D-dimer as a significant marker of deep vein thrombosis in patients with subclinical or overt Cushing's syndrome

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Abstract. Venous thromboembolism (VTE) is frequently associated with hypercortisolemia. This retrospective single-center study aimed to clarify the significance of plasma D-dimer levels for VTE screening in patients with subclinical or overt Cushing's syndrome (soCS). A total of 72 consecutive treatment-naïve patients with soCS diagnosed at Kobe University Hospital between 2002 and 2014 were enrolled. Patients with both lower extremity ultrasound and D-dimer measurement data (n = 19) were recruited in study 1 and divided into 2 groups, a deep vein thrombosis (DVT) (-) group (n = 12) and DVT (+) group (n = 7) for a comparison of the associated factors. The age and D-dimer levels were higher in the DVT (+) group than in the DVT (-) group ($p = 0.04$ and 0.02, respectively). A receiver operating characteristic analysis found that D-dimer level ≥2.6 µg/mL correlated with the presence of DVT (sensitivity, 100%; specificity, 91.7%). Next, patients with D-dimer measurement data (n = 36) were recruited in study 2 and divided into 2 groups according to D-dimer level: D-dimer (-), <1 µg/mL group (n = 23) and D-dimer (+), ≥1 µg/mL group (n = 13); the groups were compared with respect to various VTE-related risk factors. A logistic regression analysis revealed that elevated cortisol level after low-dose dexamethasone suppression was a significant risk factor for D-dimer elevation (OR = 1.21, $p = 0.02$). In conclusion, these data demonstrate that a D-dimer level ≥2.6 µg/mL is an indicator of DVT in treatment naïve patients with soCS and suggests that relatively high autonomous cortisol secretion may be associated with thrombus formation.

Keywords: Cushing's syndrome, Venous thromboembolism, D-dimer

CUSHING'S SYNDROME (CS) comprises signs and symptoms that reflect the prolonged and inappropriately strong exposure of tissues to glucocorticoids. This condition is associated with a cluster of cardiovascular risk factors that strongly impact morbidity and mortality, including visceral obesity with insulin resistance, dyslipidemia, systemic hypertension, and hypercoagulability [1]. Venous thromboembolism (VTE) is a frequent complication in patients with CS especially during and after surgery [2, 3] and causes significant clinical manifestations. VTE is associated with pulmonary embolism, a potentially lethal complication, and therefore thromboprophylaxis has been recommended for patients with active CS [4]. In this regard, it is import-

ant to predict the risk of VTE in CS patients to ensure an appropriate indication of thromboprophylaxis.

In a hypercortisol state, an impaired hemostatic system was found to associate significantly with several biomarkers such as elevated levels of von Willebrand factor (VWF), factor VIII, factor IX, fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) [4-6]. Recently, VWF gene promoter polymorphisms have been reported to increase the risk of VTE *via* elevated levels of VWF in CS patients [7]. Although the risk of VTE is as high as 14.6 per 1000 person-years [2], the optimal VTE prediction marker in patients with CS remains to be determined. Disturbances in the hemostatic coagulation system have also been observed in patients with subclinical Cushing's syndrome [8, 9]. Therefore, we included patients with subclinical CS in this study.

As the majority of VTE is associated with deep vein thrombosis (DVT) [10, 11], evaluation of DVT is important to VTE screening. Although contrast venography is the most reliable exam for diagnosing DVT

[12], this method cannot be used for routine screening. Therefore, D-dimer, a cross-linked fibrin degradation product, is commonly used as a marker for DVT [13]. Although elevated D-dimer levels are not specific for DVT [14-16], D-dimer measurement has gained a prominent role as a rapid, simple, and inexpensive test for excluding DVT [17].

In this study, we investigated the role of D-dimer as a DVT prediction marker in patients with subclinical or overt CS (soCS). Furthermore, we analyzed the factors associated with elevated D-dimer levels in these patients to understand how inappropriate cortisol secretion might contribute to a hypercoagulated state.

Materials and Methods

Subjects

We performed a retrospective cross-sectional analysis of 72 consecutive naïve patients with soCS (mean age, 55.3 ± 14.7 years; 14 men and 58 women) who had been hospitalized in Kobe University Hospital during the period from 2002–2014. Written informed consent was obtained from all patients. The diagnosis of soCS was based on general diagnostic criteria as previously described [18, 19]. The etiologies of soCS include Cushing's disease [20], ectopic adrenocorticotropic hormone (ACTH) syndrome (EAS), CS due to adrenal adenoma (aCS), and subclinical CS due to adrenal adenoma (SCS). CD and EAS were classified as ACTH-dependent, whereas aCS and SCS were ACTH-independent. Three patients who used anti-coagulant medications were excluded. Patients who were pregnant, had undergone recent surgery, suffered from any malignancy, or received estrogen therapy were also excluded. Anthropometric parameters such as the body mass index (BMI) and systolic (SBP) and diastolic pressures (DBP) were collected for each patient along with biochemical test date, including the fasting plasma glucose (FPG), low density lipoprotein cholesterol (LDL-chol), and hemoglobin A1c (HbA1c; NGSP) levels. The disease duration (Duration) was estimated according to the onset of soCS-related clinical symptoms.

Study design

As shown in Fig. 1, 19 naïve patients with soCS for whom both the D-dimer levels and lower extremity ultrasound (LEU) data were available were recruited to Study 1. Regarding the indication for LEU, only patients with elevated D-dimer levels or leg edema
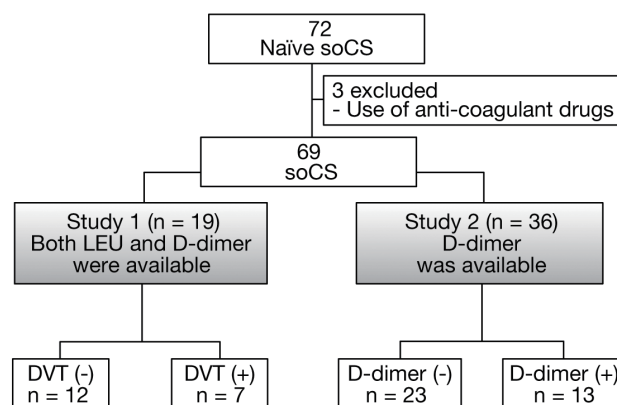

Fig. 1 Designs of Studies 1 and 2

underwent LEU before 2012; subsequently, all patients have undergone LEU. These patients were divided into 2 groups: DVT (-), indicating a negative LEU finding for DVT (n = 12) or DVT (+), indicating a positive finding for DVT (n = 7). For Study 2, patients for whom the D-dimer levels were available (n = 36) were divided into 2 groups: D-dimer (-), indicating D-dimer levels <1 µg/mL (n = 23) or D-dimer (+), indicating D-dimer levels ≥1 µg/mL (n = 13).

Hormone assays

The serum cortisol (F) levels were measured *via* an enzyme immunoassay (EIA; TOSOH, Tokyo, Japan) and the 24-h urinary free cortisol levels (UFC) were measured *via* radioimmunoassay (RIA; TFB, Tokyo, Japan). The low-dose dexamethasone suppression test (LDDST) was performed as described previously [18, 21, 22].

Measurements of the coagulation and fibrinolysis variables

The plasma D-dimer levels were measured using LPIA-ACE D-dimer II (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The interassay coefficients of variation for D-dimer concentrations of 1.3 and 13.6 µg/mL were 8% and 1%, respectively. The limit of detection for D-dimer was 0.5 µg/mL. The normal range of D-dimer levels was <1 µg/mL. Blood collection for D-dimer measurement was performed on the day of admission (n = 9), at the outpatient clinic (n = 1), or during a hospital stay (n = 9). The activated partial thromboplastin time (aPTT) was measured using HemosIL SynthASil (Instrumentation Laboratories, Tokyo, Japan). Fibrinogen was measured using HemosIL Fib (Instrumentation Laboratories).

Statistical analysis

All data are described as means ± standard deviations (SD). Comparisons between 2 groups were made with Student's *t* test. The chi-square test was used to examine differences between categorical variables. A multiple logistic regression analysis was used to evaluate the independent valuables associated with the D-dimer levels. The tested factors included the night F, morning F after the low-dose dexamethasone suppression test (LDDST F), UFC, and LDL-chol, followed by a variance inflation factor (VIF) analysis. P values <0.05 were considered statistically significant. Data were processed using an SPSS software package (Dr. SPSS II for windows; NANKODO Co., Ltd., Tokyo, Japan).

Results

Study 1: Elevated D-dimer levels were associated with DVT in soCS patients

Nineteen patients who had undergone LEU and D-dimer measurements were divided into 2 groups: DVT (-) (n = 12) and DVT (+) (n = 7; Fig. 1). There were no differences between these groups in terms of the ACTH-dependency, soCS etiology, gender, BMI, SBP, DBP, and Duration (Table 1). The age and D-dimer levels were significantly higher in the DVT (+) group relative to the DVT (-) group (65.0 ± 13.3 years *vs*. 48.3 ± 19.1 years, $p = 0.04$ and 6.33 ± 4.20 μg/mL *vs*. 0.88 ± 1.64 μg/mL, $p = 0.02$; Table 1, Fig. 2a). Although no patients with D-dimer levels <1 μg/mL manifested DVT according to LEU, 7 of the 10 patients (70.0%) with D-dimer levels ≥1 μg/mL manifested DVT (Fig. 2a). During the clinical course, no patients developed DVT after the first LEU. Fig. 2b shows the D-dimer values with the highest areas under the receiver operating characteristic (ROC) curves. The selected D-dimer cut-off value for DVT detection was ≥2.6 μg/mL (sensitivity 100%, specificity 91.7%; 95% confidence interval (CI), 0.79–1.07; Fig. 2b). The preoperative and postoperative D-dimer levels were evaluated in 13 patients. The postoperative D-dimer levels were significantly higher (8.7 ± 7.9 days post-operation) than the preoperative levels (6.36 ± 5.40 μg/mL *vs*. 1.62 ± 2.25 μg/mL, $p = 0.02$). There was no association between the collection timing and D-dimer elevation in these subjects ($p = 0.11$).

Study 2: A high LDDST F level is a risk factor for an elevated D-dimer level in soCS

We also analyzed whether D-dimer was associated with parameters related to the hemostatic system, metabolic state, or cortisol secretion such as aPTT, fibrinogen, LDL-chol, HbA1c, morning F, night F, LDDST F, and UFC. Thirty-six patients with D-dimer level data were divided into 2 groups: D-dimer (-) (n = 23) or D-dimer (+) (n = 13; Fig. 1c). As expected, the D-dimer levels were higher in D-dimer (+) than in D-dimer (-) (3.59 ± 2.89 μg/mL *vs*. 0.04 ± 0.17 μg/mL, $p <0.01$; Table 2). No significant differences were found between the 2 groups in terms of ACTH-dependency, soCS etiology, gender, age, BMI, SBP, and DBP (Table 2). As shown in Table 3, the hemostatic system parameters aPTT and fibrinogen did not significantly differ between the groups. Regarding the other risk factors of VTE, the LDL-chol levels [23] were significantly higher in the D-dimer (+) group than in the D-dimer group (-) (142.2 ± 37.5 μg/dL *vs*. 109.9 ± 33.1 μg/dL, $p = 0.01$), whereas no difference seen in the HbA1c level was observed between the groups (6.3 ± 2.1% *vs*. 6.3 ± 1.0%, $p = 0.27$). Representative parameters of the

Table 1 Clinical characteristics of the patients in Study 1

	DVT (-)	DVT (+)	p value
n	12	7	
ACTH-dependent / -independent	3 / 9	2 / 5	0.87
CD / EAS / aCS / SCS	3 / 0 / 6 / 3	1 / 1 / 4 / 1	0.52
Gender (Female / Male)	10 / 2	7 / 0	0.25
Age (years) [*]	48.3 ± 19.1	65.0 ± 13.3	0.04
BMI (kg/m^2)	25.0 ± 4.1	23.8 ± 5.9	0.65
Duration (years)	3.5 ± 3.1	1.6 ± 1.7	0.10
SBP (mmHg)	135.2 ± 20.5	138.0 ± 23.8	0.81
DBP (mmHg)	70.6 ± 25.8	79.0 ± 13.7	0.38

CD, Cushing's disease; EAS, Ectopic ACTH syndrome; aCS, Cushing's syndrome due to adrenal adenoma; SCS, Subclinical Cushing's syndrome due to adrenal adenoma. Data are expressed as means ± SD. *p<0.05
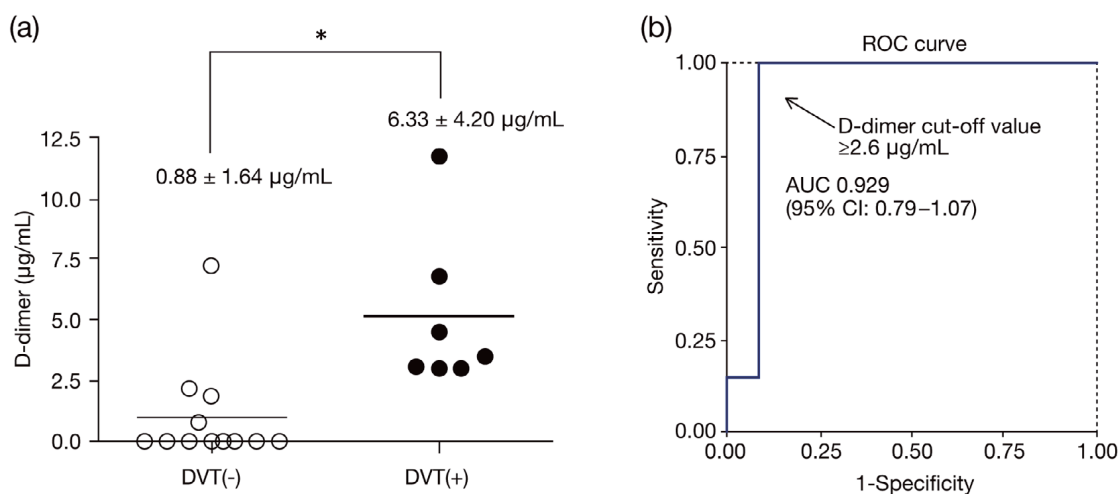
Fig. 2 (a) D-dimer levels in patients with soCS with or without DVT
      * *p* <0.05
      (b) ROC analysis of the D-dimer values for DVT detection in patients with soCS
      Area under the curve = 0.929

Table 2   Clinical characteristics of the patients in Study 2

	D-dimer (-)	D-dimer (+)	*p* value
D-dimer (µg/mL)**	0.04 ± 0.17	3.59 ± 2.89	<0.01
n	23	13	
ACTH-dependent / -independent	4 / 19	5 / 8	0.16
CD / EAS / aCS / SCS	4 / 0 / 11 / 8	4 / 1 / 7 / 1	0.18
Gender (Female / Male)	19 / 4	11 / 2	0.88
Age (years)	50.5 ± 16.6	55.7 ± 18.9	0.40
BMI (kg/m²)	24.9 ± 3.6	23.3 ± 4.6	0.25
SBP (mmHg)	134.7 ± 15.8	134.7 ± 25.4	1.00
DBP (mmHg)	76.6 ± 21.8	78.5 ± 16.6	0.80

CD, Cushing's disease; EAS, Ectopic ACTH syndrome; aCS, Cushing's syndrome due to adrenal adenoma; SCS, Subclinical Cushing's syndrome due to adrenal adenoma; SBP, Systolic blood pressure; DBP, Diastolic blood pressure. Data are expressed as means ± SD. ** *p* <0.01

Table 3   Comparison of the hemostatic, metabolic, and biochemical parameters of patients with soCS according to a D-dimer cut-off value of 1 µg/mL

	D-dimer (-)	D-dimer (+)	*p* value
aPTT (sec)	28.7 ± 7.1	25.4 ± 4.9	0.12
Fibrinogen (µg/dL)	268.8 ± 53.1	259.1 ± 126.0	0.78
LDL-chol (µg/dL)*	109.9 ± 33.1	142.2 ± 37.5	0.01
HbA1c (%)	6.9 ± 2.1	6.3 ± 1.0	0.27
Morning F (µg/dL)	17.2 ± 6.2	23.8 ± 11.2	0.07
Night F (µg/dL)**	15.6 ± 6.4	26.7 ± 9.9	<0.01
LDDST F (µg/dL)**	15.9 ± 7.0	24.2 ± 6.6	<0.01
UFC (µg/24h)*	210.9 ± 220.1	546.2 ± 404.8	0.02

aPTT, activated partial thromboplastin time; LDDST F, F after low-dose dexamethasone suppression test; UFC, urinary free cortisol. Data are expressed as means ± SD. * *p* <0.05, ** *p* <0.01

Table 4 Independent risk factors associated with the elevated D-dimer levels in patients with soCS (step-wise logistic regression analysis)

	OR	95% CI	*p* value
LDDST F*	1.22	1.04-1.44	0.02
UFC			0.16
Night F			0.23
LDL-chol			0.47

OR, odds ratio; *p <0.05

cortisol secretion state such as morning F, night F, and LDDST F correlated with the D-dimer levels (r = 0.36, *p* = 0.03; r = 0.42, *p* = 0.02; and r = 0.659, *p* <0.01, respectively); however, no correlation was observed between the D-dimer levels and UFC (r = 0.32, *p* = 0.07). The night F, LDDST F, and UFC were higher in the D-dimer (+) group than in the D-dimer (-) group. The D-dimer (+) group also tended to have a higher morning F level. To clarify the independent factors associated with elevated D-dimer levels, we performed a step-wise logistic regression analysis (parameters: LDL-chol, night F, LDDST F, and UFC) and found that the LDDST F levels were independently associated with elevated D-dimer levels (odds ratio (OR) = 1.22, 95% CI 1.04–1.44; Table 4).

Discussion

In the present study, we first showed that a D-dimer level ≥2.6 μg/mL indicates the presence of DVT in patients with soCS. We also demonstrated that a high LDDST F level was associated with elevated D-dimer levels. To prevent VTE in these patients, early VTE (particularly DVT) screening and diagnosis are very important [10, 11]. VTE is a frequent complication in patients with CS, especially during and after surgery [2, 3], and thromboprophylaxis has been recommended for patients with active CS. However, thromboprophylaxis carries some inherent risks such as bleeding events. Therefore, clarification is needed to determine which patients would beneficial from thromboprophylaxis. In this regard, our D-dimer cut-off value is instrumental to further examinations, including LEU, and decisions regarding thromboprophylaxis. The association between the LDDST F and elevated D-dimer levels suggests an underlying mechanistic insight into hypercoagulability in soCS.

Although contrast venography is the most sensitive and accurate test for diagnosing DVT, it is too invasive to use as a screening method [12]. Therefore, LEU is generally used for DVT diagnosis; this technique has a sensitivity of 97–100% and a specificity of 98–99% for proximal thrombosis [24, 25] but reduced accuracy for distal thrombosis (sensitivity, 70% and specificity, 60%) [12]. We found that 7 of the 19 patients with soCS exhibited DVT (37%), which was higher than a previously reported incidence [2]. The higher prevalence of DVT might be because of the indication bias from LEU. However, all the patients complicated with DVT showed elevated D-dimer levels, indicating this biomarker could be useful to detect DVT. High prevalence of VTE has been shown in ACTH-dependent CS than -independent after surgery. However, overall incidence rates before surgery were similar between them [2]. No difference was seen between ACTH-dependent and -independent in study 1, is compatible with the previous report.

Our study was limited by its retrospective nature and relatively small number of patients. In addition, the number of patients for whom D-dimer level and LEU data were available was limited. Therefore, selection bias, particularly for patients who had undergone LEU, cannot be ruled out. Furthermore, LEU screening might miss the presence of distal thrombosis (sensitivity, 70%; specificity, 60%) in a comparison with contrast venography, which is a gold standard modality. We could not prepare a control group for this study.

Many biomarkers are used to diagnose DVT, including Factor VIII, soluble fibrin (SF), thrombin generation, inflammatory cytokine production, and D-dimer. In the present study, we focused on D-dimer in patients with soCS because D-dimer measurement is rapid, simple, and inexpensive [17, 26]. On the other hand, elevated D-dimer levels are not specific for DVT and can also be present in the settings of infection, inflammation, cancer, surgery, trauma, ischemic heart disease, and stroke [16]. Therefore a combination of D-dimer measurements and an imaging technique such as LEU or contrast venography is required for a definitive diagnosis. In this study, we excluded patients who had undergone recent surgery, suffered from any malignancy, or used estrogen therapy. Regarding D-dimer, it would be intriguing to test the usefulness of other biomarkers for predicting DVT in patients with soCS.

In study 1, the D-dimer levels in DVT-complicated patients with soCS were significantly higher than those in patients without DVT. The ROC curve analysis revealed that the D-dimer cut-off value for DVT detection was ≥2.6 μg/mL (sensitivity, 100% and spec-

ificity, 91.7%). In the general Japanese population, the D-dimer cut-off value for DVT in detection was reported to be 7.87 µg/mL (measured using the same kit as in the current study) [26], suggesting that cut-off value was much lower in patients with soCS from the present study. The D-dimer cut-off values different among Japan, Europe, and North America because of the use of different measurement kits [27]. A 2-fold D-dimer cut-off value has been recommended when comparing assays used in Japan with those used in Europe and North America [26], thus indicating that the cut-off value for DVT in patients with soCS might be ≥1.3 µg/mL as measured by an assay used in Europe and North America.

Regarding the mechanistic insight, patients with CS have been reported to present with elevated plasma levels of clotting factors, including VWF and factor VIII, which increases the plasma D-dimer levels [6, 28, 29]. On the other hand, the levels of PAI-1, the primary inhibitor of the fibrinolytic system, were found to be elevated in patients with CS [6, 30, 31]. PAI-1 attenuates D-dimer elevation by inducing a coagulation cascade, suggesting that the elevated PAI-1 levels in CS might reduce the D-dimer levels. In this regard, it is possible that elevated D-dimer levels might be masked in CS. This could explain the finding that the cut-off value for DVT was lower in patients with soCS than in the general population.

There has been controversy regarding the D-dimer levels in patients with CS. The D-dimer levels were found to be elevated [6] or unchanged [32] relative to those in healthy control subjects. No changes in the D-dimer levels in patients with soCS were observed at 28 and 77 days after medical treatment [28]. In the present study, we could not compare the patient data with data from healthy control subjects, and therefore it is unknown whether the D-dimer levels were elevated relative to those in control subjects. On the other hand, it is well known that surgery and subsequent bed rest increase the D-dimer levels. In this study 1, we analyzed only naïve CS patients. In these patients, thirteen were sub-analyzed the postoperative D-dimer levels, showing significantly increase relative to the preoperative levels. The cut-off value of 2.6 µg/mL was based on the preoperative data; therefore, further study is needed to validate the effectiveness of this cut-off value during the postoperative period.

We next investigated the factors associated with elevated D-dimer levels in Study 2. In the most commonly used assay in Europe and North America, D-dimer concentrations <0.5 µg/mL are considered the cut-off value for excluding DVT [13]. As the cut-off value differs between Japan and the Western countries, we used a cut-off value of 1 µg/mL in study 2 in accordance with Japanese references. Indeed, no patients with D-dimer levels <1 µg/mL manifested DVT. In study 2, the serum LDL-chol, LDDST F, night F, and UFC levels were significantly higher in the D-dimer (+) group than in the D-dimer (-) group. Hypercholesterolemia has been reported as a risk factor of VTE in the general population (OR = 1.2; 95% CI 0.67–2.0) [33]. Given our data, this factor may also be applicable in patients with soCS.

The effects of glucocorticoid on the coagulation system have been demonstrated *via* dexamethasone treatments, which were found to increase the plasma concentrations of factor VIII, factor IX, and fibrinogen in healthy volunteers [34]. Elevated UFC levels have been reported as a risk factor of VTE in patients with CS [29]. Intriguingly, a logistic regression analysis followed by a VIF analysis that included LDL-chol, LDDST F, night F, and UFC revealed that only LDDST F was an independent risk factor associated with elevated D-dimer levels. Factor VIII and PAI-I have been shown to correlate with the night F levels [29, 31]. Taken together with our data, autonomic cortisol secretion, rather than excess cortisol secretion, might contribute to the abnormal anticoagulation state in patients with soCS.

Although anti-thrombotic prophylaxis has been recommended for patients with CS, particularly during the active disease phase and after transsphenoidal surgery [2], there are currently no established guidelines for antithrombotic therapy. The present study suggests that the D-dimer measurement is a useful marker for predicting DVT in patients with soCS and that the DVT detection cut-off value might differ among these patients relative to the general population. A prospective intervention study to clarify the effects of anti-thrombotic prophylaxis on the prognosis of patients with CS will be necessary to establish the significance of anti-thrombotic prophylaxis, and our cut-off value might be instrumental for patient selection.

In conclusion, these data demonstrate that a D-dimer level ≥ 2.6 µg/mL suggests the presence of DVT in patients with soCS. Elevated D-dimer levels are positively associated with the serum cortisol levels after LDDST, suggesting that relatively autonomous cortisol

secretion, rather than the daily total secretion, might associate with thrombus formation.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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