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Coagulation Profile in Patients with Different Etiologies for Cushing Syndrome: A Prospective Observational Study

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Abstract

Previous studies reported a higher prevalence of venous-thromboembolic events among patients with Cushing disease (CD) compared to those with ACTH-independent Cushing syndrome (CS) from adrenal sources. The objective of the current study was to evaluate the coagulation profile of patients with CS from different etiologies. A prospective observational study was conducted at a clinical research center. The study included adult patients admitted for evaluation of suspected CS (n = 85), that were divided into 3 groups: CD (n = 22), ACTH-independent CS from an adrenal tumor/hyperplasia (adrenal CS, n = 21), and a control group consisting of subjects with negative screening for CS (rule-out CS, n = 42). Coagulation profiles were drawn before and 8.5 ± 4.3 months after surgery (trans-sphenoidal or adrenalectomy, n = 18), and included fibrinogen, Factor VIII (FVIII), von Willebrand factor antigen (vWF:Ag), plasminogen activator inhibitor-1 (PAI-1), antithrombin III (ATIII), Protein C (PC), Protein S (PS), α 2-antiplasmin (α 2AP), and aPTT measurements. Patients with CD had higher baseline mean cortisol levels, ATIII activity and vWF:Ag levels compared with adrenal CS. Differences in ATIII activity and vWF:Ag levels remained even after controlling for BMI, and ATIII after also controlling for 24-h urinary free cortisol collections. Our study showed for the first time the differences in coagulation profiles between various etiologies of CS. We assume that the higher cortisol burden among CD patients may explain the differences found in the coagulation profile as well as the higher risk for VTE compared with primary adrenal CS patients.

Keywords

cushing syndrome; hypercoagulability; hypercortisolism; thrombus

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Conflicts of Interest

The authors declare no conflict of interest.

Introduction

Cushing syndrome (CS) is traditionally divided into adrenocorticotropin (ACTH)-dependent CS, caused by a pituitary adenoma (Cushing disease, CD) or more rarely ectopic ACTH production, and ACTH-independent CS, caused by adrenal tumors [1]. CS is a hypercoagulable state, associated with an increased incidence of both arterial [2] and venous thrombo-embolic events (VTEs) [3,4]. Systematic review of VTE occurrence in CS patients [4] revealed a pre-operative incidence of 2.5–3.1/1 000 patient years, high compared with the general population. Similarly, patients who are treated with systemic (high-dose) glucocorticoids have also an increased VTE risk [5, 6].

The hypercoagulable state in CS has been attributed to an increase in endogenous procoagulants [e. g., von Willebrand factor (vWF), Factor VIII (FVIII), and Fibrinogen], and antifibrinolytics [plasminogen activator inhibitor-1 (PAI-1) and α 2-antiplasmin (α 2AP)] [7, 8], despite a concomitant increase in endogenous anticoagulants [Proteins C and S, and antithrombin III (ATIII)] [9, 10]. Additional laboratory findings in patients with CS and VTE were shortened activated thromboplastin time (aPTT), indicating activated coagulation, and high D-dimer levels [7].

Although thromboprophylaxis decreased the VTE event rate from 20 to 6% [11], post-operative VTE events, despite treatment, were reported in 4.4% of CS patients [4, 11]. Interestingly, two studies showed differences in the VTE incidence between patients with CD and those with primary adrenal disease. Boscaro et al. [11] performed retrospective analysis of VTE rates among patients with CS. After excluding patients with adrenocortical carcinoma, 69% of the patients experiencing VTE were diagnosed with CD whereas only 31 % had other causes for CS. In another study, Stuijver et al. [3] described 374 patients with CS, 37 of them (10 %) had VTE events, most of them before surgery. However, among the 12 patients with post-operative VTE, all had CS from a pituitary source.

The reason for the difference in VTE rate between CD and adrenal CS is not clear, and none of the prior studies on hypercoagulability in CS had enough power to compare the coagulation profiles of patients with CD to those with adrenal sources for CS. Moreover, 2 recent reviews raised the need for further studies, in order to tailor possible thromboprophylactic treatment for patients with CS [12, 13].

In the current analysis, we aimed to investigate the coagulation disturbances in patients with CS of different etiologies. We have also followed a subset of the patients to assess coagulation profile dynamics post-operatively.

Patients and Methods

We conducted a prospective observational study, including consecutive patients, aged 18 years, that were admitted to the National Institutes of Health for evaluation of suspected CS between 2013 and 2015. All patients were recruited through clinical protocols 97-CH-0076 (pituitary tumors), 00-CH-0160 (ACTH independent CS) and 95-CH-0059 (primary pigmented nodular adrenocortical disease), conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and gave written

informed consent. For the current analysis we included 3 groups of patients: the CD group included patients with an histopathologically proven pituitary adenoma that positively stained for ACTH; the adrenal CS group included patients that underwent adrenalectomy due to confirmed autonomous cortisol secretion from the adrenal glands; finally, the control group included patients that were screened for CS, and were found to be negative for endogenous CS (rule-out CS).

All patients were hospitalized and underwent screening tests for CS, including serial 24-h urinary free cortisol (UFC) collections and diurnal plasma cortisol measurements. Patients diagnosed with CS had all clinically overt CS and positive screening tests for CS. They then underwent further investigation in order to locate the source of hypercortisolemia, including plasma ACTH measurement, and if necessary Liddle's, high dose (8 mg) dexamethasone suppression, and corticotropin releasing hormone (CRH) stimulation tests. Patients with suspected CD underwent a pituitary magnetic resonance imaging (MRI) scan, and those with suspected adrenal CS underwent adrenal CT scan. In case of uncertainty regarding CD diagnosis (according to the pituitary MRI), a CRH stimulation test, or an 8 mg dexamethasone suppression test, and/or an inferior petrosal sinus sampling (IPSS) were performed to exclude ectopic ACTH secretion. Patients then went on to surgical intervention, either adrenalectomy or trans-sphenoidal surgery (TSS), as appropriate. All pathological reports were reviewed. Patients were followed-up with repeated serial 24-h UFCs measurement and diurnal plasma cortisol testing. In order to compare coagulation factor dynamics after effective intervention, we included only patients cured of CS according to their 24 h UFC levels and midnight plasma cortisol levels (18/22 patients with full post-operative tests).

Since our research protocol was approved before the recent reports on VTE events in CS patients [3, 9], we did not prescribe thromboprophylactic treatment regularly during hospitalization, and did not routinely recommend on thromboprophylaxis after discharge. Hence, 30/42 patients operated (71.4 %) received thromboprophylaxis during the hospitalization (63.6 vs. 76.2 % in the CD and ACTH independent adrenal CS groups, respectively, $p = 0.2$). However, none of the patients were treated during any laboratory evaluation, either baseline or follow-up. In addition, none of the patients was treated with a vitamin K antagonist during evaluation.

Coagulation profile included fibrinogen (reference range, 177–466 mg/dl), FVIII (41–184 IU/dl), von Willebrand factor antigen (vWF:Ag, 50–197 IU/dl), and PAI-1 plasma levels (3–86 IU/ml), ATIII (57–134 %), Protein C (59–144 %), Protein S (55–134 %) and α_2AP (75–132 %) activities, and aPTT measurements (25.3–37.3 s). Blood samples for coagulation profile were drawn before intervention and 6–12 months following surgery (either trans-sphenoidal surgery or adrenalectomy). We have also compared rate of blood subtype O between groups due to its known effect on coagulation factors levels [12].

Laboratory methods

Hormones

Plasma cortisol levels (reference range for morning levels, 5–25 µg/dl) were measured using a fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL, USA) with an intra-assay coefficient of variation (CV) of 2.1 and an inter-assay CV of 4.1 %. Plasma ACTH levels (reference range for a random sample, 0–46 pg/ml) were measured using Nichols Advantage chemiluminescent kit (Nichols Institute, San Clemente, CA, USA) with an intra-assay and inter-assay CVs of 6.2 and 11 %, respectively.

Coagulation elements

Antigen levels of FVIII, PAI-1, vWF:Ag, and fibrinogen were measured, whereas activity was measured for proteins C and S, a2AP, and ATIII. Factor VIII antigen levels were measured assays were done by correction of the aPTT at various dilutions of patient sample mixed with factor VIII depleted plasma (George King Biomedical, Overland Park, KS, USA), vWF:Ag assays were done by immunoturbidometric measurements, fibrinogen measurements by the method of Clauss, and Protein C, Protein S, ATIII, a2AP, and PAI-1 were analyzed by a chromogenic assays. All coagulation factors analyses were done on a StaRevolution analyzer (Stago Diagnostica, Parsippany, NJ, USA).

Statistical analysis

Statistical calculations were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Normality of variables distribution was tested using the Shapiro-Wilk test. All coagulation elements were normally distributed except for AT-III and protein C. Results are expressed as mean $\pm$ standard deviation (SD), or median (inter quartile range, IQR) for non-normally distributed variables. The independent Student's t-test was used to analyze differences in numerical variables, and the chi-square test was employed to analyze differences in categorical variables, whereas nonparametric tests were used for the analyses of non-normally distributed variables, as appropriate. Comparisons were performed between patients with either CD or ACTH-independent adrenal CS and rule-out CS patients at baseline, and a paired analysis was performed for comparisons before and after surgery. The Pearson product was used for analysis of correlations between variables, and Spearman's rho– for the correlation analyses of AT-III and protein C. A linear regression analysis was performed in order to eliminate the effect of BMI and/or UFC levels on coagulation elements. The p-value for significance was set at less than 0.05.

Results

The current analysis included 85 patients, aged 41.1 ± 16.4 years, 64 females (75.3 %). Twenty-two patients (25.9 %) had CD, 21 (24.7 %) were operated for ACTH-independent adrenal adenoma or hyperplasia. The control group consisted of 42 (49.4 %) rule-out CS subjects. Demographic and biochemical characteristics according to the study groups are depicted in ►Table 1, and the pathological diagnoses for the adrenal CS group are detailed in ►Table 2. Prevalence of hypertension, smoking status, type II diabetes mellitus and with CD was diagnosed with sigmoid sinus thrombosis 6 months following TS cardiovascular

disease did not differ between the groups (►Table 1). All the patients that were re-evaluated following surgery received glucocorticoids as a replacement therapy. One patient S, whereas none of the patients with ACTH-independent adrenal CS were diagnosed with VTE.

Patients with CS of any etiology vs. controls – baseline

Patients diagnosed with either CD or CS due to adrenal adenoma or hyperplasia had higher 24-h UFC levels (330.3 ± 662.0 vs. 31.2 ± 15.7 $\mu\text{g}/24$ h, $p = 0.009$) and midnight plasma cortisol levels (11.9 ± 8.8 vs. 2.4 ± 1.3 $\mu\text{g}/\text{dl}$, $p < 0.001$) compared with the control group, respectively. Mean levels of each coagulation element according to study group at baseline are shown in ►Table 3. The elements are classified as procoagulants and antifibrinolytics, which act to form and maintain the clot, and anticoagulants – which oppose their action by inhibiting clot formation. Patients with CS of any etiology had higher baseline levels of the endogenous procoagulants FVIII (132.9 ± 68.9 vs. 106.7 ± 45.2 IU/dl, $p = 0.04$) and vWF:Ag (103.2 ± 47.2 vs. 83.5 ± 27.3 IU/dl, $p = 0.02$), and higher activity of the antifibrinolytic enzyme $\alpha 2\text{AP}$ ($86.2 \% \pm 17.5$ vs. $79.3 \% \pm 15.1$, $p = 0.047$), compared with the control group, respectively. Moreover, median protein C activity was higher among CS patients of any etiology compared with the control group [120.5% (47.6) vs. 105.0% (40.0), median (IQR), $p = 0.04$], and similar trend was found for ATIII [84.0% (29.8) vs. 78.0% (22.0), $p = 0.07$]. Also, patients with CS had shorter aPTT compared with the controls (30.2 ± 2.7 vs. 32.1 ± 3.3 s, $p = 0.008$).

CD vs. ACTH independent adrenal CS – pre-surgery

Patients with CD had higher baseline 24-h UFC levels (628.2 ± 865.8 vs. 76.0 ± 52.2 $\mu\text{g}/24$ h, $p = 0.01$), midnight plasma cortisol levels (16.5 ± 10.1 vs. 8.0 ± 4.7 $\mu\text{g}/\text{dl}$, $p = 0.003$) and morning cortisol levels (17.9 ± 8.9 vs. 11.4 ± 5.0 $\mu\text{g}/\text{dl}$, $p = 0.007$) compared with ACTH-independent adrenal CS patients, respectively. Subjects with CD had higher vWF:Ag levels (119.8 ± 55.8 vs. 87.5 ± 31.2 IU/dl, $p = 0.03$) and ATIII activity [93.3% (28.9) vs. 128.5% (52.8), median (IQR), $p = 0.01$] compared with those with primary adrenal disease, respectively.

Patients with CS of any etiology – post-surgery

Eighteen patients were evaluated 8.5 ± 4.3 months, following surgery (13 patients with CD and 5 – primary adrenal CS). Mean UFC decreased following surgical intervention to 28.6 ± 28.2 $\mu\text{g}/24\text{h}$ ($p = 0.03$), and midnight cortisol to 1.5 ± 0.9 $\mu\text{g}/\text{dl}$ ($p < 0.001$) compared with baseline levels. In terms of the coagulation elements, the activity of the endogenous anticoagulants ATIII ($p = 0.006$) and Protein C (0.02) decreased compared with pre-surgical measurements, mainly among patients with CD ($p = 0.02$ and $p = 0.03$, respectively), and similar trend was found for Protein S (0.07). Coagulation elements profiles for both groups before and after intervention are shown in ►Fig. 1.

Correlation analysis

Analysis of data both before and after surgery revealed positive correlations between 24-h UFC levels, and the endogenous procoagulants vWF:Ag ($r = 0.4$, $p = 0.01$) and FVIII ($r = 0.5$, $p = 0.001$, ►Fig. 2a); the endogenous anticoagulants ATIII ($r = 0.5$, $p < 0.001$,

Spearman's; ►Fig. 2b), Protein C ($r = 0.4$, $p = 0.008$, Spearman's), and Protein S ($r = 0.5$, $p < 0.001$); and negative correlation was found with aPTT ($r = -0.5$, $p = 0.001$, ►Fig. 2c). The change in 24-h UFC after surgery correlated positively with FVIII ($r = 0.8$, $p < 0.001$), and Protein C ($r = 0.6$, $p = 0.02$) levels. The decrease in ACTH levels positively correlated with the procoagulant vWF:Ag ($r = 0.5$, $p = 0.04$, Spearman's), and with the antifibrinolytic PAI-1 ($r = 0.6$, $p = 0.02$). Body mass index correlated positively with baseline ACTH ($r = 0.3$, $p = 0.02$), Protein S ($r = 0.3$, $p = 0.03$), fibrinogen ($r = 0.5$, $p < 0.001$), and PAI-1 levels ($r = 0.3$, $p = 0.005$).

After adjusting the comparisons for 24-h UFC levels, aPTT remained significantly shorter among patients with CS due to both CD and ACTH-independent adrenal CS compared with controls ($p = 0.03$), but other comparisons were nonsignificant. Comparison of coagulation profiles between patients with CD and adrenal CS were adjusted for BMI and UFC levels, corrected p-values are shown in ►Table 3.

Discussion

In the current study, we aimed to evaluate coagulation profile of patients with CS from different etiologies, and indeed we found higher levels of the procoagulant vWF:Ag and of the anticoagulant ATIII among patients with ACTH-secreting pituitary adenomas compared with those with primary adrenal CS. Levels of 24-h UFC positively correlated with activity of the anticoagulants ATIII, Protein C and S, and with plasma levels of the procoagulants vWF:Ag and FVIII, and correlated negatively with aPTT. Moreover, decrease in 24-h UFC after intervention positively correlated with decrease in FVIII levels and Protein C activity. Since patients with CD had higher 24-h UFC collections compared with primary adrenal CS, the coagulation profile differences can be partially attributed to the 24-h UFC levels. However, the difference in ATIII remained significant after adjusting for UFC measurements.

The differences in hypercortisolemia levels between patients with CD and those with hypercortisolemia from adrenal adenoma or hyperplasia might stem from the impetus for their medical investigation. Whereas patients with CD are usually admitted for evaluation of florid CS, the hypercortisolemia of patients with primary adrenal disease may be revealed during screening due to adrenal incidentalomas [14] or as a part of systemic syndrome management, such as primary pigmented nodular adrenocortical disease, Carney's complex, and other bilateral adrenocortical hyperplasias [15]. Hence, the different risk for venous thromboembolic events between CD and adrenal CS might be related to the timing of diagnosis, and not to other factors.

In regard to the comparison between CS patients and controls, our study results are supported by former reports [7]. In past studies, when compared with healthy controls patients with CS had higher levels of the endogenous procoagulants FVIII [11,16–19], vWF [8,11,16,20,21], and Fibrinogen [8,11, 16, 18, 19, 22,23]; higher levels of the antifibrinolytics PAI-1 [8,10,11,16,19,23] and $\alpha 2$ -antiplasmin [8,17,19], and of the anticoagulants Proteins C and S [10] and ATIII [10, 23]. The decrease in ATIII and Protein C activities following surgery in our study can be attributed to the resolution of

hypercortisolism, as supported by their positive correlation with 24-h UFC levels, and by prior studies [8]. However, emerging inflammatory state is thought to reduce Protein C and ATIII activities [24], and might also contribute to their dynamics. Interestingly, PAI-1 levels did not show correlation with UFC levels or midnight cortisol as shown before [7, 23], although its decrease following surgery correlated with ACTH decrease.

Abdominal adiposity is an important consequence of CS, which by itself might directly affect several coagulation elements [7, 25]. In a study assessing hypercoagulability in CD patients, BMI correlated with aPTT, FVIII, PAI-1, vWF, and α 2AP [19]. In our study, we found correlations between BMI and several coagulation elements. However, multivariate analysis showed that the differences between CS patients and controls; and between patients with CD and adrenal CS remained significant or had similar trend after adjustment for most variables, apart from Protein C.

The difference in coagulation elements profile between patients with CD and ACTH-independent CS may be explained by an inflammatory state. ACTH is derived from the pro-opiomelanocortin (POMC) molecule, which was shown to be capable of modulating the immune system through both ACTH and β -endorphin [26]. Thus, the hypercoagulability derived from an inflammatory state [27], may hypothetically link the ACTH levels to the coagulation profiles in different CS etiologies.

To summarize the conclusions that can be drawn from our study results, the strong positive correlation between UFC and FVIII and vWF:Ag levels demonstrate the influence of hypercortisolism on the clot formation by pushing the intrinsic coagulation cascade forward, as also demonstrated by the shortened aPTT among patients with CS compared to controls. Finally, fibrinolysis by plasmin is also inhibited by increased levels of α 2-antiplasmin, further altering the complex balance between clot formation and dissolution.

This was a prospective comparative study, all CD and adrenal CS diagnoses were pathologically proven, and we were able to show and explain several significant differences between the groups' coagulation profiles, even after controlling for UFC levels and BMI. Nevertheless, our study has several limitations: The small number of postoperative coagulation profiles measurements limits our ability to assess coagulation profile changes following resolution of hypercortisolemia. The adrenal CS group included patients with early stages of CS, thus their 24-h UFC levels were heterogenous. Finally, the control group consisted of "rule-out CS" patients, screened due to their clinical manifestations (e. g., weight gain, metabolic syndrome). Since these phenomena have been associated with increased VTE risk, some masking of the differences in coagulation profiles between CS patients and controls cannot be ruled out.

In conclusion, our data show for the first time the differences in coagulation components between patients with CS of different etiologies. Some of these differences are driven by higher cortisol levels among CD patients compared to those with adrenal CS.

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References

- [1]. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. *Lancet* (London, England) 2015; 386: 913–927
- [2]. Colao A, Pivonello R, Spiezia S, Faggiano A, Ferone D, Filippella M, Marzullo P, Cerbone G, Siciliani M, Lombardi G. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *J Clin Endocrinol Metab* 1999; 84: 2664–2672 [PubMed: 10443657]
- [3]. Stuijver DJF, van Zaane B, Feelders RA, Debeij J, Cannegieter SC, Hermus AR, van den Berg G, Pereira AM, de Herder WW, Wagenmakers MAEM, Kerstens MN, Zelissen PMJ, Fliers E, Schaper N, Drent ML, Dekkers OM, Gerdes VEA. Incidence of venous thromboembolism in patients with Cushing's syndrome: a multicenter cohort study. *J Clin Endocrinol Metab* 2011; 96: 3525–3532 [PubMed: 21880802]
- [4]. Van Zaane B, Nur E, Squizzato A, Dekkers OM, Twickler MTB, Fliers E, Gerdes VEA, Büller HR, Brandjes DPM. Hypercoagulable state in Cushing's syndrome: a systematic review. *J Clin Endocrinol Metab* 2009; 94: 2743–2750 [PubMed: 19454584]
- [5]. Johannesdottir Sa, Horváth-Puhó E, Dekkers OM, Cannegieter SC, Jørgensen JOL, Ehrenstein V, Vandenbroucke JP, Pedersen L, Sørensen HT. Use of Glucocorticoids and Risk of Venous Thromboembolism: A Nationwide Population-Based Case-Control Study. *JAMA Intern Med* 2013; 173: 1–10 [PubMed: 23877079]
- [6]. Stuijver DJF, Majoor CJ, van Zaane B, Souverein PC, de Boer A, Dekkers OM, Büller HR, Gerdes VEA. Use of oral glucocorticoids and the risk of pulmonary embolism. *CHEST J* 2013; 143: 1337
- [7]. Van Der Pas R, Leebeek FWG, Hofland LJ, De Herder WW, Feelders RA. Hypercoagulability in Cushing's syndrome: Prevalence, pathogenesis and treatment. *Clin Endocrinol (Oxf)* 2013; 78: 481–488 [PubMed: 23134530]
- [8]. Manetti L, Bogazzi F, Giovannetti C, Raffaelli V, Genovesi M, Pellegrini G, Ruocco L, Iannelli A, Martino E. Changes in coagulation indexes and occurrence of venous thromboembolism in patients with Cushing's syndrome: results from a prospective study before and after surgery. *Eur J Endocrinol* 2010; 163: 783–791 [PubMed: 20696792]
- [9]. Barbot M, Daidone V, Zilio M, Albiger N, Mazzai L, Sartori MT, Frigo AC, Scanarini M, Denaro L, Boscaro M, Casonato S, Ceccato F, Scaroni C. Perioperative thromboprophylaxis in Cushing's disease: What we did and what we are doing? *Pituitary* 2015; 18: 487–493 [PubMed: 25239557]
- [10]. Kastelan D, Dusek T, Kraljevic I, Polasek O, Giljevic Z, Solak M, Salek SZ, Jelcic J, Aganovic I, Korsic M. Hypercoagulability in Cushing's syndrome: the role of specific haemostatic and fibrinolytic markers. *Endocrine* 2009; 36: 70–74 [PubMed: 19381886]
- [11]. Boscaro M, Sonino N, Scarda A, Barzon L, Fallo F, Sartori MT, Patrassi GM, Girolami A. Anticoagulant prophylaxis markedly reduces thromboembolic complications in Cushing's syndrome. *J Clin Endocrinol Metab* 2002; 87: 3662–3666 [PubMed: 12161492]
- [12]. Isidori AM, Minnetti M, Sbardella E, Graziadio C, Grossman A. Mechanisms in endocrinology: The spectrum of haemostatic abnormalities in glucocorticoid excess and defect. *Eur J Endocrinol* 2015; 3: EJE-15–0308
- [13]. Coelho MCA, Santos CV, Vieira Neto L, Gadelha MR. Adverse effects of glucocorticoids: coagulopathy. *Eur J Endocrinol* 2015; 173: M11–M21 [PubMed: 25971647]
- [14]. Zeiger MA, Thompson GB, Duh Q-Y, Hamrahian AH, Angelos P, Elaraj D, Fishman E, Kharlip J. American association of clinical endocrinologists and american association of endocrine surgeons medical guidelines for the management of adrenal incidentalomas: executive summary of recommendations. *Endocr Pract.* 15: 450–453 [PubMed: 19632968]

- [15]. Correa R, Salpea P, Stratakis C. a. Carney complex: an update. *Eur J Endocrinol* 2015; 173: M85–M97 [PubMed: 26130139]
- [16]. Patrassi GM, Sartori MT, Viero ML, Scarano L, Boscaro M, Girolami A. The fibrinolytic potential in patients with Cushing's disease: a clue to their hypercoagulable state. *Blood Coagul Fibrinolysis* 1992; 3: 789–793 [PubMed: 1489900]
- [17]. Patrassi GM, Dal Bo Zanon R, Boscaro M, Martinelli S, Girolami A. Further studies on the hypercoagulable state of patients with Cushing's syndrome. *Thromb Haemost* 1985; 54: 518–520 [PubMed: 4082087]
- [18]. Dal Bo Zanon, Fornasiero R, Boscaro L, Cappellato M, Fabris G, Girolami FA Increased factor VIII associated activities in Cushing's syndrome: a probable hypercoagulable state. *Thromb Haemost* 1982; 47:116–117 [PubMed: 6808692]
- [19]. Van Der Pas R, De Bruin C, Leebeek FWG, De Maat MPM, Rijken DC, Pereira AM, Romijn JA, Netea-Maier RT, Hermus AR, Zelissen PMJ, De Jong FH, Van Der Lely AJ, De Herder WW, Lamberts SWJ, Hofland LJ, Feelders RA. The hypercoagulable state in Cushing's disease is associated with increased levels of procoagulant factors and impaired fibrinolysis, but is not reversible after short-term biochemical remission induced by medical therapy. *J Clin Endocrinol Metab* 2012; 97:1303–1310 [PubMed: 22278425]
- [20]. Fatti LM, Bottasso B, Invitti C, Coppola R, Cavagnini F, Mannucci PM. Markers of activation of coagulation and fibrinolysis in patients with Cushing's syndrome. *J Endocrinol Invest* 2000; 23:145–150 [PubMed: 10803470]
- [21]. Casonato A, Pontara E, Boscaro M, Sonino N, Sartorello F, Ferasin S, Girolami A. Abnormalities of von Willebrand factor are also part of the prothrombotic state of Cushing's syndrome. *Blood Coagul Fibrinolysis* 1999; 10: 145–151 [PubMed: 10357009]
- [22]. Tauchmanová L, Rossi R, Biondi B, Pulcrano M, Nuzzo V, Palmieri E-A, Fazio S, Lombardi G. Patients with subclinical Cushing's syndrome due to adrenal adenoma have increased cardiovascular risk. *J Clin Endocrinol Metab* 2002; 87: 4872–4878 [PubMed: 12414841]
- [23]. Erem C, Nuhoglu I, Yilmaz M, Kocak M, Demirel A, Ucuncu O, Onder Ersoz H. Blood coagulation and fibrinolysis in patients with Cushing's syndrome: increased plasminogen activator inhibitor-1, decreased tissue factor pathway inhibitor, and unchanged thrombin-activatable fibrinolysis inhibitor levels. *J Endocrinol Invest* 2009; 32: 169–174 [PubMed: 19411818]
- [24]. Allen KS, Sawheny E, Kinasewitz GT. Anticoagulant modulation of inflammation in severe sepsis. *World J. Crit care Med* 2015; 4: 105–115 [PubMed: 25938026]
- [25]. Mertens I, Considine RV, Van der Planken M, Van Gaal LF. Hemostasis and fibrinolysis in non-diabetic overweight and obese men and women. Is there still a role for leptin? *Eur J Endocrinol* 2006; 155: 477–484 [PubMed: 16914603]
- [26]. Heijnen CJ, Zijlstra J, Kavelaars A, Croiset G, Ballieux RE. Modulation of the immune response by POMC-derived peptides. I. Influence on proliferation of human lymphocytes. *Brain Behav Immun* 1987; 1: 284–291 [PubMed: 2839258]
- [27]. Levi M, van der Poll T. Inflammation and coagulation. *Crit Care Med* 2010; 38: S26–S34 [PubMed: 20083910]

Fig. 1. Coagulation factors profile before and after intervention in patients with CD (blue bars) vs. adrenal Cushing's (green bars).

Fig. 2. Correlation analyses between 24-h urinary free cortisol (UFC) levels (Log10) with Factor VIII levels **a**, antithrombin III activity **b**, and aPTT **c** measurements among patients with Cushing syndrome.

Table 1

Patients' demographic and baseline biochemical characteristics according to study groups.

	CD	Adrenal	Control	p-Value		
				CD vs. Adrenal	CD vs. control	Adrenal vs. control
Female gender n (%)	17/22 (77.3%)	17/21 (81.0%)	30/42 (71.4%)	0.8	0.6	0.4
Age (years)	26.9 ± 14.1	47.1 ± 14.0	42.1 ± 15.0	< 0.001	< 0.001	0.2
BMI (kg/m ²)	37.2 ± 10.1	33.5 ± 6.8	29.9 ± 7.7	0.3	0.009	0.1
Medical history n (%)						
Hypertension	6/22 (27.3%)	9/21 (42.9%)	17/42 (40.5%)	0.3	0.3	0.9
Type II DM	4/22 (18.2%)	4/21 (19.0%)	5/42 (11.9%)	0.9	0.5	0.4
Blood subtype O	9/20 (45.0%)	9/18 (50.0%)	3/13 (23.1%)	0.7	0.2	0.1
ACTH (pg/ml)	73.0 ± 57.7 (n = 22)	7.3 ± 6.4 (n = 17)	20.1 ± 12.5 (n = 37)	< 0.001	< 0.001	< 0.001
PM cortisol levels (µg/dl)	16.5 ± 10.1 (n = 19)	8.0 ± 4.7 (n = 21)	2.3 ± 1.3 (n = 40)	0.003	< 0.001	< 0.001
UFC (µg/24-h)	628.2 ± 865.8 (n = 19)	76.0 ± 52.2 (n = 19)	31.6 ± 15.8 (n = 38)	0.01	0.008	0.002

Data are presented as mean ± SD, unless mentioned otherwise

CD: Cushing disease; DM: Diabetes mellitus; CVD: Cardiovascular disease; ACTH: Adrenocorticotrophic hormone; UFC: Urinary free cortisol

Table 2

Pathological diagnoses of patients with Cushing's syndrome of adrenal source.

Pathological diagnosis	n (%)
Adrenal adenoma	5 (23.8)
Micronodular adrenal hyperplasia	1 (4.8)
Macronodular adrenal hyperplasia	9 (42.9)
PPNAD	6 (28.6)
Total	21 (100)

PPNAD: Primary pigmented nodular adrenocortical disease

Table 3

Comparison of baseline coagulation elements according to study groups.

	CD		Adrenal CS		Control		p-Value		
	n	Result	n	Result	n	Result	CD vs. Adrenal	CD vs. control	Adrenal vs. control
Factor VIII (IU/dl)	20	144.1 ± 78.9	21	122.1 ± 57.7	41	105.4 ± 49.6	0.3	0.06	0.2
vWF:Ag (IU/dl)	20	119.8 ± 55.8	21	87.5 ± 31.2	41	83.1 ± 28.7	0.03 [‡]	0.01	0.6
Fibrinogen (mg/dl)	20	343 ± 120.1	21	342.2 ± 67.3	41	328.4 ± 82.5	0.9	0.6	0.5
Antithrombin III activity (%)	20	93.3 (28.9)	21	74.5 (26.0)	41	78.0 (22.0)	0.01 [*] , [‡]	0.004 [*]	0.9 [*]
Protein C activity (%)	20	128.5 (52.8)	21	118.0 (55.0)	37	105 (40.0)	0.1 [*]	0.009 [*]	0.4 [*]
Protein S activity (%)	20	94 ± 19.0	21	84 ± 24.4	40	83.9 ± 30.0	0.2	0.2	0.9
α2-antiplasmin activity (%)	20	88.6 ± 17.0	21	83.9 ± 18.1	41	79.6 ± 16.2	0.4	0.05	0.3
PAI-1 (IU/ml)	19	21 ± 22.0	21	23.9 ± 17.3	40	25.5 ± 23.8	0.6	0.5	0.8
aPTT (seconds)	19	29.8 ± 3.1	21	30.5 ± 2.3	42	32.2 ± 3.3	0.5	0.009	0.03

Data are presented as mean ± SD, except for protein C and antithrombin III, which are presented as median (interquartile range)

^{*} Mann-Whitney U-test

[‡] The difference remained statistically significant after controlling for body mass index

[‡] The difference remained statistically significant after controlling for UFC collections

BMI: Body mass index; UFC: 24-h urinary free cortisol; vWF: von Willebrand factor; PAI-1: Plasminogen activator inhibitor-1; aPTT: Activated activated partial thromboplastin time