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ORIGINAL ARTICLE

The degree of urinary hypercortisolism is not correlated with the severity of cushing's syndrome

Valentina Guarnotta[1] · Marco C. Amato[1] · Rosario Pivonello[2] · Giorgio Arnaldi[3] · Alessandro Ciresi[1] · Laura Trementino[3] · Roberto Citarrella[1] · Davide Iacuaniello[2] · Grazia Michetti[3] · Chiara Simeoli[2] · Annamaria Colao[2] · Carla Giordano[1]

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Abstract Cushing syndrome (CS) is characterized by increased morbidity and mortality compared to the general population. However, there are patients who have more clinical aggressive forms than others. Aim of the study is to evaluate whether the degree of hypercortisolism, defined by the number of times urinary free cortisol (UFC) levels exceed the upper limit of the normal range (ULN), is related to the worsening of phenotypic features, as well as metabolic and cardiovascular parameters, in a cohort of CS patients. A cross-sectional study was conducted on 192 patients with active CS, consecutively presenting at the outpatients' clinic of the University Hospitals of Ancona, Naples, and Palermo. Patients were grouped into mild (UFC not exceeding twice the ULN), moderate (2–5 times the ULN), and severe (more than 5 times the ULN) hypercortisolism. Thirty-seven patients (19.3 %) had mild, 115 (59.8 %) moderate, and 40 (20.9 %) severe hypercortisolism. A significant trend of increase among the three groups was demonstrated for 8-, 16-, and 24-h serum cortisol levels ($p < 0.001$) and serum cortisol after low dose of dexamethasone suppression test ($p = 0.001$). No significant trend of increase was found regarding phenotype and comorbidities. The degree of hypercortisolism by itself does not appear to be a sufficient parameter to express the severity of CS. Therefore, estimating the severity of CS according to biochemical parameters remains a challenge, while the clinical phenotype and the associated comorbidities might be more useful to assessing the severity of the CS.

Keywords Cushing syndrome severity · Urinary free cortisol · Cushing syndrome comorbidities · Degree of hypercortisolism

Introduction

Cushing syndrome (CS) is caused by prolonged exposure to inappropriately high levels of cortisol. It is the consequence of a pituitary or extrapituitary ACTH-secreting tumor (respectively 70–75 and 5–10 % of cases), which in turn stimulate cortisol overproduction from adrenal glands, while in few patients it may depend on an adrenal tumor or hyperplasia (10–20 % of cases) [1–4].

CS is associated with increased mortality rate compared to general population mainly due to cardiovascular disease, direct consequence of the several comorbidities associated with CS, but also to infectious diseases, and consequent sepsis as result of the immunosuppression induced by hypercortisolism [5–12]. However, the mortality rate seems to be strongly dependent by the disease status. Patients with persistent or recurrent disease show a dramatically increased mortality compared to the general population, while patients who obtain the surgical remission seem to have a mortality rate similar to that of general population, even though some discrepancies have been reported [13]. Indeed, different retrospective studies reported a maintained increased mortality compared to general population,

✉ Carla Giordano
carla.giordano@unipa.it

[1] Dipartimento Biomedico di Medicina Interna e Specialistica (Di.Bi.M.I.S), Sezione di Endocrinologia, Diabetologia e Malattie Metaboliche, Università degli Studi di Palermo, Palermo, Italy

[2] Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy

[3] Clinica di Endocrinologia e del Metabolismo, Azienda Ospedaliero-Universitaria Ospedali Riuniti di Ancona, Ancona, Italy

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even in patients who achieved the remission [14–16], due to the persistence of several comorbidities and strongly dependent on the duration of exposure to glucocorticoid excess.

Therefore, an immediate therapeutic action is required in patients with CS in order to improve life expectancy [17].

Obviously, patients do not have the same degree of the disease. A subgroup of patients shows an early onset, with a different severity of complications at diagnosis, suggesting the interaction of many factors in inducing the aggressiveness of the disease. However, all these pathogenetic factors are not completely known. Maybe, the degree and duration of hypercortisolism have a crucial role, even though other factors might have an influence in determining CS complications [18, 19].

To date, in agreement with the Endocrine Society guidelines, the recommended parameters to diagnose CS hypercortisolism are measurement of 24-h urinary free cortisol (UFC), late-night salivary cortisol (LNSC), and serum cortisol after low dose (1 mg) of dexamethasone suppression test (LDDST) [20]. However, in some clinical trials, patients with CD have been grouped into patients with mild, moderate, severe, and very severe hypercortisolism according to UFC levels, evaluated in terms of the number of times they exceed the upper limit of the normal range (ULN) [21, 22]. This arbitrary grouping might represent a way to differentiate patients according to the severity of CD, even though no studies have been conducted to demonstrate its effectiveness.

Therefore, the aim of this study was to evaluate whether the degree of hypercortisolism, based on the ULN of UFC levels, is related to severity of clinical, metabolic, and cardiovascular picture, in a large cohort of patients with active CS.

Materials and methods

Patients and study design

The current study is based on a retrospective analysis of data collected from 192 patients (males 19.8 % and females 80.2 %) with active CS (71.8 % with pituitary and 28.2 % with adrenal dependent disease), recruited at the Endocrinology Units of the University Hospitals of Ancona, Naples, and Palermo between 2000 and 2013.

The diagnosis of CS was based, in accordance with the international criteria, on high daily UFC levels (at least three samples), absent cortisol suppression after LDDST (<1.8 μg/dl), and lack of cortisol rhythm (midnight cortisol levels <7.5 μg/dl) [20]. In addition, the diagnosis of CD was based on high or inappropriately normal serum ACTH levels, magnetic resonance imaging confirmation of a pituitary tumor, or an ACTH central-peripheral gradient >2 at baseline and/or >3 after corticotrophin-releasing hormone (CRH) or desmopressin (DDAVP) stimulation at inferior petrosal sinus sampling [20]. Exclusion criteria were subclinical CS, adrenal, or pituitary cancer. No women taking oral contraceptives were included.

This study was approved by the Institutional Review Boards at each center. At the time of hospitalization, all patients provided written informed consent for the scientific use of their data. The identity of the participation remained anonymous during database analysis.

All patients at the diagnosis had undergone a complete hormonal evaluation of the pituitary–adrenal axis. Three 24-h urine samples had been collected to calculate the mean UFC (mUFC) for each patient. Then, for all patients with high mUFC, the serum cortisol after low dose of dexamethasone (1 mg) and the 8-, 16-, and 24-h serum cortisol and ACTH levels had been performed.

Patients were subdivided into three groups according to mUFC. Mild hypercortisolism was defined by mUFC levels ≤33th percentile (approximately equivalent to UFC level not exceeding 2 times the ULN). Moderate hypercortisolism was defined by a level of mUFC more than 34th to 66th percentile (approximately equivalent to UFC level more than 2–5 times the ULN). Severe hypercortisolism was defined by a mUFC $\geq 67^{th}$ percentile (approximately equivalent to UFC level more than five times the ULN).

Clinical parameters such as BMI, systolic and diastolic blood pressure, measured according to international criteria [23], waist circumference (WC), measured at the midpoint between the lower rib and the iliac crest, and waist/hip ratio (WHR) were retrospectively obtained. The mean duration of the disease was established by patient interview, patients' clinical pictures, and beginning of the weight increase.

Some phenotypic parameters such as muscle hypotrophy, moon face, facial plethora, buffalo hump, and purple striae evaluated by clinical examination, were drawn out. As comorbidities, the study included the cardiovascular complications such as coronary heart disease, vascular disease (central and peripheral), and thrombotic events, infections, hypokalemia, the metabolic complications such as metabolic syndrome and impairment of glucose metabolism, the depression and the bone complications such as osteoporosis/osteopenia and vertebral and non-fractures. All these data had been obtained by patient interviews, laboratory, and instrumental data.

After an overnight fast, lipids [total cholesterol (C), HDL-C, LDL-C, and triglycerides (TG)], HbA1c, glycaemia, insulin, coagulation parameters, calcium, phosphorus, parathyroid hormone (PTH), and hepatic and renal functions had been evaluated. The insulin sensitivity was

indirectly estimated using basal insulin and glucose values to calculate the homeostatic model of insulin resistance (HOMA2-IR) [glycaemia (mmol/l) × insulinemia (mU/ml)/22.5] [24]. In a subgroup of 70 patients who had undergone oral glucose tolerance test (OGTT) the Matsuda index of insulin sensitivity (ISI Matsuda) [10000/glucose (mg/dl) × insulin (mU/ml) × glucose mean x insulin mean] [25], the oral disposition index (DIo) [($\Delta Insulin_{0-30}$/$\Delta Glucose_{0-30}$) × (1/fasting insulin)] [26], and the area under the curve for insulin ($AUC_{2\text{-h insulin}}$) and glucose ($AUC_{2\text{-h glucose}}$) were calculated.

The HOMA-β was calculated as [(360 × Insulin)/(Glucose—63) % (Glucose in mg/dl)] [23]. The visceral adiposity index (VAI) was calculated according to gender, where TG levels were expressed in mmol/l and HDL levels were expressed in mmol/l:

- Males $VAI = [WC/39.68 + (1.88 \times BMI)] \times (TG/1.03) \times (1.31/HDL)$
- Females $VAI = [WC/36.58 + (1.89 \times BMI)] \times (TG/0.81) \times (1.52/HDL)$ [27].

The metabolic syndrome (MS) was diagnosed according to the NCEP ATP III criteria, whereas DM and prediabetes were diagnosed according to the ADA criteria [28].

All patients included in the study who had surgery, had a subsequent histological confirmation of the CS.

Hormone and biochemical assays

Serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), creatinine, bone markers such as calcium, phosphorus, PTH, clotting factors, insulin, glycaemia, HbA1c, and lipids were measured by standard methods for each center. LDL-C levels were calculated using the Friedewald formula [total cholesterol − (HDL + (TG/5)]. UFC levels had been detected by electrochemiluminescence immunoassay (ECLIA) (Elecsys cortisol reagent kit with prior dichloromethane extraction to reduce the amount of cortisol metabolites and conjugates) in patients from Ancona and Palermo, and by Immulite, solid-phase chemiluminescent enzyme immunoassay (DPC, Los Angeles, CA, USA) in patients from Naples. A correction of UFC for creatinine concentration had been performed dividing UFC by urinary creatinine excretion. The normal range for UFC was 10–110, 35–135, and 36–137 μg/24 h, respectively for Ancona, Naples, and Palermo.

Serum cortisol was evaluated using the electrochemiluminescent (ECLIA) automated method Access (Beckman Coulter, Brea, CA, USA) for Palermo and Ancona, by Immulite, solid-phase chemiluminescent enzyme immunoassay (DPC, Los Angeles, CA, USA) for Naples. The intra- and inter-assay coefficients of variation for serum and urinary cortisol were 1.2 and 1.4 % for Ancona and Palermo and 6.04 and 7.78 % for Naples. The conversion factors for the International System (SI) were as follows: glucose mg/dl versus mmol/l: 0.0555; insulin mUI/ml versus pmol/l: 6.945; total cholesterol and HDL-C mg/dl versus mmol/l: 0.0259; triglycerides mg/dl versus mmol/l: 0.0113; cortisol ng/ml versus nmol/l: 2.76; UFC microg/24 h versus nmol/24 h: 2.76.

Statistical analysis

The Statistical Packages for Social Science SPSS version 17 (SPSS, Inc.) was used for data analysis. The normality of quantitative variables was tested with the Shapiro–Wilk test. Baseline characteristics were presented as mean ± SD for continuous variables, while rates and proportions were calculated for categorical data. The ANOVA trend analysis for quantitative variables and χ^2 for trend for categorical variables were carried out for the three groups: mild, moderate, and severe. Simple univariate correlations among continuous variables with non-normal distribution were determined by Spearman test. A p value <0.05 was considered to be statistically significant.

Results

Patients were classified as having a mild (19.2 %), moderate (59.8 %), and severe hypercortisolism (20.8 %).

Clinical and phenotypic characteristics and the duration of disease of all patients are shown in Table 1. No significant trend was found among the three groups of patients for the above-mentioned parameters.

Comorbidities of patients are shown in Table 2. No significant trend was observed for cardiovascular and thrombotic complications, infections, hypokalemia, psychiatric, bone, and metabolic comorbidities.

The biochemical parameters of all patients are shown in Table 3. No significant trend was observed for lipids, glucose, insulin sensitivity and secretion indexes, and clotting parameters among the three groups of patients.

A significant trend of increase was observed in 8-, 16-, and 24-h serum cortisol and similarly in serum cortisol after LDDST (Fig. 1). A strong correlation between the ULN of UFC and 8 h cortisol (Rho 0.339, $p < 0.001$) (Fig. 1a), ULN of UFC and 16 h cortisol (Rho 0.336 $p < 0.001$) (Fig. 1b), ULN of UFC and 24 h cortisol (Rho 0.400, $p < 0.001$) (Fig. 1c), and ULN of UFC and serum cortisol after LDDST (Rho 0.308, $p < 0.0001$) (Fig. 1d) was found.

Comparing patients with adrenal and pituitary disease not significant differences were found for the ULN of UFC (p 0.173), 8 h cortisol (p 0.936), 16 h cortisol (0.830), 24 h

Table 1 Clinical and phenotypic parameters of all 192 Cushing patients grouped according to the degree of hypercortisolism, in mild, moderate, and severe

	Mild ($N = 37$) Mean ± SD	Moderate ($N = 115$) Mean ± SD	Severe ($N = 40$) Mean ± SD	p
Clinical parameters				
Age (year)	45.59 ± 17.64	43.71 ± 16.25	38.83 ± 17.40	0.075
BMI (Kg/m^2)	31.29 ± 8.52	30.87 ± 5.0	29.62 ± 5.23	0.316
WC (cm)	100.76 ± 19.72	105.80 ± 15.42	95.55 ± 16.01	0.140
WHR	0.98 ± 0.08	1.02 ± 0.09	0.98 ± 0.06	0.863
Duration of disease (months)	33.38 ± 24.6	28.8 ± 28.37	33.65 ± 26.98	0.513
	Subjects (%)	Subjects (%)	Subjects (%)	
Gender				
Female	30 (81.1 %)	93 (80.9 %)	31 (77.5 %)	0.680
Male	7 (18.9 %)	22 (19.1 %)	9 (22.5 %)	0.700
Pituitary dependent	24 (64.9 %)	81 (70.4 %)	33 (82.5 %)	0.082
Adrenal dependent	13 (35.1 %)	34 (29.6 %)	7 (17.5 %)	
Phenotypic parameters				
Moon face	24 (64.9 %)	54 (47.0 %)	22 (55 %)	0.416
Facial Plethora	18 (48.6 %)	63 (54.8 %)	20 (50 %)	0.978
Buffalo hump	16 (43.2 %)	43 (37.4 %)	11 (27.5 %)	0.148
Purple striae	14 (37.8 %)	35 (30.8 %)	19 (47.5 %)	0.348

Table 2 Comorbidities of all 192 Cushing patients grouped according to the degree of hypercortisolism, in mild, moderate, and severe

	Mild ($N = 37$) Subjects (%)	Moderate ($N = 115$) Subjects (%)	Severe ($N = 40$) Subjects (%)	P
Cardiovascular and thrombotic complications				
Coronary heart disease	8 (21.6 %)	26 (22.6 %)	7 (17.5 %)	0.648
Thromboembolic events	6 (16.5 %)	7 (6.1 %)	3 (7.5 %)	0.180
Peripheral vascular disease	6 (16.2 %)	7 (6.1 %)	2 (5 %)	0.172
Cerebral vascular disease	3 (8.1 %)	9 (7.8 %)	4 (10 %)	0.757
Psychiatric complications				
Depression	7 (18.9 %)	23 (20.0 %)	12 (30 %)	0.231
Infections	6 (16.2 %)	9 (7.8 %)	2 (5 %)	0.087
Hypokalemia	7 (18.9 %)	16 (13.9 %)	7 (17.5 %)	0.586
Bone complications				
Osteoporosis/osteopenia	11 (29.7 %)	27 (23.5 %)	16 (40 %)	0.292
Non-vertebral fractures	6 (16.2 %)	12 (10.4 %)	2 (5 %)	0.107
Vertebral collapse	2 (5.4 %)	16 (13.9 %)	3 (7.5 %)	0.806
Metabolic complications				
Metabolic syndrome***	22 (59.5 %)	69 (60 %)	19 (47.5 %)	0.276
High Blood pressure***	24 (64.9 %)	88 (76.5 %)	25 (62.5 %)	0.773
High Triglycerides***	12 (32.4 %)	42 (36.5 %)	12 (30 %)	0.804
Low HDL Cholesterol***	17 (45.9 %)	58 (50.4 %)	12 (30 %)	0.145
Increased WC***	27 (73 %)	95 (82.6 %)	27 (67.5 %)	0.524
Hypercholesterolemia	15 (40.5 %)	50 (47 %)	17 (42.5 %)	0.873
Impaired fasting glucose (IFG)	1 (2.7 %)	7 (6.1 %)	0	0.521
Impaired glucose tolerance (IGT)	2 (5.4 %)	18 (15.7 %)	3 (7.5 %)	0.822
IFG + IGT	0	1 (0.9 %)	1 (2.5 %)	0.276
Diabetes mellitus	12 (32.4 %)	35 (30.4 %)	14 (35 %)	0.797

*** According to Adult Treatment Panel (ATP) III criteria

Table 3 Biochemical parameters of all 192 Cushing patients grouped according to the degree of hypercortisolism, in mild, moderate, and severe

	Mild (N = 37) Mean ± SD	Moderate (N = 115) Mean ± SD	Severe (N = 40) Mean ± SD	*p*
Metabolic and general parameters				
Total Cholesterol (mmol/l)	5.30 ± 0.86	5.15 ± 0.97	5.32 ± 1.05	0.911
HDL Cholesterol (mmol/l)	1.32 ± 0.39	1.34 ± 0.37	1.41 ± 0.37	0.343
LDL cholesterol (mmol/l)	3.12 ± 0.85	3.04 ± 0.83	3.04 ± 1.01	0.681
Triglycerides (mmol/l)	1.68 ± 0.97	1.61 ± 0.82	1.65 ± 1.16	0.901
Fasting glucose (mmol/l)	5.95 ± 2.65	6.07 ± 2.18	5.25 ± 1.39	0.143
Fasting insulin (UI/ml)	11.34 ± 0.67	13.66 ± 6.85	11.87 ± 5.41	0.745
GOT (mg/dl)	25.4 ± 17.1	19.8 ± 7.05	18.10 ± 4.75	0.124
GPT (mg/dl)	34.63 ± 21.7	32.3 ± 18.17	35.4 ± 19.75	0.936
Calcium (mg/dl)	9.6 ± 0.54	9.45 ± 0.51	9.35 ± 0.46	0.174
Phosphorus (mg/dl)	3.81 ± 0.4	3.63 ± 0.7	3.45 ± 0.6	0.115
PTH (pg/ml)	44.1 ± 24.13	48.01 ± 30.76	45.62 ± 26.27	0.832
Creatinin (mg/dl)	0.95 ± 0.27	0.87 ± 0.28	0.89 ± 0.23	0.613
HbA1c (%)	6.5 ± 1.3	6.5 ± 1.32	6.31 ± 1.17	0.651
HbA1c (mmol/mol)	48 ± 0.9	48 ± 0.9	45 ± 0.1	0.651
VAI	2.69 ± 2.02	2.63 ± 1.72	2.40 ± 2.13	0.492
AUC $_{2h\ glucose}$* (mmol/l 120 min)	1042.63 ± 321.33	993.51 ± 378.48	1064.17 ± 368.7	0.800
Insulin sensitivity indexes				
Homa 2-IR	1.66 ± 0.6	2.03 ± 1.03	1.68 ± 0.79	0.995
ISI Matsuda*	4.83 ± 3.35	4.05 ± 3.53	3.98 ± 1.66	0.522
Insulin secretion indexes				
Homa β	129.71 ± 68.31	136.45 ± 80.25	141.65 ± 55.85	0.479
AUC $_{2h\ insulin}$* (nmol/l 120 min)	7739.12 ± 4830.2	10842.8 ± 6951.5	7959.2 ± 3660.6	0.845
Oral Disposition Index (DIo)*	3.10 ± 5.74	1.58 ± 1.55	1.68 ± 2.15	0.216
Clotting parameters				
INR	0.99 ± 0.08	0.94 ± 0.09	1.67 ± 2.86	0.149
aPTT (sec)	28.2 ± 3.81	28.3 ± 4.69	27.1 ± 4.45	0.482
ATIII (%)	107.1 ± 12.18	120.69 ± 39.69	118.0 ± 17.21	0.415
D-Dimer (ng/ml)	206.5 ± 75.6	159.9 ± 147.5	370.7 ± 424.2	0.359
Fibrinogen (mg/dl)	378.2 ± 96.16	347.3 ± 106.1	336.1 ± 106.6	0.232
Hormonal parameters				
UFC (nmol/24 h)	4057.9 ± 1297.8	9095.3 ± 2299.9	24.138 ± 5872.5	<0.001

* In a subgroup of 70 patients without known diabetes

cortisol (0.764), and serum cortisol after LDDST (0.139) (data not shown).

Discussion

Currently, many studies show a relation between hypercortisolism and some conditions such as cognitive function impairment, depression, cardiovascular disease, infections, and metabolic syndrome [29–32]. However, little is known about a possible association between the degree of hypercortisolism and the aggressiveness of the syndrome.

This study shows that the degree of hypercortisolism evaluated by UFC levels, based on the number of times they exceed the ULN, is not correlated with the prevalence and severity of cardiovascular and thrombotic complications, infections, bone and psychiatric comorbidities, and impairment of phenotypical and metabolic parameters including insulin sensitivity and secretion indexes and VAI. This is an interesting finding because it might be expected that high UFC levels would correlate with the prevalence and intensity of phenotypic features and complications.

The results of the current study confirm and expand those of a previous series, showing that UFC levels alone

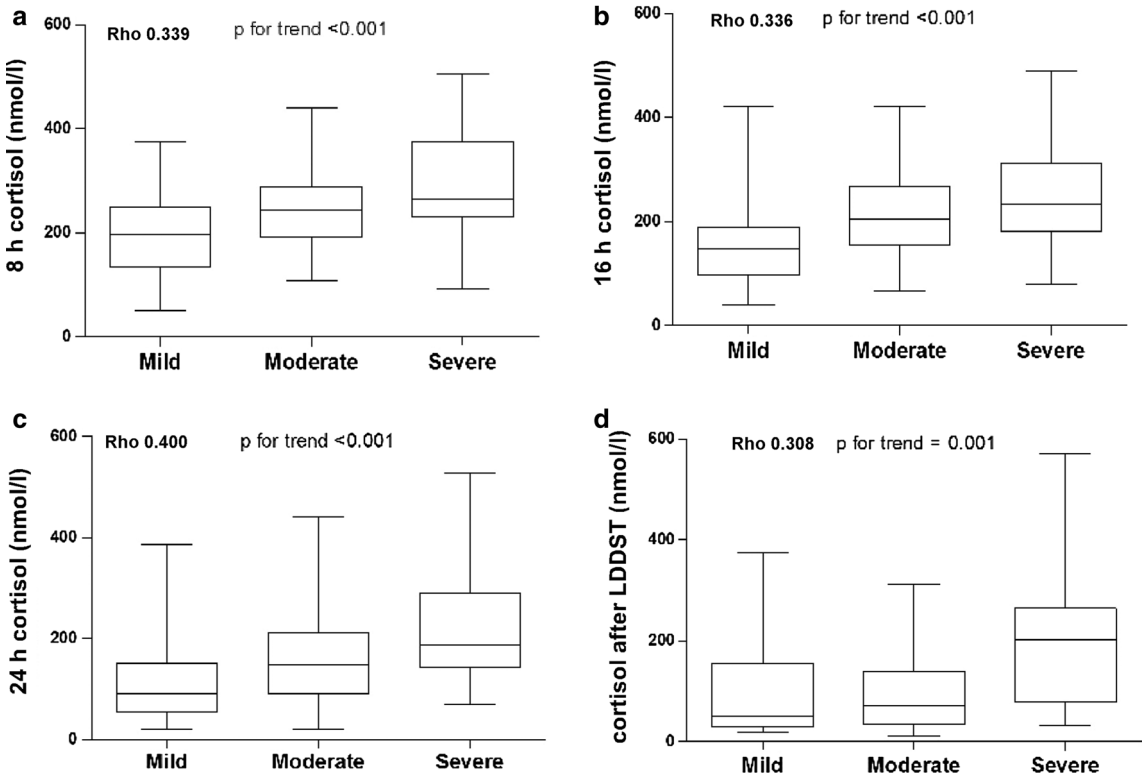

Fig. 1 Spearman correlation among 8-, 16-, 24-h serum cortisol, cortisol after LDDST and the ULN of UFC. **a** Correlation between the ULN of UFC and 8 h cortisol (Rho 0.339, $p < 0.001$). **b** ULN of UFC and 16 h cortisol (Rho 0.336 $p < 0.001$). **c** ULN of UFC and 24 h cortisol (Rho 0.400, $p < 0.001$). **d** ULN of UFC and serum cortisol after LDDST (Rho 0.308, $p < 0.0001$)

are not suggestive of CS severity [12, 33]. However, in the study of Petersenn et al. patients had a pituitary disease, while in our study also patients with adrenal disease were evaluated.

As known, UFC provides a good assessment of cortisol secretion over the 24-h period [34]. Measurement of 24-h UFC excretion has the advantage of being unaffected by short-term fluctuations in cortisol and by varying plasma protein binding capacities [35]. 24-h UFC reflects overall daily cortisol production and, compared to serial blood sampling, is relatively easy to collect in a large study population. An important advantage of this method is its reliability [36], although it is crucially dependent on the quality and performance of the particular analytical procedure applied. Indeed, several conditions may result in falsely elevated values such as alcoholism, high fluid intake, pregnancy, psychiatric disorders, or in falsely low levels such as cyclic CS or severe renal function [37, 38]. In this study, UFC levels were analyzed at three different laboratories, by different techniques, ECLIA and Immulite analytical procedures, even though the samples belonging to the same patients were measured in the same laboratory.

In the present study, the concordance between ULN of UFC and serum cortisol after LDDST and ULN of UFC and cortisol circadian rhythm (8-, 16-, 24-h cortisol) has been demonstrated, showing a significant correlation between these parameters. Unfortunately, concordance with LNSC has not been performed. However, previous studies have reported a significant correlation between UFC and LNSC [39–41], showing even a better performance of LNSC compared to UFC in the diagnosis of Cushing's syndrome.

In agreement with these results, it has been reported that some CS complications are not related to the degree of hypercortisolism, but influenced by a combination of factors, especially by the duration of glucocorticoid level exposure. Recently, it has been observed that diabetes in patients with CS is not strictly dependent on UFC and serum cortisol levels, but related to other parameters such as familial history of diabetes, age, and likely duration of disease [42]. In another study conducted by Zilio et al., no correlation between UFC and onset of venous thromboembolic events were found [43].

However, some authors found a correlation between hypercortisolism and CS complications. A significant association of 17 hydroxysteroid progesterone with severe infections in a cohort of patients with ectopic production of ACTH was found [44]. Interestingly, the association of cardiovascular complications with hypercortisolism in CS was documented in a series of clinical investigations [45–

47], showing a strong relationship between these factors. In addition, an association of hypercortisolism with diabetes has been suggested in a recent study evaluating the prevalence of CS in outpatients with poor controlled type 2 diabetes [48].

A further confirmation of the complex and heterogeneous variables implicated in CS aggressiveness comes from the evidence that patients cured from CS maintain increased cardiovascular risk factors of the active disease, even in the long term. In a study performed in 25 patients with CD before and one year after successful treatment, the persistence of visceral obesity, hypertension, glucose intolerance, and dyslipidemia was observed, despite clinical and biochemical remission [9]. Similarly, in another study performed in 15 patients in remission for at least 5 years, the persistence of visceral obesity, hypertension, impairment of glucose tolerance or diabetes mellitus, and dyslipidemia were found. Thus, patients with CS in remission might have a higher incidence of the metabolic syndrome and vascular disease, due to the persistence of an insulin resistance state and cardiac damage [49, 50], but also of psychiatric disorders, compared to the general population [51–53].

From the data obtained in the current study, it might be suggested that the degree of hypercortisolism is not the only parameter that has a role in influencing the severity of CS, but also the duration of hypercortisolism and the sensitivity to glucocorticoid excess.

Cortisol secretion can be variable, cyclical, and dependent on many factors, making it difficult to assess the "amount" of cortisol production. In this connection, it is known that there are many factors that influence glucocorticoid action such as glucocorticoid receptor (GR) polymorphisms [18] and the 11 beta-hydroxysteroid-dehydrogenase type 1 (11b-HSD1) enzyme [54]. GR polymorphisms have been demonstrated to impair glucocorticoid sensitivity, determining metabolic complications and bone alterations even in patients with subclinical CS [55]. Some GR polymorphisms such as N363S, BCL1, and ER22/EK are associated with diabetes, hypertension, and dyslipidemia, while others, such as A3669G, seem to be protective in CS [18]. Even the 11b-HSD1 enzyme has a prominent role in the regulation of glucocorticoid activity, catalyzing the in vivo conversion of inactive to active glucocorticoids. Transgenic mice overexpressing 11b-HSD1 in their white adipose tissue are obese, hypertensive, dyslipidemic, and insulin resistant [56]. Further, 11b-HSD1 knockout mice are protected from these metabolic abnormalities [57, 58].

The duration to cortisol exposure might be the prevalent factor influencing the severity of CS, even though it is a not well-defined parameter due to a subtle onset of the disease. In the current study a rough estimate of the duration of disease was performed. However, no association with UFC levels was found, even though it might be expected that the mild hypercortisolism would be associated with a long duration of disease, while the severe with a short duration of disease.

In conclusion, the results of the current study show how difficult is defining CS severity based on UFC. Serum, salivary, and urinary cortisol values are the current parameters used to define hypercortisolism "amount", despite their assays limits. The degree of hypercortisolism by itself seems not to be a sufficiently exhaustive parameter to assess the severity of active CS. Predicting the aggressiveness of active CS at the diagnosis remains even now a challenge, due to the difficulty in estimating the duration of the cortisol exposure and in finding any prognostic hormonal markers.

Therefore, currently, the presence of comorbidities seem to be the most predictive factors for assessing the aggressiveness of the active CS.

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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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