fatty tissue), may contribute to influencing long-term CV risk in SH patients [33]. As regard, after a long time follow-up we interestingly found a significant reduction of visceral adiposity, expressed clinically as waist circumference, in those patients surgically treated, due to resolution of autonomous cortisol excess production.

Table 6 Univariate and backward-stepwise analysis model for CV event

	Univariate analysis				Multivariate analysis backward-stepwise cox analysis			
	Unstandardized coefficients (95% CI)	Standard error	Standardized β	p	Unstandardized coefficients (95% CI)	Standard error	Standardized β	p
DST-Csl (nmol/L)	0.0002 (0.0001–0.001)	0.0001	0.048	0.234	–	–	–	–
UFC (nmol/L)	0.00002 (–0.002–0.002)	0.0001	0.008	0.848	–	–	–	–
BMI (kg/m ²)	–0.001 (–0.05–0.002)	0.002	–0.032	0.453	–	–	–	–
WC (cm)	0.001 (–0.01–0.003)	0.001	0.027	0.534	–	–	–	–
NC (cm)	0.0001 (–0.003–0.003)	0.001	–0.007	0.865	–	–	–	–
Glycemia (mg/dL)	0.001 (0.0001–0.002)	0.001	0.079	0.055	0.001 (0.0003–0.002)	0.001	0.103	0.008
LDL-C (mg/dL)	–0.001 (–0.001–0.0001)	0.0001	–0.087	0.037	–0.001 (–0.0015–0.0020)	0.0003	–0.106	0.006
Total-C (mg/dL)	0.0001 (0.00004–0.00009)	0.0001	–0.05	0.204	–	–	–	–
TGLs (mg/dL)	0.00003 (0.0003–0.0004)	0.0002	0.007	0.866	–	–	–	–
UA (mg/dL)	0.013 (–0.04–0.030)	0.009	0.054	0.191	–	–	–	–
G-SBP (mmHg)	0.001 (0.0001–0.0002)	0.0002	0.118	0.004	0.001 (0.001–0.001)	0.0002	0.127	0.001
G-DBP (mmHg)	0.0002 (–0.001–0.001)	0.0004	0.013	0.762	–	–	–	–
D-SBP (mmHg)	0.001 (–0.002–0.006)	0.002	0.089	0.332	–	–	–	–
D-DBP (mmHg)	–0.001 (–0.002–0.006)	0.003	–0.047	0.622	–	–	–	–
N-SBP (mmHg)	–0.001 (–0.008–0.004)	0.002	–0.037	0.715	–	–	–	–
N-DBP (mmHg)	–0.001 (–0.005–0.003)	0.003	–0.030	0.776	–	–	–	–
c-IMT (mm)	0.037 (–0.117–0.193)	0.078	–0.020	0.637	–	–	–	–

DST Csl cortisol levels after 1 mg of dexamethasone suppression test, *UFC* urinary free cortisol, *ACTH* Adreno Cortico Tropic Hormone, *Total-C* total cholesterol, *LDL-C* low density lipoprotein cholesterol, *TGLs* triglycerides, *BMI* body mass index, *WC* waist circumference, *NC* neck circumference, *UA* uric acid, *G-SBP* global average systolic blood pressure, *G-DBP* global average diastolic blood pressure, *D-SBP* daytime average systolic blood pressure, *D-DBP* daytime average diastolic blood pressure, *N-SBP* night-time average systolic blood pressure, *N-DBP* night-time average diastolic blood pressure, *c-IMT* carotid intima media thickness

Moreover, Maurice et al. found in patients with Cushing's Syndrome higher left ventricular mass index associated to higher levels of epicardial adipose tissue, suggesting pathophysiological relationship between epicardial fat tissue, left ventricular function, and cortisol secretion [34].

Leptin is directly responsible to sympathetic activation leading to development or maintenance of arterial hypertension [35]. Overstimulation of sympathetic nerve function [36] and sodium-retaining action of cortisol can contribute to the alteration of the circadian BP rhythm, explaining higher prevalence of non-dipping pattern [9, 12], as well as the restoration of BP rhythm after treatment of cortisol excess.

GCs can affect glucose metabolism by multifactorial mechanisms, demonstrated by in vitro and in vivo studies [37], as increased hepatic glucose production (directly or indirectly antagonizing metabolic actions of insulin), decreased insulin-dependent glucose uptake into peripheral tissues, especially skeletal muscle tissue (reduced expression and phosphorylation of insulin receptor substrate-1, phosphatidylinositol 3-kinase, and protein kinase B/Atk, reduced migration of glucose trans-porter GLUT-4 to cell surface, reduced glycogen synthesis, stimulated muscle proteolysis), inhibition of insulin secretion from pancreatic β -cells (suppressed expression of GLUT-2 and glucokinase, increased glucose oxidation and decreased protein kinase's A and C activation) [38].

Table 7 Univariate and backward-stepwise analysis model for cardiovascular mortality

	Univariate analysis				Multivariate analysis backward-stepwise cox analysis			
	Unstandardized coefficients (95% CI)	Standard error	Standardized β	p	Unstandardized coefficients (95% CI)	Standard error	Standardized β	p
DST-Csl (nmol/L)	0.0002 (−0.0001–0.0002)	0.00007	0.014	0.725	–	–	–	–
UFC (nmol/L)	−0.000004 (−0.0001–0.00006)	0.00005	−0.033	0.427	–	–	–	–
BMI (kg/m ²)	0.0001 (−0.02–0.001)	0.001	−0.013	0.752	–	–	–	–
WC (cm)	0.0001 (−0.001–0.001)	0.0001	−0.013	0.764	–	–	–	–
NC (cm)	−0.00008 (−0.001–0.001)	0.001	−0.005	0.898	–	–	–	–
Glycemia (mg/dL)	0.001 (0.001–0.002)	0.0002	0.205	<0.001	0.001 (−0.001–0.001)	0.00002	0.184	0.001
LDL-C (mg/dL)	−0.00002 (0.0001–0.0002)	0.0001	−0.008	0.850	–	–	–	–
Total-C (mg/dL)	0.0002 (0.0001–0.0002)	0.0001	0.015	0.718	–	–	–	–
TGLs (mg/dL)	−0.00002 (0.0001–0.0002)	0.0001	−0.001	0.973	–	–	–	–
UA (mg/dL)	−0.004 (−0.011–0.003)	0.004	−0.042	0.305	–	–	–	–
G-SBP (mmHg)	−0.00003 (0.0001–0.0002)	0.0001	−0.001	0.974	–	–	–	–
G-DBP (mmHg)	0.0000007 (−0.0002–0.001)	0.001	0.002	0.967	–	–	–	–
D-SBP (mmHg)	0.0003 (−0.002–0.001)	0.001	−0.042	0.644	–	–	–	–
D-DBP (mmHg)	−0.00009 (−0.003–0.023)	0.001	−0.007	0.942	–	–	–	–
N-SBP (mmHg)	0.0001 (−0.002–0.003)	0.001	0.017	0.866	–	–	–	–
N-DBP (mmHg)	0.001 (−0.002–0.003)	0.001	0.042	0.688	–	–	–	–
c-IMT (mm)	−0.0238 (−0.087–0.1)	0.033	−0.030	0.477	–	–	–	–

DST Csl cortisol levels after 1 mg of dexamethasone suppression test, *UFC* urinary free cortisol, *ACTH* Adreno Cortico Tropic Hormone, *LDL-C* low density lipoprotein cholesterol, *Total-C* total cholesterol, *TGLs* triglycerides, *BMI* body mass index, *WC* waist circumference, *NC* neck circumference, *UA* uric acid, *G-SBP* global average systolic blood pressure, *G-DBP* global average diastolic blood pressure, *D-SBP* daytime average systolic blood pressure, *D-DBP* daytime average diastolic blood pressure, *N-SBP* night-time average systolic blood pressure, *N-DBP* night-time average diastolic blood pressure, *c-IMT* carotid intima media thickness

Second aim of our study was to evaluate if the surgical treatment of cortisol excess (by laparoscopic adrenalectomy) could prevent and reduce CV risk, compared with SH patients undergoing an optimized pharmacological treatment in order to reduce metabolic modifications. As regard it, the debate is still open: while National Institutes of Health and French Society of Endocrinology do not extensively recommend surgery [39], Italian Association of Clinical Endocrinologists and American Association of Clinical Endocrinologists suggest surgical approach [14, 40], because cardiometabolic comorbidities, such as worsening hypertension, abnormal glucose tolerance, dyslipidemia or osteoporosis, are not completely controlled with an intensified pharmacological therapy. Some authors

have suggested that surgery should be considered in SH patients in the presence of at least two comorbidities among obesity, arterial hypertension, type 2 diabetes and dyslipidemia [41]. Comparing surgical and conservatively management of SH patients, Chiadini et al. found that medical therapy has relevant side-effects and minor beneficial effects, whereas surgical treatment is associated to significant improvement (more than half of cases) of hypertension, diabetes, obesity and risk of fractures [42–44].

As regard it, in an interesting review Iacobone et al. [21] confirmed the beneficial effects of surgery for several metabolic abnormalities in SH patients, despite methodological limitations of evaluated studies (heterogeneity definition of SH, different endpoints). About technical aspects,

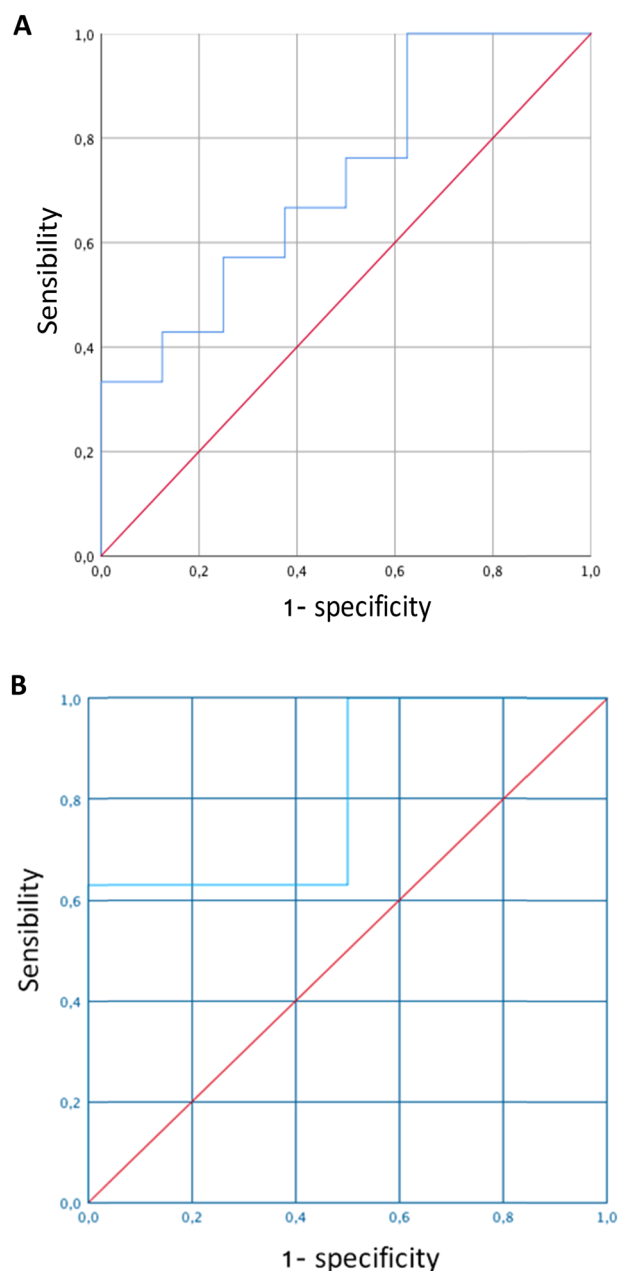


Fig. 2 The receiver operating characteristic (ROC) curve analysis shows (a) the diagnostic performance of urinary free cortisol (UFC) at baseline to discriminate Office Systolic Blood pressure (O-SBP) reduction after surgery in SSH patients; the best cutoffs for UFC was set at 109.65 nmol/24 h (AUC 0.720 ± 0.106 , $p < 0.05$; sensibility 76.2%, specificity 37.5%); (b) the association between UFC at baseline and the reduction of waist circumference after surgery in SSH patients. The best cutoffs for UFC was set at 132 nmol/24 h (AUC 0.815 ± 0.153 , $p < 0.05$; sensibility 63%, specificity 50%)

in large study population conducted in specialized and experienced Centers, de La Villeon demonstrated that laparoscopic adrenalectomy, besides being valid and adequate in order to improve several metabolic and CV abnormalities, is also safe and risk-free, with low morbidity and no significant mortality [45].

In previous study [9] we observed in selected patients with NFAI and SH undergoing to surgical treatment, after a follow-up of 12 months, the significant reduction in terms of prevalence of obesity, hypertension, MS, and reduction of 24-h average-SBP, as well as reduction of non-dipper pattern and mean number of antihypertensive medications used. Same results were observed by Debono [15] and Patrova [15], with relevant relationship between mortality in patients with autonomous cortisol secretion with cortisol levels post-DXT, age and tumor size; moreover, it was observed increased mortality, beyond CVEs, related to infection disease, as well as and higher incidence of osteoporosis.

In this study, we have expanded our population, added new information on personal history of CVEs (detecting CVEs prior to enrollment), and extended timing of follow-up to 15 years. During follow-up, in SSH patients we have observed a significant reduction of several metabolic and CV parameters as WC, serum behaviors of triglycerides and LDL-cholesterol, up to reduced prevalence of dyslipidaemia, hypertriglyceridemia, MS and type 2 diabetes; whereas in MSH group we have found significant increase of WC and serum uric acid.

Besides the overt damage previously described, there's more emerging interest on subclinical atherosclerosis markers in SH. In a previous study, we described that subjects with Cushing's Syndrome had higher values of IMT and ABI, compared with healthy and essential hypertensive subjects [46]. Hence, it has also been discovered that in SH patients, arterial stiffness, IMT, flow mediated dilation, and atherosclerotic plaques, are independently associated with severity and duration of excessive cortisol exposure [47–52]. Once subclinical cortisol excess has permanently removed (by surgical treatment), it is clear the potential positive effect of pre-existing vascular damage. About it, in this study during follow-up we found significant thickening of c-IMT (from 0.93 ± 0.3 to 1.0 ± 0.2 ; $p < 0.01$) and an increased incidence of plaques (from 21 to 30%, $p < 0.01$) in subject undergoing medical management (MSH patients), while in SSH patients we did not observe any worsening in vascular indexes in SSH patients. Moreover, using ROC curve analysis, we have found that UFC values higher than 110 and 132 nmol/24 h are able to predict before surgery the significative amelioration of relevant anthropometric and vascular parameters, as reduction of visceral obesity and office systolic blood pressure control in SSH patients.

Several registries and studies have widely demonstrated that patients with SH are at increased risk of CVEs [5–19]. In an Italian multicenter study directed by Morelli in 2014 [19], it has been shown that SH is an independent risk factor for the occurrence of new CVE over time, with an annual rate of CV events of 3.1%, that is substantially similar in population affected by type 2 diabetes, hypertension and dyslipidemia.

Di Dalmazi et al. [7] discovered that the survival rate for all-cause mortality was lower in AI patients with autonomous secretion of cortisol, especially in those with worsening cortisol secretion during follow-up (evaluated by cortisol levels after 1mg-DST) compared with nonsecreting masses (57 vs. 91%). In this report, factors associated with increased mortality were age and mean cortisol levels after 1mg-DST, but not previous coronary heart disease.

In our study, we observed either higher CVEs prevalence (MI, stroke, revascularization procedures) at diagnosis of SH, as well as higher prevalence of MS and DM, either significant reduction of CVEs and mortality in subjects treated with surgical removal of cortisol excess. Main independent factors associated to CV mortality and CVEs were mean value of 24-h average-SBP, plasma levels of LDL-cholesterol and serum glucose, underling the role of these metabolic and vascular alterations strictly related to autonomous cortisol secretion in the development of CV and metabolic complications.

Regarding it, our study confirms and extends literature evidences: cortisol excess in SH is associated to worse lipid and glicometabolic profile, and a globally increased recurrence of MS. As regard, in our casuistry those patients with lack of suppression cortisol levels after DST ($>1.8 \mu\text{g/dL}$ or $>50 \text{ nmol/L}$), identified by Guideline as “possible autonomous cortisol secretion”, in presence of coexistence of CV co-morbidities (arterial hypertension poorly treated with medications, glucose intolerance or type 2 diabetes mellitus, MS, obesity, dyslipidemia) show higher prevalence of main CVEs and mortality, similar to patients with cortisol levels after DSM test $>5.0 \mu\text{g/dL}$ or $>138 \text{ nmol/L}$), highlighting the additive action of CV comorbidities associated to autonomous cortisol secretion into development of main CV complications. SH patients who received a pharmacological management of their comorbidities, compared with patients who went to laparoscopic adrenalectomy showed a dramatic recurrence of major CVE, with unadjusted RR for CVE in MSH respect NFA and SSH of 2.56 and 2.10, respectively ($p < 0.001$).

After multivariate analysis, cortisol secretion was not a major factor that influenced CV events and CV mortality as shown in Tables 5 and 6. This result could be addressed to two different aspects. First, we have taken into account the overall population (SH was represented by 25% of patients enrolled); second, in the statistic model we have analyzed the continuous variables and not the excessive cortisol secretion status as a dichotomic variable. These two factors may have reduced the statistical power of cortisol secretion influencing CV events and mortality in our analysis.

Because of high prevalence of subclinical organ damage and metabolic abnormalities in SH patients, future efforts should not be in addressing to surgery only those patients with overt CV damage, or whit previously major CVE; but

once clearly biochemically and clinically defined SH, physicians should be inclined to identify subclinical damage, an essential part of hypertension-mediate organ damage in SH patients [51, 52]. Thus, it will be desirable to recognize those subjects who will get maximum benefit from laparoscopic adrenalectomy and will prevent a certain worsening of their metabolic and CV profile.

In our case series, none of our patients who went laparoscopic adrenalectomy had peri and post-procedural complications. Our experience agrees with literature that surgical approach is effective on reducing CV abnormalities and recurrence of new CVE. Probably, for the pathological mechanisms mentioned above, surgical approach has been demonstrated as really way to reduce hypertensive overload, expressed in terms of average 24 h-blood pressure and non-dipping pattern. Moreover, during follow-up, SSH patients used a significantly reduced number of anti-hypertensive medications (2.3 ± 1.0 vs 1.5 ± 0.4 ; $p < 0.05$).

Future studies should be designed in prospective randomized controlled model and should require a long-term follow up to determine real effects on cumulative rate survival and life of these patients.

In conclusion, in our experience patients with SH, especially if associated to CV comorbidities, should be encouraged to strict follow-up and treatment, optimization of pharmacological treatment, until laparoscopic adrenalectomy if indicated, because of its promising effect on CV, heart and cerebro-vascular disease.

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Compliance with ethical standards

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

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