Cyclic Subclinical Hypercortisolism: A Previously Unidentified Hypersecretory Form of Adrenal Incidentalomas

Rafael B. Giorgi,[1] Marcelo V. Correa,[1] Flávia A. Costa-Barbosa,[1] and Claudio E. Kater[1]

[1]*Division of Endocrinology, Department of Medicine, Universidade Federal de São Paulo (UNIFESP) Medical School, 04039-032 São Paulo, SP, Brazil*

ORCiD numbers: 0000-0003-4581-3403 (R. B. Giorgi).

Purpose: Most adrenal incidentalomas (AIs) are nonfunctioning adenomas (NFAs), but up to 30% may secrete cortisol autonomously without clinical evidence of Cushing syndrome (CS), which nevertheless may increase cardiovascular mortality. This subclinical hypercortisolism (SCH) is confirmed by cortisol resistance to a dexamethasone suppression test (DST). Cyclic cortisol secretion occurs in classic CS but was not reported in SCH.

Objective: Investigate cyclic cortisol production/autonomy in AIs using sequential DSTs.

Methods: A total of 251 patients with AI underwent 487 DSTs over 12 years; patients with at least three DSTs were selected. DSTs were validated by measuring serum dexamethasone. Cyclic SCH was defined when at least two abnormal and two normal DSTs were documented.

Results: A total of 44 patients had three or more DSTs during follow-up: 9 of 44 patients (20.4%) had all negative test results (post-DST cortisol ≤1.8 µg/dL) and were classified as NFA; another nine patients had all positive results (cortisol >1.8 µg/dL) and were classified as sustained SCH. The remaining 26 (59.2%) had discordant responses: 8 of 44 (18.3%) had at least two positive and two negative tests, matching the criterion for cyclic SCH, whereas 18 of 44 (40.9%) had only one discordant test and were classified as possibly cyclic SCH. Eleven of 20 (55%) patients initially classified as NFA did not maintain their cortisol pattern.

Conclusions: Extended follow-up with repeated DSTs uncovered an unusual subset of AIs with cyclic SCH. Recurring production of cortisol may affect determination of AI subtypes if based on just one DST. Lack of recognition of this phenomenon makes follow-up of patients with AI misleading because even cyclic SCH may result in potential cardiovascular risk.

Adrenal incidentalomas (AIs) are recognized as a public health problem, reaching a prevalence of 5% in the general population and 10% in the elderly population. These figures are related to both the improvement in imaging techniques and the widespread access and use of these procedures [1–3]. Although most AIs are nonfunctioning adenomas (NFAs), some may

Abbreviations: AI, adrenal incidentaloma; CS, Cushing syndrome; CV, cardiovascular; DHEAS, dehydroepiandrosterone sulfate; DST, dexamethasone suppression test; LC-MS/MS, liquid chromatography–tandem mass spectrometry; NFA, nonfunctioning adenoma; SCH, subclinical hypercortisolism.

have minor autonomous steroid secretion [4, 5]. Subclinical hypercortisolism (SCH) (or autonomic cortisol secretion, as preferred by others [4]), is defined as an autonomous hypersecretion of cortisol with no clear phenotypic manifestations of Cushing syndrome (CS) [4, 6]. SCH (the term used herein) has emerged in recent years as the most common cause of functioning AI, reaching a prevalence of up to 30% depending on how it is defined [7]. Due to the long-term cortisol overexposure, SCH is associated with metabolic derangements and unfavorable cardiovascular outcomes [8–11]. Although NFA has also been associated with a high prevalence of metabolic abnormalities [11], a current guideline suggests that no follow-up is necessary when hypercortisolism is ruled out after the initial evaluation [4]. This recommendation has recently been contested [12].

All forms of clinically overt CS (*e.g.*, Cushing disease, ectopic ACTH syndrome, and adrenal neoplasia) can present with cyclic cortisol secretion [13]. This intriguing wax and wane pattern can mislead the investigation, increasing false-negative scores and the possibility of misdiagnosis [5, 14]. There is no consensus to define cortisol cyclicity in CS [15]. Several researchers have identified anomalous cortisol secretion in SCH and a substantial conversion rate to hypercortisolism in patients initially classified as NFA [5, 11, 15]. However, truly cyclic cortisol secretion has not been documented in AI.

In the current study, we examined whether a cyclic pattern of cortisol secretion may be present in AI, analogous to what is observed in CS. In addition, we compared the prevalence of metabolic abnormalities in the subsets of AI to justify why patients initially classified as NFA may also have a high prevalence of cardiovascular (CV) manifestations.

1. Materials and Methods

We reviewed the medical records of 251 consecutive patients with AI followed from 2006 to 2017 in a single reference center (Adrenal and Hypertension Unit, Division of Endocrinology, Federal University of Sao Paulo, Brazil). The study was approved by the ethical committee of the Federal University of Sao Paulo. Consent was obtained from each patient after a full explanation of the purpose and nature of all procedures used. The study population was 74% female (n = 186) and 26% male (n = 65); patients ranged in age from 19 to 87 years (median, 60 years). On the first visit, every patient underwent an overnight 1-mg dexamethasone suppression test (DST) as part of the investigation that also included plasma renin activity, serum aldosterone, and 24-hour urinary metanephrines (when appropriate) to exclude overt endocrine hyperfunction. Dedicated adrenal CT or MRI was performed to confirm the presence of an adrenal mass and to rule out malignancy.

For the DST, patients were provided with an instruction sheet and appropriate material for outpatient testing. Patients were instructed to swallow two 0.5-mg tablets of Decadron® (Ache Laboratories, Guarulhos, São Paulo, Brazil) at 11:00 PM with a glass of water and to be at the laboratory the next morning no later than 9:30 AM for a fasting blood sample collection. All participants underwent a careful interview regarding the use of medications that could potentially interfere with cortisol and dexamethasone measurement and/or metabolism, such as estrogen, anticonvulsants, and synthetic glucocorticoids; patients who had used these medications in the past 6 months were excluded.

Serum cortisol and dexamethasone were determined in all samples by in-house RIAs [16, 17] as well as baseline ACTH and dehydroepiandrosterone sulfate (DHEAS) (in most patients) by commercially available kits. Our in-house cortisol RIA has been used routinely for more than 40 years and was recently validated against liquid chromatography–tandem mass spectrometry (LC-MS/MS) [18].

We considered a valid DST whenever post-DST serum dexamethasone levels were >140 ng/dL [17]. We defined an abnormal cortisol suppression to a valid DST whenever post-DST cortisol levels were >1.8 μg/dL [19]. SCH was characterized by an abnormal cortisol response to DST (in the absence of clear manifestations of hypercortisolism) plus low or suppressed levels of ACTH and/or DHEAS.

Patients were followed with at least one DST yearly; in patients with abnormal results, tests were repeated every 6 months. Only patients with at least three DST assessments were considered for the study.

Based on these criteria, four distinct cortisol secretory profiles emerged: (i) NFA (when all three or more DST results were normal), (ii) sustained SCH (when all three or more DST results were abnormal), (iii) cyclic SCH (when at least two abnormal and two normal DST were observed randomly and not progressively, which characterizes conversion to SCH), and (iv) possibly cyclic SCH (if at least one test was discordant among three or more tests performed).

To ensure the variability of the cortisol response to DST in the cyclic and possibly cyclic SCH subgroups, we calculated the amplitude of cortisol responses (*i.e.*, the difference between the highest and the lowest post-DST serum cortisol value in the same patient) [20]. We used two additional post-DST cortisol cutoffs to compare the prevalence of SCH: the more specific 5 µg/dL and our own receiver operating characteristic curve–defined intermediate cutoff level of 2.5 µg/dL [21, 22].

Hypertension, type 2 diabetes, and dyslipidemia were established whenever patients were already on specific treatments or as defined (on two distinct occasions along follow-up) by systolic blood pressure ≥130 and/or diastolic blood pressure ≥80 mm Hg, hemoglobin A1c values ≥6.5%, and cholesterol or triglyceride levels beyond target according to individual cardiovascular risks, respectively [23–25].

A. Statistical Analysis

We used the MedCalc software package (version 18.2.1; MedCalc (MedCalc Software, Ostend, Belgium)) to perform statistical analysis. Continuous variables are presented as mean ± SD; categorical variables are presented as median and range and as absolute frequency and percentage. Comparisons among two or more groups were assessed by the Kruskal-Wallis nonparametric ANOVA complemented by Dunn test for pairwise comparisons. A *P* value <0.05 was considered statistically significant.

2. Results

Of the initial 251 patients with AI, 44 (17.5%) met the study criterion. The remaining 207 (82.5%) were excluded for one or more of the following reasons: (i) 187 patients had fewer than three DST assessments during follow-up, (ii) 14 patients had invalid DSTs (post-DST serum dexamethasone levels <140 ng/dL), and (iii) six patients lacked the complete data set.

Table 1 shows clinical and hormonal data of the selected patients separated by subgroups. Age and sex at diagnosis were similar among the four subgroups. Based on the 1.8 µg/dL post-DST cutoff, NFA was observed in 9 of 44 patients (20.5%); sustained SCH was present in another nine patients (20.5%); and the remaining 26 patients (59%) were classified as cyclic SCH (n = 8, 18%) and possibly cyclic SCH (n = 18, 41%). The median number of DSTs performed during follow-up was four (range, 3 to 8), with a median time interval between two tests of 9 months (range, 3 to 17 months).

The cyclic and possibly cyclic SCH subgroups had significantly longer follow-up periods than the sustained SCH subgroup (6.0 ± 2.3 and 6.5 ± 2.3 years *vs* 3.0 ± 1.3 years, *P* < 0.05), but the follow-up periods were not different from the NFA subgroup (4.1 ± 1.5 years) (Table 1).

Cortisol response to DST was significantly higher in the sustained SCH subgroup than in the NFA subgroup (3.3 *vs* 1.1 µg/dL; *P* < 0.05). During the latency periods (no secretory activity), the cyclic and possibly cyclic SCH subgroups had post-DST cortisol responses that were not different from the NFA subgroup (1.2 and 1.3 µg/dL, respectively). Moreover, during the active cortisol secretory periods, the cyclic and possibly cyclic SCH subgroups had post-DST cortisol responses similar than the sustained SCH subgroup (2.8 and 2.6 µg/dL,

Table 1. Clinical and Hormonal Data of Four Subgroups of Patients With Adrenal Incidentalomas

Variable	Patient Subgroups				
	NFA (n = 9)	Sustained SCH (n = 9)	Cyclic SCH (n = 8)	Possibly cyclic (n = 18)	P
Sex (M/F)	4/5	0/9	2/6	4/14	NS
Age at diagnosis, y	55 (47–82)	60 (52–79)	54 (43–64)	61 (37–71)	NS
Follow-up, y	4.1 ± 1.5	3.0 ± 1.3[a]	6.5 ± 2.3	6.0 ± 2.3	<0.05
Hypertension, %	44.4	66.7	62.5	72.2	NS
DM, %	44.4	55.5	50.0	72.2	NS
Dyslipidemia, %	33.3	33.3	75.0	61.1	NS
Normal F response on first DST, %	100	0	50	39	
Post-DST F, µg/dL	1.1 (1.0–1.5)		↓:[b] 1.2 (1.0–1.6)	1.3 (1.0–1.7)	NS
		3.3 (2.6–8.7)	↑:[c] 2.8 (2.1–16.0)	2.6 (2.0–27.9)	NS
F amplitude, µg/dL	0.3 (0.1–0.8)[d]	3.9 (2.4–17.2)	1.9 0.8–15.1)	1.7 (0.4–26.9)	<0.05
Serum dex, ng/dL	405 (210–586)		↓: 347 (278–1889)	381 (226–982)	NS
		323 (152–587)	↑: 753 (191–1268)	413 (176–1281)	NS

Age, serum Cortisol (F) and serum Dexamethasone (Dex) values and amplitude in median and range. Follow up in mean ±SD.

Abbreviations: dex, dexamethasone; DM, diabetes mellitus; NS, not significant.

[a]$P < 0.05$, SCH vs cyclic and possible cyclic.

[b]↓: Latent phase (post-DST F ≤ 1.8).

[c]↑: Active phase (post-DST F > 1.8).

[d]$P < 0.05$ NFA vs sustained, cyclic, possible cyclic, and SCH.

respectively). Post-DST serum dexamethasone levels were not significantly different among all subgroups (Table 1).

The sustained SCH subgroup had a significantly higher amplitude of post-DST cortisol response than the NFA subgroup (3.9 *vs* 0.3 µg/dL, $P < 0.05$), whereas the cyclic and possibly cyclic SCH subgroups had similar amplitudes (1.9 and 1.7 µg/dL).

At initial evaluation, a normal cortisol response was observed in 20 of the 44 patients. Of these, nine patients maintained the NFA classification, but 11 were reclassified as cyclic SCH (n = 4) and possibly cyclic SCH (n = 7). Thus, only 45% of patients primarily classified as NFA maintained their normal cortisol profile and functional status after successive evaluations.

Most of the patients (59%) in the SCH and NFA groups in the first evaluation were reclassified to the cyclic or possible cyclic groups after three evaluations (Fig. 1).

We observed a lower but not significant prevalence of hypertension, type 2 diabetes, and dyslipidemia in the NFA subgroup as compared with the other subgroups (Table 1). In addition, the prevalence of a cyclic secretory pattern was clearly unveiled when the more sensitive DST cutoffs of 1.8 and 2.5 µg/dL were applied, whereas the more specific cutoff of 5 µg/dL conceals the phenomenon (Fig. 2)

3. Discussion

Results from this preliminary study systematically reveal the presence of cyclic cortisol secretion in adrenal incidentalomas during prolonged follow-up. This phenomenon has been repeatedly demonstrated in various forms of classic CS [13, 15, 26, 27]. Although possibly underestimated due to the lack of a clear definition, the prevalence of cyclicity has been observed in 20% to 40% of endogenous CS [15]. Most cases of CS are ACTH-secreting pituitary adenomas occurring predominantly in adults [15, 26–29]. However, cyclic cortisol production has also been reported in well-differentiated neuroendocrine tumors, adrenal adenoma, and rare forms of the pigmented variant of micronodular adrenocortical hyperplasia [30–35].
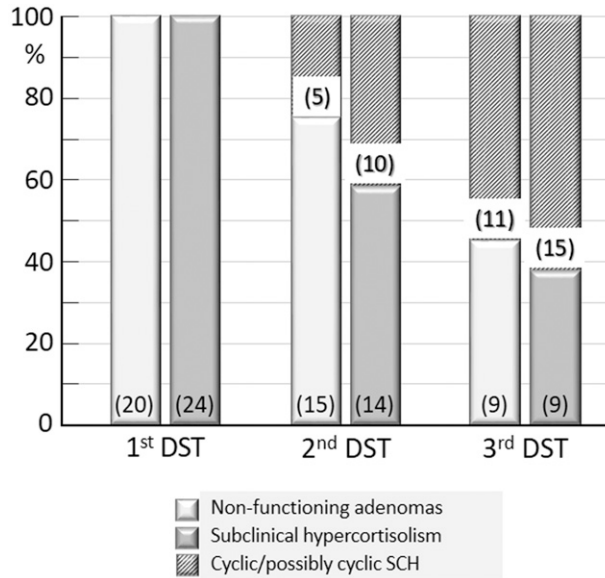
Figure 1. Changes in AI subgrouping across consecutive DST evaluations.

Based on only one altered DST during follow-up, Vassilatou *et al*. [5] have identified intermittent cortisol secretion in 15% of patients with AI, but the possibility of a cyclic pattern was not specifically evaluated.

Moreover, cycles of hypercortisolism occur irregularly, with phases of interlude ranging from days to years [27]. Several studies have demonstrated that cycle lengths of cortisol hypersecretion vary from 12 hours to 85 days, which renders the investigation complex, requiring successive evaluations of the cortisol profile to ascertain this fluctuation [14, 15, 28].

Our data show that only 9 out of 20 patients (45%) initially diagnosed as NFA maintained the same cortisol secretory profile during follow-up; higher figures (69%) have been shown by others [11, 36]. This difference can be partially explained by the larger number of tests performed in our study. Also, Papanastasiou *et al*. [11] did not establish additional criteria for divergent test responses during follow-up, restraining classification exclusively to NFA or SCH.
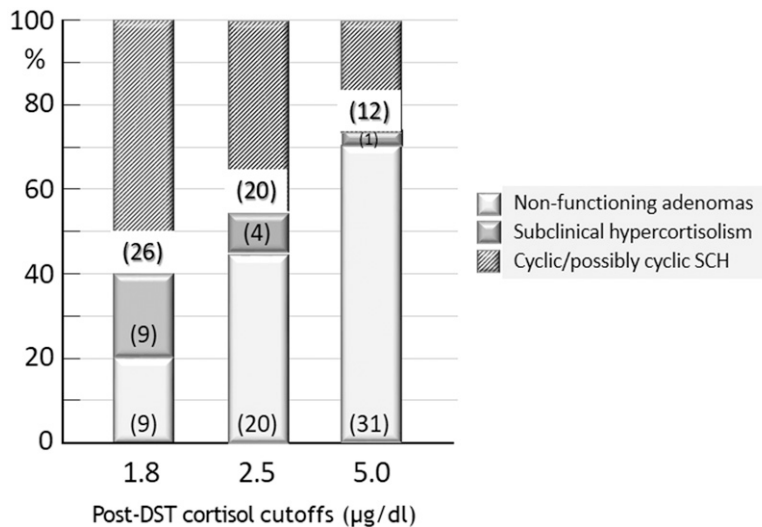

Figure 2. Prevalence of AI subgroups according to different post-DST cortisol cutoffs.

Because there is no literature consensus regarding hormonal cyclicity, we adopted an arbitrary criterion in which a minimum of four DSTs was required, with two normal and two abnormal random, nonprogressive results. However, we also included patients with three DSTs; we argued that this would be the minimum number of assessments needed to confirm sustained SCH or NFA. If a discordant measure was observed, an extended follow-up would be necessary.

Several studies agree that even "benign" NFA may be associated with increased CV risk [11, 37–39]. Three possibilities may support this observation: (i) excessive secretion of unusual adrenocortical steroids (other than cortisol) by an "apparent" NFA [40], (ii) the post-DST cortisol cutoff of 1.8 µg/dL still being too high to allow inclusion of patients with minor and yet autonomous cortisol production [38], and (iii) a cyclic cortisol pattern that explains intermittent hypercortisolism among a number of patients primarily classified as NFA, as suggested by the present results. All three possibilities encompass a subset of patients primarily classified as NFA that may behave as SCH or produce an anomalous steroid mixture that poses major CV risks. Regarding the generally accepted post-DST cortisol cutoff of 1.8 µg/dL, Androulakis *et al.* [38] suggested even lower cutoffs (*e.g.*, 1.39 or 1.11 µg/dL) that would improve discrimination between patients with or without increased CV risk.

Although a typical evaluation for endogenous hypercortisolism includes 24-hour urinary free cortisol, overnight DSTs, and nighttime plasma and salivary cortisol measurements, the 1-mg oral DST is the most widely used single test to scrutinize SCH [4, 41]. At initial investigation, basal ACTH and DHEAS measurements added strength to discriminate our patients with SCH from those with NFA [4, 42, 43]. All tests proved effective in diagnosing CS when hypercortisolemia is pronounced and sustained. However, their utility in patients with mild and cyclic or episodic hypercortisolemia has not been evaluated [14].

A few caveats must be considered regarding interpretation of DST data: CBG variations, poor sleep quality and excess activity after dexamethasone administration, reduced dexamethasone absorption (malabsorptive conditions), and the concomitant use of drugs that may interfere with the CYP3A4 enzyme, altering dexamethasone metabolism [36]. To avoid these setbacks, every DST performed in our patients was preceded by a systematic drug questionnaire and the determination of serum dexamethasone to validate the test.

Even in validated tests, we could not rule out the possibility that there might have been some false-positive or false-negative DST responses among the cyclic and possibly cyclic SCH subgroups due to minor hypersecretion and the inability of the predefined test cutoff to discriminate borderline values [14]. A possible limitation of our study was the use of RIA instead of LC-MS/MS to determine post-DST cortisol levels. However, our in-house RIA has recently been validated against LC-MS/MS by comparing 318 pairs of serum cortisol values obtained from patients with adrenal incidentalomas and normal subjects, disclosing a significant ($P < 0.001$) positive correlation of 0.9345 [18].

Due to this study's retrospective design, we were not able to program the timing of the tests to characterize the cycle lengths of cortisol production. Therefore, repeated tests must be used during a prolonged follow-up period of months to years to confirm such a pattern, even though test reproducibility is uncertain [15, 44, 45]. Also, despite the large population of patients with an adrenal incidentaloma seen in our service, most patients did not observe the proper follow-up for diverse reasons. This fact negatively affected the size of our sample as per the study inclusion criterion.

The present paper is a proof-of-concept study in which we identified a cyclic pattern of cortisol hypersecretion (or autonomy) of adrenal incidentalomas, comparable to that reported in CS. It reinforces the observation that even NFA may be associated with increased cardiovascular risk and other complications. As new therapies arise (*e.g.*, safe medications or radiofrequency nodule ablation arise), we can probably offer better outcomes to these patients. Further studies are needed to validate this cyclic cortisol profile of AI as an actual entity, like the examination of possible atrophy of the adjacent nontumoral cortical tissue and suppression of immunohistochemical expression of CYP17 and Dehydroepiandrosterone sulfotransferase (DHEA-ST) secretion. Moreover, genetic studies directed toward clock genes may help to elucidate the nature of this cyclicity.

In summary, an extended follow-up with repeated overnight DST allowed the recognition of a subset of patients with AI with a cyclic cortisol secretory pattern. These data raise the possibility (supported by recent reports that patients with NFA may behave clinically as SCH, with increased CV morbidity and mortality) of misclassification of AI as NFA or SCH based on only one DST or other related tests, prompting treatment and care recommendations that may not be appropriate. More well-controlled, prospective, and long-term studies are needed to confirm our hypotheses and to ascertain the clinical implications of this newly uncovered secretory profile as well as which are the best diagnostic criteria and which is the most appropriate protocol to follow with these patients.

Acknowledgments

References and Notes

1. Terzolo M, Osella G, Alì A, Borretta G, Cesario F, Paccotti P, Angeli A. Subclinical Cushing's syndrome in adrenal incidentaloma. *Clin Endocrinol (Oxf).* 1998;**48**(1):89–97.

2. Libè R, Dall'Asta C, Barbetta L, Baccarelli A, Beck-Peccoz P, Ambrosi B. Long-term follow-up study of patients with adrenal incidentalomas. *Eur J Endocrinol.* 2002;**147**(4):489–494.

3. Bovio S, Cataldi A, Reimondo G, Sperone P, Novello S, Berruti A, Borasio P, Fava C, Dogliotti L, Scagliotti GV, Angeli A, Terzolo M. Prevalence of adrenal incidentaloma in a contemporary computerized tomography series. *J Endocrinol Invest.* 2006;**29**(4):298–302.

4. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, Tabarin A, Terzolo M, Tsagarakis S, Dekkers OM. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. *Eur J Endocrinol.* 2016;**175**(2):G1–G34.

5. Vassilatou E, Vryonidou A, Michalopoulou S, Manolis J, Caratzas J, Phenekos C, Tzavara I. Hormonal activity of adrenal incidentalomas: results from a long-term follow-up study. *Clin Endocrinol (Oxf).* 2009;**70**(5):674–679.

6. Terzolo M, Stigliano A, Chiodini I, Loli P, Furlani L, Arnaldi G, Reimondo G, Pia A, Toscano V, Zini M, Borretta G, Papini E, Garofalo P, Allolio B, Dupas B, Mantero F, Tabarin A; Italian Association of Clinical Endocrinologists. AME position statement on adrenal incidentaloma. *Eur J Endocrinol.* 2011; **164**(6):851–870.

7. Terzolo M, Bovio S, Reimondo G, Pia A, Osella G, Borretta G, Angeli A. Subclinical Cushing's syndrome in adrenal incidentalomas. *Endocrinol Metab Clin North Am*. 2005;**34**(2):423–439, x.

8. Reincke M. Subclinical Cushing's syndrome. *Endocrinol Metab Clin North Am*. 2000;**29**(1):43–56.

9. Terzolo M, Pia A, Reimondo G. Subclinical Cushing's syndrome: definition and management. *Clin Endocrinol (Oxf).* 2012;**76**(1):12–18.

10. Barzon L, Fallo F, Sonino N, Boscaro M. Development of overt Cushing's syndrome in patients with adrenal incidentaloma. *Eur J Endocrinol.* 2002;**146**(1):61–66.

11. Papanastasiou L, Alexandraki KI, Androulakis II, Fountoulakis S, Kounadi T, Markou A, Tsiavos V, Samara C, Papaioannou TG, Piaditis G, Kaltsas G. Concomitant alterations of metabolic parameters, cardiovascular risk factors and altered cortisol secretion in patients with adrenal incidentalomas during prolonged follow-up. *Clin Endocrinol (Oxf).* 2017;**86**(4):488–498.

12. Morelli V, Scillitani A, Arosio M, Chiodini I. Follow-up of patients with adrenal incidentaloma, in accordance with the European society of endocrinology guidelines: could we be safe? *J Endocrinol Invest.* 2017;**40**(3):331–333.

13. Alexandraki KI, Kaltsas GA, Isidori AM, Akker SA, Drake WM, Chew SL, Monson JP, Besser GM, Grossman AB. The prevalence and characteristic features of cyclicity and variability in Cushing's disease. *Eur J Endocrinol.* 2009;**160**(6):1011–1018.

14. Friedman TC, Ghods DE, Shahinian HK, Zachery L, Shayesteh N, Seasholtz S, Zuckerbraun E, Lee ML, McCutcheon IE. High prevalence of normal tests assessing hypercortisolism in subjects with mild and episodic Cushing's syndrome suggests that the paradigm for diagnosis and exclusion of Cushing's syndrome requires multiple testing. *Horm Metab Res*. 2010;**42**(12):874–881.

15. Meinardi JR, Wolffenbuttel BH, Dullaart RP. Cyclic Cushing's syndrome: a clinical challenge. *Eur J Endocrinol*. 2007;**157**(3):245–254.

16. Vieira JGH, Russo EMK, Germek OA, Antunes SL. A radioimmunoassay method for measurement of serum cortisol. *Rev Bras Patol Clin*. 1979;**15**(3):125–130.

17. Hayashi LF, Noguti KO, Nakamura OH, Kater CE, Vieira JGH. Development of a RIA for serum dexamethasone as an aid to diagnose Cushing's syndrome. *J Bras Patol Med Lab*. 2011;**47**(5):505–510.

18. Huayllas MKP, Netzel BC, Singh RJ, Kater CE. Serum cortisol levels via radioimmunoassay vs liquid chromatography mass spectrophotometry in healthy control subjects and patients with adrenal incidentalomas. *Lab Med*. 2018;**49**(3):259–267.

19. Wood PJ, Barth JH, Freedman DB, Perry L, Sheridan B. Evidence for the low dose dexamethasone suppression test to screen for Cushing's syndrome: recommendations for a protocol for biochemistry laboratories. *Ann Clin Biochem*. 1997;**34**(Pt 3):222–229.

20. Debono M, Harrison RF, Chadarevian R, Gueroult C, Abitbol JL, Newell-Price J. Resetting the abnormal circadian cortisol rhythm in adrenal incidentaloma patients with mild autonomous cortisol secretion. *J Clin Endocrinol Metab*. 2017;**102**(9):3461–3469.

21. Nieman LK, Biller BMK, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2008;**93**(5):1526–1540.

22. Costa-Barbosa F, Hayashi L, Oliveira K, Kater CE. Performance of the overnight 1mg dexamethasone suppression test coupled with serum dexamethasone measurement in identifying subclinical hypercortisolism among patients with an adrenal incidentaloma. *Endocr Rev*. 2016;**37**(2, Suppl).

23. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, MacLaughlin EJ, Muntner P, Ovbiagele B, Smith SC Jr, Spencer CC, Stafford RS, Taler SJ, Thomas RJ, Williams KA Sr, Williamson JD, Wright JT Jr. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines [published correction appears in Hypertension. 2018;71:e136–e139]. *Hypertension*. 2018;**71**(6):1269–1324.

24. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. *Diabetes Care*. 2018;**41**(Suppl 1):S13–S27.

25. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, Grunberger G, Guerin CK, Bell DSH, Mechanick JI, Pessah-Pollack R, Wyne K, Smith D, Brinton EA, Fazio S, Davidson M. American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. *Endocr Pract*. 2017;**23**(Suppl 2):1–87.

26. Albiger NM, Scaroni CM, Mantero F. Cyclic Cushing's syndrome: an overview. *Arq Bras Endocrinol Metabol*. 2007;**51**(8):1253–1260.

27. Jahan S, Hasanat MA, Hussain E, Danesh R, Sultana N, Hasan M, Fariduddin M. Cyclic Cushing syndrome, an enigma in diagnosis: a case report. *Am J Med Case Rep*. 2016;**4**(2):65–70.

28. Vasiliu I, Scripcariu V, Poeata I, Bodescu I, Idriceanu J, Manolachie A, Fadur A, Cristea C, Mogos V, Vulpoi C. Periodic Cushing's disease: difficult patient, difficult management. *Endocr Abstracts* 2014;**35**:41.

29. Shapiro MS, Shenkman L. Variable hormonogenesis in Cushing's syndrome. *Q J Med*. 1991;**79**(288):351–363.

30. Arnaldi G, Mancini T, Kola B, Appolloni G, Freddi S, Concettoni C, Bearzi I, Masini A, Boscaro M, Mantero F. Cyclical Cushing's syndrome in a patient with a bronchial neuroendocrine tumor (typical carcinoid) expressing ghrelin and growth hormone secretagogue receptors. *J Clin Endocrinol Metab*. 2003;**88**(12):5834–5840.

31. Chajek T, Romanoff H. Cushing syndrome with cyclical edema and periodic secretion of corticosteroids. *Arch Intern Med*. 1976;**136**(4):441–443.

32. Hannah J, Lippe B, Lai-Goldman M, Bhuta S. Oncocytic carcinoid of the kidney associated with periodic Cushing's syndrome. *Cancer*. 1988;**61**(10):2136–2140.

33. Shapiro MS, Gutman A, Bruderman I, Myers B, Griffel WB. Cushing's syndrome associated with a bronchial adenoma: possible periodic hormonogenesis. *Isr J Med Sci*. 1975;**11**(9):919–924.

34. Terzolo M, Alì A, Pia A, Bollito E, Reimondo G, Paccotti P, Scardapane R, Angeli A. Cyclic Cushing's syndrome due to ectopic ACTH secretion by an adrenal pheochromocytoma. *J Endocrinol Invest*. 1994; **17**(11):869–874.

35. Blau N, Miller WE, Miller ER Jr, Cervi-Skinner SJ. Spontaneous remission of Cushing's syndrome in a patient with an adrenal adenoma. *J Clin Endocrinol Metab*. 1975;**40**(4):659–663.

36. Bansal V, El Asmar N, Selman WR, Arafah BM. Pitfalls in the diagnosis and management of Cushing's syndrome. *Neurosurg Focus*. 2015;**38**(2):E4.

37. Lopez D, Luque-Fernandez MA, Steele A, Adler GK, Turchin A, Vaidya A. "Nonfunctional" adrenal tumors and the risk for incident diabetes and cardiovascular outcomes: a cohort study. *Ann Intern Med*. 2016;**165**(8):533–542.

38. Androulakis II, Kaltsas GA, Kollias GE, Markou AC, Gouli AK, Thomas DA, Alexandraki KI, Papamichael CM, Hadjidakis DJ, Piaditis GP. Patients with apparently nonfunctioning adrenal incidentalomas may be at increased cardiovascular risk due to excessive cortisol secretion. *J Clin Endocrinol Metab*. 2014;**99**(8):2754–2762.

39. Sereg M, Szappanos A, Toke J, Karlinger K, Feldman K, Kaszper E, Varga I, Gláz E, Rácz K, Tóth M. Atherosclerotic risk factors and complications in patients with non-functioning adrenal adenomas treated with or without adrenalectomy: a long-term follow-up study. *Eur J Endocrinol*. 2009;**160**(4): 647–655.

40. Huayllas MK, Singh RJ. Netzel B, Kater CE. Are "non-functioning" adrenal incidentalomas actually quiescent masses? *Endocr Rev*. 2017;**38**(3, Suppl).

41. Newell-Price J, Trainer P, Besser M, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev*. 1998;**19**(5):647–672.

42. Dennedy MC, Annamalai AK, Prankerd-Smith O, Freeman N, Vengopal K, Graggaber J, Koulouri O, Powlson AS, Shaw A, Halsall DJ, Gurnell M. Low DHEAS: a sensitive and specific test for the detection of subclinical hypercortisolism in adrenal incidentalomas. *J Clin Endocrinol Metab*. 2017;**102**(3): 786–792.

43. Chiodini I. Clinical review: diagnosis and treatment of subclinical hypercortisolism. *J Clin Endocrinol Metab*. 2011;**96**(5):1223–1236.

44. Mullan KR, Atkinson AB, Sheridan B. Cyclical Cushing's syndrome: an update. *Curr Opin Endocrinol Diabetes Obes*. 2007;**14**(4):317–322.

45. Jang YS, Lee IS, Lee JM, Choi SA, Kim GJ, Kim HS. Diagnosis of cyclic Cushing syndrome using the morning urine free cortisol to creatinine ratio. *Korean J Intern Med (Korean Assoc Intern Med)*. 2016; **31**(1):184–187.