Activity of selected coagulation factors in overt and subclinical hypercortisolism

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Abstract. It is universally acknowledged that glucocorticoids are hormones that exert a significant effect on hemostasis. The aim of this study was to analyze the activities of coagulation factors VIII, von Willebrand (vW), IX, X, and XI, in patients with overt and subclinical hypercortisolism, as well as to examine possible associations between activities of these factors and the degree of hypercortisolism. Thirty endogenous hypercortisolemic patients were included in the study. Twelve of them were diagnosed with overt Cushing’s syndrome (OCS), and eighteen with subclinical Cushing’s syndrome (SCS). Healthy, age- and sex-matched volunteers comprised the control group. Activities of coagulation factors VIII, IX, X, and XI were examined using a coagulometric method, and von Willebrand factor (vWF) using an immunoturbidimetric method. Mean activities of examined coagulation factors were significantly higher in OCS patients in comparison to healthy controls. SCS patients had significantly higher mean vWF activities versus controls; and a clear trend toward higher mean activities of other factors in SCS patients versus controls was recorded (but no significant differences). Furthermore, statistically significant positive correlations were found between activities of factor IX and: morning serum cortisol concentrations, 24-hour urinary cortisol excretion values, cortisol concentrations in the overnight suppression test with 1 mg of dexamethasone. Activities of factors X and XI positively correlated with cortisol levels in the overnight suppression test. In endogenous hypercortisolemic patients the coagulation pathway is hyperactivated as indicated by increased activities of coagulation factors. These disorders are evident among patients with overt hypercortisolism.

Key words: Coagulation factors, Cushing’s syndrome, Subclinical Cushing’s syndrome

IT IS UNIVERSALLY acknowledged that glucocorticoids are hormones that exert a significant effect on hemostasis [1-4]. Effects of these hormones on coagulation and fibrinolysis parameters have been shown repeatedly. The result is an increased incidence of thromboembolic complications [4-6].

Hemostasis disorders observed in hypercortisolemic subjects are multifaceted. In overt hypercortisolemic patients a trend has been confirmed toward increased serum concentrations of coagulation factors V, VIII, von Willebrand (vW), IX and XI [7-15]. However, statistically significant differences were recorded mainly for factors VIII, IX, and vW.

Patients with subclinical hypercortisolism have been studied much less extensively. So far, at this stage of the disease, increased concentrations were stated for: fibrinogen (which plays a crucial role not only in the plasma coagulation cascade but also in primary hemostasis by stimulating platelet aggregation), and homocysteine – a confirmed venous as well as arterial thrombosis risk factor; also increased endogenous anticoagulation system activity was recorded, i.e. proteins C and S [16-18]. Active protein C, with the involvement of cofactor protein S, degrades and inactivates coagulation factors VIIIa and Va by cleaving specific arginine residues (what leads to their partial proteolysis) [19].

Until now, activity of coagulation factors in subclinical Cushing’s syndrome (SCS) patients has not been investigated. Increases in factors’ activities seem to play a major role in stimulating the coagulation system and might explain the over-stimulation of the endogenous anticoagulation system. Similarly, no studies have been published in which possible associations...
were analyzed between the activity of coagulation factors and hormonal parameters indicating the degree of hypercortisolism.

In this study we aimed at determining activities of coagulation factors VIII, IX, X, XI, and vW in overt and subclinical endogenous hypercortisolism, as well as investigating associations between activities of these factors and the degree of hypercortisolism.

**Patients and Methods**

Thirty endogenous hypercortisolemic patients were enrolled into this prospective study, 24 women and 6 men, mean age 56.5 ± 10.3 years, who were hospitalized in or monitored at the outpatient clinic of the Department of Endocrinology and Internal Medicine of the Medical University of Gdańsk. The following were excluded from the study: subjects with a connective tissue disease, acute or chronic symptomatic infection, neoplasm, renal failure, affective disorder, taking oral contraceptives or hormonal replacement therapy, patients who underwent a thromboembolic event in preceding 6 months, persons prescribed with anticoagulants. Enrolled participants did not use drugs altering glucocorticoid metabolism, *i.e.* synthetic steroids, drugs influencing dexamethasone metabolism (CYP3A4 inducing and/or inhibiting agents), drugs altering transcortin (CBG) levels, and drugs interfering in urinary cortisol excretion.

All patients were diagnosed with endogenous hypercortisolism. Twelve participants had overt Cushing’s syndrome (OCS): 9 met the criteria of an ACTH (adrenocorticotropic hormone) -dependent Cushing’s syndrome – 7 of them were diagnosed with a corticotropinoma of the hypophysis, in 2 relapse of a previously resected corticotropinoma was diagnosed. In 3 patients ACTH-independent Cushing’s syndrome due to a hormonally active adrenal tumor was stated. The second study group comprised 18 patients who met the criteria of SCS in the course of an adrenal cortex adenoma.

OCS diagnosis was made in patients with clinical hypercortisolism features (plethora, moon face, buffalo hump, central obesity, proximal muscle atrophy), and, abnormal hormonal tests results, *i.e.* cortisol secretion was suppressed neither in the overnight test with 1 mg of dexamethasone, nor low-dose (0.5 mg orally every 6 h for 2 days) dexamethasone test (cortisol levels over 50 nmol/L); there was no circadian rhythm of cortisol secretion (late evening to morning serum cortisol % ratio exceeded 50%); 24-hour urinary free cortisol excretion was elevated. Also, in adrenal CS patients low ACTH values were recorded, while in pituitary-dependent CS patients inadequately high morning ACTH concentrations and a typical CRH (corticotropin releasing hormone) test response were found (increase in ACTH level by 35 - 50% or cortisol by 15 - 20%). Hormonal characteristics of study samples are shown in Table 1.

Patients were enrolled into the study only if the diagnosis of hypercortisolism raised no doubts, and was clear considering laboratory as well as imaging examinations. In all adrenally dependent hypercortisolemic patients a mass in the gland was found using a targeted adrenal CT scan; in case of all Cushing’s disease subjects pituitary magnetic resonance imaging revealed gland’s microadenoma.

SCS was diagnosed in accordance with the definition, *i.e.* in patients with an incidental adrenal mass found in an abdominal CT scan, who did not have typical physical features of CS and exhibited endogenous hypercortisolism in hormonal tests. So far, SCS diagnosis does not have universally accepted hormonal criteria [20-24]. Cortisol value of 140 nmol/L and above in the overnight suppression test with 1 mg of dexamethasone was a sufficient criterion to diagnose SCS [23, 24]. Patients with cortisol values between

<table>
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<tr>
<th>Laboratory examination</th>
<th>OCS</th>
<th>SCS</th>
<th>Normal ranges</th>
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<tbody>
<tr>
<td></td>
<td>ACTH-dependent</td>
<td>ACTH-independent</td>
<td></td>
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<tr>
<td>Serum cortisol, nmol/L: morning</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Serum cortisol, nmol/L: night</td>
<td>515.3</td>
<td>160.5</td>
<td>487.3</td>
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<tr>
<td>Serum cortisol in an overnight suppression test (1mg DXM), nmol/L</td>
<td>239.4</td>
<td>105.3</td>
<td>454.7</td>
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<tr>
<td>24-hour urinary free cortisol excretion, nmol/24h</td>
<td>771.9</td>
<td>606.2</td>
<td>852.3</td>
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<tr>
<td>Plasma ACTH pg/mL: morning</td>
<td>55.7</td>
<td>29.5</td>
<td>12.3</td>
</tr>
<tr>
<td>Serum DHEA-S, µg/dL</td>
<td>150.4</td>
<td>162.7</td>
<td>22.3</td>
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</table>

Data are presented as mean and standard deviation (SD). DXM, dexamethasone; ACTH, adrenocorticotropic hormone; DHEA-S, dehydroepiandrosterone sulfate.
50 and 140 nmol/L in this test must have met at least one other criterion: lack of circadian cortisol secretion rhythm (late evening to morning serum cortisol % ratio exceeded 50%), decreased morning ACTH concentration (≤10 pg/mL), and/or increased free cortisol urinary excretion [23, 24]. Furthermore, in all SCS patients cortisol levels were not suppressed to less than 50 nmol/L in the low-dose dexamethasone test (0.5 mg every 6 hours for 2 days).

All hormonal assays were performed in the same laboratory using freely available kits. ACTH concentration was determined with a solid-phase, two-site sequential chemiluminescent immunometric assay Immulite®1000ACTH manufactured by Siemens. Serum and urinary cortisol concentrations were determined with a Chemiluminescent Microparticle Immunoassay Cortisol Reagent Kit on Abbott’s Architect analyzer.

Blood was drawn in the morning, after fasting, from a cubital vein.

To evaluate metabolic disorders, body mass index (BMI) was calculated in all hypercortisolemic patients based on weight and height measurements, blood pressure was examined, as well as the following laboratory parameters were determined by routine procedures: lipid profile (mean concentration of total cholesterol concentration was 206.4 ± 35.5 mg/dL; triglycerides 132.5 ± 50.0 mg/dL; mean LDL 131.8 ± 29.2 mg/dL), fasting glucose (mean level was 101.1 ± 15.7 mg/dL), 2 hour oral glucose tolerance test (OGTT). In the hypercortisolemic group (both OCS and SCS) 19 patients (63%) were overweight or obese (mean BMI 28.1 ± 4.1 kg/m²), 14 patients (47%) had impaired glucose tolerance or impaired fasting glycaemia. None of the patients fulfilled diabetes criteria, or presented complications of hyperglycaemia.

A control group comprised 30 healthy individuals matched for age, sex (24 women and 6 men, mean age 49.2 ± 11.6) and BMI.

Coagulation factors’ VIII, IX, X, XI activity was determined using a coagulometric method with a mixture of an appropriate factor-deficient plasma and patient’s plasma (on a Siemens BCS analyzer) with designated reagents. The result was interpreted using a calibration curve obtained from dilutions of standard human plasma. vWF activity was determined using an immunoturbidimetric method (on the BCS analyzer using designated reagents). In the presence of ristocetin vWF present in a sample lead to stabilized platelet agglutination (ristocetin cofactor activity measurement by platelet agglutination). The agglutination process resulted in a change of plasma optic density, which was measured in the analyzer.

In all subjects, activated partial thromboplastin time (aPTT) and international normalized ratio (INR) were determined using standard methods.

All statistical calculations were performed with the use of the STATA 13.1 software (StataCorp, Texas, USA). Prior to analysis, data were screened for potential errors. Standard descriptive statistics were calculated. Some variables were transformed before parametric analyzes. Comparisons between groups were tested by ANOVA. Scheffe post-hoc test was used when indicated. Correlations were evaluated using Spearman rank correlation method. Multivariate analysis was performed using multiple linear regression. The level of statistical significance was set at 0.05.

The study was approved by the ethics committee of the Medical University of Gdańsk (NKBBN/43-598/2013).

**Results**

Mean activities of coagulation parameters of hypercortisolemic patients and healthy controls are shown in Table 2. A comparison of coagulation factors’ VIII, IX, X, XI, and vW activities between hypercortisolemic patients and controls revealed statistically significant differences for all examined parameters (respectively: $p=0.007$, $p=0.002$, $p=0.038$, $p=0.001$, $p=0.000$).

<table>
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<th>Table 2</th>
<th>Activities of examined coagulation factors in hypercortisolemic patients and healthy controls.</th>
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<tr>
<td></td>
<td>Factor VIII (%)</td>
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<td></td>
<td>Mean</td>
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<tr>
<td>CS</td>
<td>176.0</td>
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<tr>
<td>Controls</td>
<td>145.8</td>
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<tr>
<td>p-values</td>
<td>0.007</td>
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Data are presented as mean and standard deviation (SD). CS, Cushing’s syndrome (OCS and SCS); vW, von Willebrand factor.
examined coagulation factors’ activities and hormonal parameters indicating the degree of hypercortisolism was performed. Statistically significant correlations were found between factor IX activity and examined hypercortisolism parameters (morning cortisol concentration, 24-hour urinary cortisol excretion, overnight test cortisol concentration, respectively: $r=0.43, p=0.017; r=0.43, p=0.016; r=0.49, p=0.007$), Fig. 1.

Activities of factors X and XI correlated with cortisol level in the overnight suppression test (respectively: $r=0.40, p=0.027; r=0.42, p=0.030$), Figs. 2, 3.

Activities of coagulation factors were not analyzed depending on the etiology of hypercortisolism since examined groups were small.

**Discussion**

In overt hypercortisolemic patients a tendency toward increased concentrations and activities of plasmatic coagulation factors has been confirmed for factors V, VIII, vW, IX, and XI [7-15]. Undoubtedly, more uncertainties remain concerning subclinical hypercortisolemic patients. There are only few studies on the coagulation system in these subjects, despite the fact that SCS is the most common hormonal disorder diagnosed in individuals with an incidental adrenal mass. According to some authors, SCS prevalence lies at 5-20% of all adrenal incidentaloma patients [20, 23].

In this study we analyzed activities of coagulation factors’ VIII, IX, X, XI, and vW depending on the degree of hypercortisolism. We found statistically higher factors’ activities in: all hypercortisolemic patients, and OCS (excluding SCS) patients, versus healthy controls.

The cause of elevated concentrations and activities of coagulation factors in overt hypercortisolism is com-

<table>
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<tr>
<th>Table 3</th>
<th>Coagulation factors’ VIII, IX, X, XI, and vW activities in OCS patients, SCS patients, and controls.</th>
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<tr>
<td></td>
<td>Factor VIII (%)</td>
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<td></td>
<td>Mean</td>
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<tr>
<td>OCS</td>
<td>185.3</td>
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<tr>
<td>SCS</td>
<td>169.8</td>
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<tr>
<td>Controls</td>
<td>145.8</td>
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$p=0.000$. Higher mean activities were found in hypercortisolemic patients.

Next, activities of coagulation factors were compared depending on the degree of hypercortisolism. Mean activities of factors VIII, IX, X, XI, and vW were statistically higher in OCS patients versus controls (respectively: $p=0.029; p=0.000; p=0.030; p=0.001; p=0.005$). SCS patients had statistically higher vWF activities than controls ($p=0.001$). No significant differences in activities of other factors (VIII, IX, X, XI) were found between SCS patients and controls (respectively: $p=0.172; p=0.656; p=0.592; p=0.124$). However, a clear trend toward increased activities of all examined coagulation factors was recorded among SCS patients as shown in Table 3.

Further, coagulation factors’ activities were compared between overt and subclinical hypercortisolemic patients, see Table 3. A statistically significant difference between these two groups was found only for factor IX activity, its mean value was higher in OCS subjects ($p=0.000$).

Taking into account the possible effect of metabolic disorders on coagulation, correlations between BMI, fasting glycemia, total cholesterol and activities of coagulation factors VIII, IX, X, XI, and vW were tested in the group of patients with hypercortisolism. A significant correlation was found only between total cholesterol concentration and vWF activity ($r=-0.38; p=0.036$). However, in multivariate analysis cholesterolemia as well as other metabolic parameters did not alter the differences in activities of vWF among OCS and SCS patients.

No statistically significant relationships between metabolic parameters and other coagulation factors were found.

Subsequently, an analysis of associations between

$$p-values$$

| OCS vs Controls | 0.029 | 0.000 | 0.030 | 0.001 | 0.005 |
| SCS vs Controls | 0.172 | 0.656 | 0.592 | 0.124 | 0.001 |
| OCS vs SCS     | 0.617 | 0.000 | 0.254 | 0.167 | 1.000 |

Data are presented as mean and standard deviation. OCS, overt Cushing’s syndrome; SCS, subclinical Cushing’s syndrome.
Coagulation factors in hypercortisolism

Effect of short-term steroid use was observed in healthy controls, who did not present metabolic disorders, in that concentrations of coagulation factors II, VII, VIII, and XI were elevated [28].

In research thus far a lot of attention has been paid to vWF concentrations and activities. It was demonstrated that this factor’s promoter gene polymorphism is of major importance. It seems that haplotype 1 (GCAG) presence is associated with a particularly high thromboembolic risk [25, 32, 33]. High glucocorticoids stimulate the synthesis of coagulation factors by altering various transcription factors [25-27]. On average the stimulatory effect is absent after 12 months following successful surgery for Cushing’s syndrome [12]. A direct effect of glucocorticoids on coagulation system parameters has been confirmed in a number of studies [28-31]. A significant effect of short-term steroid use was observed in healthy controls, who did not present metabolic disorders, in that concentrations of coagulation factors II, VII, VIII, and XI were elevated [28].

In research thus far a lot of attention has been paid to vWF concentrations and activities. It was demonstrated that this factor’s promoter gene polymorphism is of major importance. It seems that haplotype 1 (GCAG) presence is associated with a particularly high thromboembolic risk [25, 32, 33]. High gluco-
corticoid levels may directly influence the structure of vWF’s multimers. Casonato and colleagues described large vWF multimers in Cushing’s syndrome patients which was connected with factor’s increased activity [7]. It is known vWF plays a dual role in coagulation processes: in primary hemostasis it is necessary for platelet adhesion to injured endothelium of a vessel, while in secondary hemostasis it binds with factor VIII as its carrier and stabilizer [34]. Therefore, elevated vWF activity, which was found in this study, may be a potent hemostasis stimulation factor.

As mentioned earlier, apart from the direct effect of hypercortisolism on coagulation factors’ synthesis, metabolic disorders seem to play an important role in the increase in thrombosis risk among Cushing’s syndrome patients. It is known that obesity and carbohydrate metabolism disorders, hyperlipidemia lead to hemostatic disturbances on various levels. A crucial trigger thereof seems to be insulin resistance – along with secondary hyperinsulinenia – which was shown to increase concentrations of coagulation factors VII, VIII, IX, X, and vW [35-38]. Therefore, the authors of this study selected the control group with a matching BMI to exclude the influence of obesity on examined parameters. Furthermore, in the group of hypercortisolemic patients correlations between BMI, fasting glycemia, total cholesterol and activities of coagulation factors VIII, IX, X, XI, and vW were tested. Despite a significant relationship between vWF activity and total cholesterol concentration in univariate analysis, the metabolic parameters had no significant effect on activities of coagulation factor among hypercortisolemic patients in multivariate analysis. In the current study direct effects of other metabolic alterations on the activities of coagulation factors were not assessed. These were examined by other researchers [35-38]. The study evaluated the end effect of cortisol – both direct and indirect – on chosen coagulation system parameters, which is most significant clinically.

Moreover, we showed a significant relation between examined coagulation parameters and hormonal hypercortisolism indicators: 24-hour urinary cortisol excretion and morning serum cortisol concentration in case of factor IX, as well as cortisol level in the overnight suppression test for factors IX, X, and XI. Activities of coagulation factors correlated positively with these hormonal parameters, which points at a relationship between coagulation and hypercortisolism.

Further, we investigated the above parameters in SCS patients and found that vWF activities were higher in these patients compared to healthy controls. As mentioned earlier, glucocorticoids’ effect on vWF activity has a complex background. In studies so far these hormones were shown to increase not only vWF synthesis by up-regulating its mRNA transcription and overexpression of abnormally large vWF multimers, but also its release from endothelial cells [4, 7, 27]. The fact that vWF is particularly strongly affected by glucocorticoids may be the reason for its increased activity already in SCS patients.

No significant differences versus controls were found for other coagulation factors’ activities. However, a clear tendency toward higher activities of all factors was observed already at this stage of the disease. What is more, in SCS patients significant associations between coagulation factors’ activities and hormonal parameters were also found.

Unfortunately, due to the lack of similar studies, it is impossible to compare our results with other authors. The majority of research performed among these patients examined cardiovascular risk and not hemostasis directly. Both Tauchmanová and Tsuchi with co-workers confirmed a significantly higher cardiovascular risk in SCS patients [16, 39]. In our previous studies we showed increased activity of protein C and its cofactor protein S, which are part of the endogenous anticoagulation system [17]. In the light of current data our conjectures about a secondary nature of this system’s activity increase due to the elevated activity of coagulation factors have been supported.

As mentioned above, our research confirms preceding reports of elevated concentrations and/or activities of chosen coagulation factors in Cushing’s syndrome patients. However, new issues are raised concerning activity of the plasmatic coagulation pathway in subclinical hypercortisolemic patients. Significantly higher mean activities of examined coagulation factors in OCS patients, a tendency toward higher activities in SCS patients as well as significant correlations between these activities and hormonal indicators of hypercortisolism all suggest the notion of an interdependence between hypercortisolism and the activation of coagulation.

Conclusions

In endogenous hypercortisolemic patients, in comparison to healthy controls, mean activities of coagu-
lation factors VIII, IX, X, XI, and vWF are increased, which indirectly indicates activation of the coagulation pathway. The extent of this activation is different in overt and subclinical hypercortisolism.

**Declaration on Conflict of Interests**

Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Disclosure**

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