HORMONES 2012, 11(1):21-30

Review

Pathogenesis of vascular complications in Cushing's syndrome

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ABSTRACT

Chronic exposure to high glucocorticoid levels in Cushing's syndrome (CS) is often associated with alterations in the hemostatic system and the expression of prothrombotic phenotypes. Increased frequency of both atherothrombotic and venous thromboembolic events (VTE) has been reported in patients with CS. In general, cardiovascular complications in these patients cause a five-fold increase in mortality compared to the normal population. Although numerous abnormalities in the hemostatic system have been detected in patients with CS, the underlying mechanisms of the prothrombotic state are not fully elucidated. High levels of factor VIII and von Willebrand factor, with evidence of enhanced thrombin generation and decreased fibrinolytic activity, have been documented in several studies. However, it is not clear to what extent these changes contribute to the shift of hemostatic balance towards the hypercoagulable state and expression of thrombophilic phenotypes. Thrombosis is usually a multicausal disease, and all three components of the so-called Virchow triad, namely 1) vascular abnormalities and endothelial dysfunction, 2) hypercoagulability and 3) stasis, may play a variable role in the pathogenesis of the prothrombotic state in CS patients. Larger studies are needed to establish strategies for prevention of cardiovascular complications in patients with Cushing's syndrome.

Key words: Cushing's syndrome, Hypercoagulability, Vascular complications

INTRODUCTION

Increased cardiovascular morbidity and mortality have been recognized in patients with CS and all contributing factors, including hemostasis, have been extensively investigated. Numerous alterations of coagulation and fibrinolysis parameters have been identified in these patients, but their causal relationship with the onset of clinically overt thrombosis still remains to be confirmed. Detecting abnormalities and screening for individual cardiovascular risk factors is important in assessing the long-term and short-term (perioperative) risk for thromboembolic events in these patients. It is also imperative to recognize that

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Received 11-04-11, Revised 04-06-11, Accepted 10-07-11

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some vascular changes in CS may be irreversible and may persist after successful operative treatment. Changes in the hemostatic system may also persist for some time after successful treatment. Since persistent and recurrent CS is often managed with drugs, their effects on the cardiovascular and hemostatic system also needs to be considered. This review summarizes the current knowledge regarding the effects of cortisol excess on the coagulation-fibrinolysis system and takes into consideration other possible mechanisms that create the prothrombotic state observed in patients with CS.

EPIDEMIOLOGIC DATA RELATING VASCULAR COMPLICATIONS TO CUSHING'S SYNDROME

Cushing's syndrome (CS) is a common condition caused by chronic exposure to excess glucocorticoids either produced endogenously, or more frequently, given exogenously for a wide range of diseases. Endogenous CS may be caused by excess ACTH production (80-85%), usually due to pituitary corticotropinoma or Cushing's disease (CD), less frequently by an extrapituitary ACTH-secreting tumor (ectopic ACTH syndrome), or very rarely by a tumor secreting CRH. CS can also be ACTH-independent (15-20%), resulting from excess secretion of cortisol by unilateral adrenocortical tumors, either benign or malignant, or by bilateral adrenal hyperplasia or dysplasia. [1] While endogenous CS is relatively rare, a remarkably high proportion (0.8-2%) of the general population is on long-term, high-dose glucocorticoid treatment.

Reported incidence of CD ranges from 1.2 to 2.4 per million people per year in newly diagnosed cases,[2,3] with a prevalence of 1.2-5.6% of all pituitary tumors.[4,5] The incidence of other causes of CS is 0.6 per million people per year for adrenal adenoma and 0.2 per million people per year for adrenal carcinoma.[2] Other causes of endogenous CS are rare. It is estimated that 2.5 million patients in the US and over half a million in the UK are under prolonged high-dose glucocorticoid treatment, therefore, cardiovascular and metabolic complications present a serious public health problem.

Few epidemiological studies have attempted to produce detailed morbidity and mortality figures for patients with CS.[2,3,6-9] Reported mortality rates are four times higher in CS than in the normal population, while mortality from cardiovascular diseases is even higher. Myocardial infarction, stroke, congestive heart failure and venous thromboembolic complications appear to be the main causes of mortality, with older age, hypertension and diabetes further contributing to the high mortality rates.[2,8,9] CS is associated with high mortality and morbidity even though biochemical cure rates are between 80% and 90%, as shown in a recent study from New Zealand.[9]

When compared to patients with nonfunctioning pituitary adenomas (NFPA), morbidity and mortality rates in CD are significantly higher and the mean age at death is significantly younger.[6] The reported standardized mortality ratio (SMR) for CD patients in remission was 1.8-3.3, whereas in patients with persistent disease it was much higher at 5.5-10.7.[6,8] Morbidity from cardiovascular diseases remains increased even after long-term remission of the disease.[7] Subjects with previous CD have a significantly greater 3-year incidence of metabolic syndrome, cardiovascular and cerebrovascular disease, suggesting that previous hypercortisolism may predispose these patients to an increased risk of metabolic syndrome and vascular complications in the future.[10] Persistent obesity and inflammatory marker increases have also been reported after long-term treatment for CS, clearly pointing to the long-term adverse consequences of hypercortisolism for increased cardiovascular risk[11].

From the early 1970s an increased risk of both unprovoked and postoperative venous thromboembolism (VTE) has been reported in patients with CS, contributing to the increased cardiovascular morbidity and mortality.[12-22] VTE not provoked by surgical intervention was observed in 1.9% and 2.5% of patients with CS,[19,20] while the risk of postoperative venous thromboembolism varied between 0% and 5.6%.[20,23-25]

In the systematic review, Van Zaane and colleagues analyzed and compared the risk of postoperative VTE in patients with CS with the risk following major surgery in the normal population. They concluded that the risk of postoperative VTE in patients with CS is high and similar to that observed after major orthopedic surgical procedures, neurosurgery, gastrointestinal and urology surgery.[23] Recent guidelines on

treatment of CS and prevention of thrombosis do not address this issue specifically,[1,26] therefore, the authors recommended routine use of thromboprophylaxis in all patients with CS undergoing transsphenoidal or adrenal surgery.[23]

In a Dutch multicentre study, high incidence of VTE was confirmed especially in the postoperative period (within 3 months after surgery) in patients with CD compared to patients operated on for NFPA and adrenal adenoma.[25] These results suggest that patients with CD are at a higher risk for postoperative VTE than patients with cortisol secreting adrenal adenomas and probably need extended anticoagulant treatment in the postoperative period.

CUSHING'S SYNDROME AND INCREASED SUSCEPTIBILITY TO THROMBOSIS: POSSIBLE MECHANISMS INVOLVED

More than 150 years ago the German pathologist Virchow, in his famous triad, identified endothelial damage, changes in the composition of the blood (blood hypercoagulability) and venous stasis as major predisposing factors for thrombosis.[27] Based on Virchow's observations, the classical view for a long time was that arterial thrombosis is mainly associated with endothelial damage, while venous thrombosis is usually considered a consequence of blood stasis or blood hypercoagulability. In recent years this view has changed significantly as it became clear that these two thrombotic complications share many common pathogenic mechanisms. Blood hypercoagulability is now identified as an important factor in the process of atherothrombosis, especially in its final phase, when the thrombus is forming on the ruptured atherosclerotic plaque.[28]

In line with the Virchow triad, it has been shown that chronic glucocorticoid excess influences endothelial function, parameters of coagulation and fibrinolysis and affects blood flow due to polycythemia, obesity or immobility, especially in the postoperative period (Table 1). Therefore, it seems that all three factors from Virchow's triad may be operating in the pathogenesis of increased thrombotic tendency in patients with CS:

1. Vascular abnormalities and endothelial dysfunction
2. Hypercoagulability
3. Stasis and other factors

1. Vascular abnormalities and endothelial dysfunction in Cushing's syndrome

While pronounced catabolic effects of cortisol excess on skeletal muscles are well recognized in Cushing's syndrome, the opposite effects have been observed in the heart and vasculature. Cortisol may induce concentric remodeling, leading to left ventricular hypertrophy and hypertrophic remodeling in small resistance arteries in patients with CS.[29,30] The exposure to increased cortisol *per se* was suggested as being the most relevant determinant of left ventricular and vascular remodeling.[29] Although high blood pressure is a common feature of CS, such vascular remodeling may develop independently of blood pressure levels.

In vasculature both hormonal factors (e.g. angiotensin II, IGF-I, insulin, aldosterone, etc.) and an increased vessel wall stress may trigger the development of smooth muscle cell hypertrophy or hyperplasia. It was previously demonstrated that aldosterone seems to possess growth-promoting and pro-fibrotic properties,[31] and excess aldosterone production is associated

Table 1. Possible mechanisms of the so-called Virchow's triad in the pathogenesis of vascular complications in Cushing's syndrome

Vascular changes	Hypercoagulability	Other
Left ventricular hypertrophy	Shortened aPTT	Stasis
Vascular remodeling (small arteries)	Increased vWF concentration	Polycythemia
Increased stiffness (carotid arteries)	Increased FVIII concentration	
Increased intima-media thickness	Increased PAI-1 activity	
High prevalence of atherosclerotic plaques	Prolonged euglobulin lysis time	
Endothelial dysfunction	Increased thrombin-antithrombin complex	
	Increased fibrinogen concentration	

with remodeling and fibrosis of small vessels.[32] It was recently demonstrated that glucocorticoids may activate rapid mineralocorticoid receptor signaling in vascular smooth muscle cells through MAPK/ERK dependent pathways.[33] This suggests that glucocorticoids may contribute to vascular disease via mineralocorticoid receptor signaling, independent of circulating aldosterone. In patients with CS, the presence of cardiac structural changes and alteration of carotid arteries (increased stiffness and intima-media thickness with high prevalence of atherosclerotic plaques) were demonstrated.[34,35] Carotid artery wall thickness and stiffness remained increased in patients with CS one year after successful treatment. Carotid wall plaques were detected in 32% of CS patients with active disease and 30% of those in remission. These findings suggest that cardiovascular morbidity persists in patients in remission due to irreversible structural changes caused by the growth-promoting effects of glucocorticoid excess on the heart and vasculature.[36]

In CS, endothelium-dependent flow-mediated vasodilatation has been reported to be impaired.[37] Several humoral markers of endothelial dysfunction such as endothelin, homocysteine, vascular endothelial growth factor, osteoprotegerin and cell adhesion molecules are also found to be elevated. These, however, decrease or normalize after successful surgery.[38-40]

2. Hypercoagulability in Cushing's syndrome

Normal hemostasis is characterized by a dynamic equilibrium between procoagulant and anticoagulant components of the hemostatic system. On the other hand, blood hypercoagulability may be considered as a state of hemostatic imbalance and a shift of equilibrium toward thrombogenesis, occurring either because of an increase in procoagulant potential or reduction of antithrombotic capability of the blood. If this imbalance progresses to a critical level, significant generation of thrombin and formation of fibrin in the circulation may occur, leading to onset of clinically overt thrombosis.

From a clinical perspective, it would be extremely useful to have a laboratory tests that accurately indicates presence of the hypercoagulable state and predicts the risk of thrombosis in an individual CS patient. Results of such a test/s should have good correlation with clinical outcomes, as this is confirmed in large studies. In several recent studies various abnormalities of coagulation and fibrinolysis have been reported in patients with CS. These findings were generally translated into conclusions that blood hypercoagulability is a common complication in these patients and that increased coagulability of the blood is the main mechanism contributing to the increased risk of both arterial and venous thrombosis. However, in the majority of these studies investigators focused on determination of a few coagulation or fibrinolysis parameters, changes of which do not mirror the state of complete hemostatic balance and do not prove the presence of overt blood hypercoagulability. In only two studies were coagulation and fibrinolytic parameters studied in relation to the occurrence of thromboembolic events, however, the number of patients with thrombosis was far too small and the results of laboratory tests were not compared between patients with and without thrombosis.[20,24]

2.a. Basic coagulation tests in patients with Cushing's syndrome

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are routine coagulation tests which reflect integrated actions of the majority of coagulation factors in extrinsic and intrinsic pathways of the blood coagulation cascade.

Significantly decreased aPTT values were found in a number of studies on patients with CS,[20,24,41-43] with an inverse correlation between aPTT and urinary free cortisol (UFC) values.[20] Normalization of decreased baseline aPTT values were obtained 3 months after successful surgical treatment, pointing the use of aPTT as a convenient routine hemostatic screening parameter for patients with CS.[41]

On the other hand, increased PT values compared to healthy controls were observed in only two studies.[20,43] More sophisticated global coagulation tests, such as overall hemostatic potential tests, have not been performed in patients with CS so far.

2.b. Fibrinogen

Fibrinogen is an inflammation marker and a well-established risk factor for cardio- and cerebrovascular disease. Fibrinogen mediates the thrombogenic effects of other risk factors such as obesity, smoking, diabetes, total cholesterol, HDL cholesterol and

triglycerides levels, most of which are associated with CS. Fibrinogen may also directly increase the risk of cardiovascular disease due to its role in platelet aggregation, plasma viscosity and fibrin formation. Increased fibrinogen levels have been shown in patients with active CS,[10,20,42,43] but also in patients with adrenal incidentalomas and subclinical Cushing's syndrome[44] and healthy men treated with short-term high dose dexamethasone.[45]

2.c. Platelet count and platelet function tests

Increased platelet counts were recently reported in patients with CS.[43] Investigation of platelet function, including bleeding time, platelet aggregation with ADP, adrenaline, collagen and ristocetin, were normal in patients with CS.[46]

Recently, significant increase in soluble P-selectin levels, a marker of platelet activation, has been demonstrated in healthy men after short-term administration of high dose dexamethasone.[47] P-selectin levels have so far not been investigated in patients with endogenous CS, although the prognostic value of P-selectin has been shown in predicting risk of acute myocardial infarction in hypertensive patients.[48]

2.d. Coagulation factors

The most striking changes in coagulation factors observed in patients with CS are increased levels of von Willebrand factor (vWF) and factor VIII, as reported in several studies.[20,41-44,49-51] Interestingly, in patients with adrenal incidentalomas higher levels of vWF were also observed.[44] A significant increase in FVIII and FXI was noted in healthy men after only 3 days of high dose dexamethasone administration,[45] this supporting the strong influence of glucocorticoids on vWF and FVIII levels.

Factor VIII levels above 160% are associated with an estimated four times increased risk of venous thromboembolism (VTE). vWF is produced and stored in the endothelial cells. When released, it mediates platelet aggregation and adhesion. Numerous clinical and experimental reports suggest that high vWF levels reflect damage to the endothelium and endothelial dysfunction. Higher vWF concentrations have been found in hypertensive compared to normotensive patients with CD.[49] Interestingly, a high prevalence of polymorphisms in the vWF gene (glucocorticoid responsive promoter region) were found to be associated with high vWF levels found in patients with CS compared to controls,[52] as well as an altered molecular organization of plasma vWF as indicated by the presence of unusually large "sticky" multimers.[50] *In vitro* studies support the clinical findings as treatment of endothelial cells with glucocorticoids induced a pro-adhesive environment, increasing production of cell adhesion molecules, tissue factor and vWF.[53]

Increased vWF, FVIII and shortened aPTT values seem to persist in the first 3 months after curative surgery for CS.[20,41,54] Boscaro and colleagues reported that implementation of anticoagulant treatment along with monitoring of coagulation factors in this vulnerable period resulted in a significant (3-fold) decrease in VTE incidence in the treated group of patients with CS compared to those patients with CS who did not receive anticoagulant treatment.[20]

2.e. Inherited thrombophilia

Several inherited conditions are associated with increased risk of venous thrombosis. Hereditary deficiency of antithrombin causes strong thrombophillia because of impaired neutralization of thrombin, while deficiency of protein C and protein S, and presence of FV Leiden or FII 20210A mutation leads to failure to control the thrombin generation. An inherited thrombophilic condition may be identified in up to 50% of patients presenting with venous thromboemolism.[55] In the general Caucasian population, prevalence of FV Leiden and FII 20210A is about 4.8% and 2.7%, respectively, while prevalence of deficiency of antithrombin, protein C or protein S is much lower. Equally high prevalence of thrombophilic mutations could be expected in CS patients, but this has not been investigated so far. Hormonal status can influence clinical expression of inherited thrombophilic mutations. Use of estrogen-containing contraceptives significantly increases thrombotic risk in carriers of thrombophilic mutations. The modifying effect of glucocorticoid excess on expression of thrombophilic mutations in light of other changes in the hemostatic system remains to be determined.

2.f. Fibrinolytic factors and activity

Decreased fibrinolytic activity in patients with CS is accompanied by increased levels of natural

fibrinolytic inhibitors and prolonged euglobuline cloth lysis time.[7,20,40,41,49]

Increased plasminogen activator inhibitor type 1 (PAI-1) activity and antigen concentrations were described.[7,20,42,43,52,56] Interestingly, prolonged euglobulin lysis time with less shortening after the venous occlusion test indicating abnormal fibrinolysis was not attributed to an impaired tissue plasminogen activator (t-PA) release but was rather the consequence of a persistent significant increase in PAI-1 activity after venous occlusion.[56] Underlying mechanisms for increased PAI-1 activity and impaired fibrinolysis in CS are both related to adverse metabolic changes, associated endothelial dysfunction and the direct action of glucocorticoids on the endothelium and liver.[56] *In vitro* glucocorticoids can reduce fibrinolytic activity by suppressing the secretion of t-PA by endothelial cells or by inducing the release of PAI-1.[57,58] The putative mechanisms by which glucocorticoids may influence these hemostatic parameters are ill-defined. It is most likely that they do so by glucocorticoid-receptor mediated up-regulation of gene transcription.[58-65]

3. Stasis and hyperviscosity in patients with Cushing's syndrome

Some patients with CS present clinically with polycythemia.[66] Increased hematocrit and secondary polycythemia may play an additional role in the pathogenesis of "hyperviscosity syndrome", predisposing to thrombotic complications. Disturbed blood flow and venous stasis are not rare in patients with CS due to decreased mobility, especially during immobilization after surgical procedures. Venous thrombi usually occur at regions of slow blood flow and often begin as small deposits in large venous sinuses in the calf, in the valve cusp pockets of the deep veins of the calf or thigh or in the parts of veins exposed to external compression. The simultaneous presence of prothrombotic conditions emphasizes the role of venous stasis for onset of thromboembolic complications. Indeed, clinical experience shows a significant proportion of venous thrombosis in patients with CS occurs peri-operatively or during immobilization, when stasis is superposed on prothrombotic alterations of the hemostatic system. Therefore, early mobilization after surgery, elastic stockings and other procedures intended to reduce venous stasis are important for prevention of thromboembolic events in patients with CS.

In the general population, previous venous thrombosis, advanced age and immobility are important independent risk factors for VTE. Patients with two or more episodes of VTE carry a very high risk of further recurrences if they are not adequately treated.[67]

4. Markers for predicting the occurrence of a thromboembolic event in patients with Cushing's syndrome

Blood hypercoagulability may be detected biochemically prior to the occurrence of a thromboembolic event. In order to identify individuals at risk who need appropriate therapy, sensitive laboratory methods have been developed for measuring peptides, enzyme-inhibitory complexes and enzymes that are liberated during activation of coagulation or fibrinolytic systems *in vivo*. These substances are usually termed hemostatic activation markers and their concentrations reflect the level of hemostatic system activation. The most commonly used markers of *in vivo* thrombin generation are thrombin-antithrombin (TAT) complexes, prothrombin fragment 1+2 and fibrinopeptide A. Activation of the fibrinolytic system may be quantitatively assessed by measurement of concentration of markers such as plasmin-α_2 antiplasmin (PAP) complexes or D-dimer (Figure 1).

Another approach is the test of global hemostatic capacity, named the endogenous thrombin potential (ETP). This is based on continuous registration, plotted into a curve, of thrombin generation *in vitro* when an appropriate procoagulant stimulus is applied.[68] However, performance of ETP is not completely standardized and there is no published data about ETP values in patients with CS. It has been shown that only extreme changes in the level of individual components of the coagulation system will affect standard coagulation assays, while even modest changes of these factors significantly affect the thrombin-generating capacity to an extent that may be expected to have clinical consequences.[69]

The levels of activation markers in patients with CS have been investigated in only a few studies. Increased thrombin-antithrombin (TAT) complexes were reported in patients with CD.[24,49] This complex is produced during inactivation of thrombin by

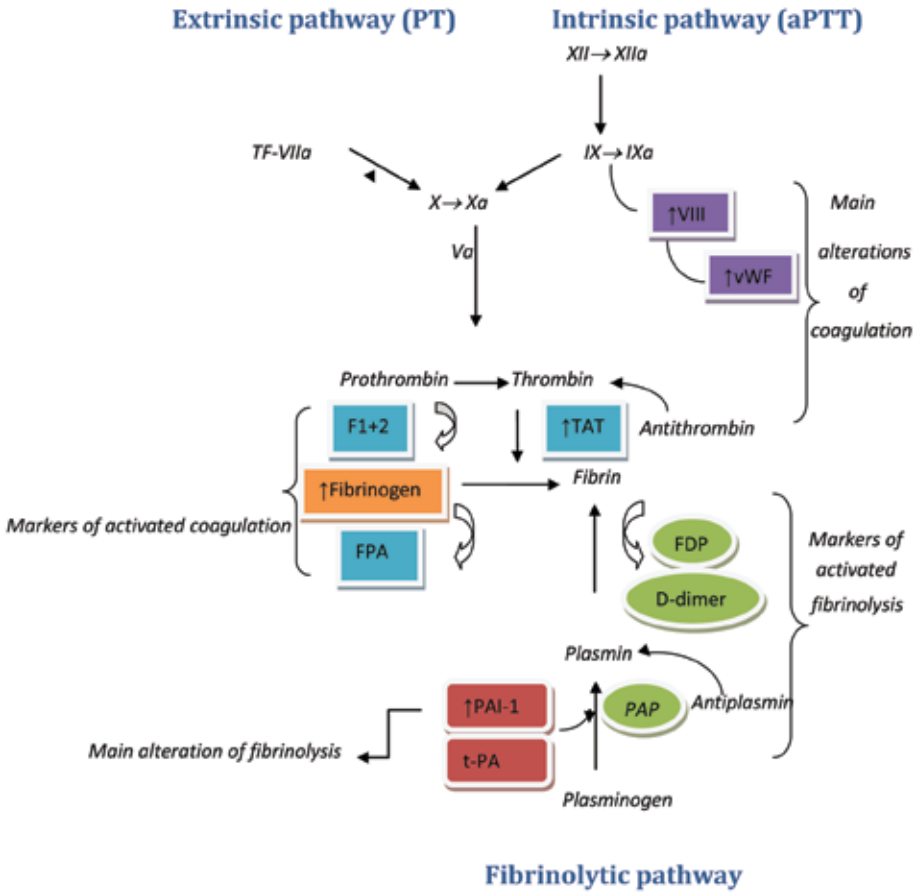

Figure 1. Main alterations in the hemostatic system and markers of activated coagulation and fibrinolysis in Cushing's syndrome: prothrombin fragment 1+2 (F1+2); thrombin-antithrombin complex (TAT), plasmin-antiplasmin complex (PAP), Fibrinopeptide A (FPA), Fibrin degradation products (FDP) and D-dimer.

antithrombin and an increased concentration is a reliable marker of enhanced thrombin generation. Interestingly, increased TAT complexes were accompanied by a vWF antigen increase, indicating simultaneous damage of endothelial cells.[49] This finding illustrates the complexity of mechanisms underlying the tendency to thrombosis in patients with CS. A study on a canine model of CS also reported an increase of TAT complexes,[70] clearly indicating a shift of the hemostatic balance in the direction of thrombosis. However, D-dimer concentrations were not significantly different between patients with active and cured CS.[24] It should also be appreciated that the levels of activation markers are influenced by factors other than thrombin generation, such as clearance times and by fibrinolytic activity. The influence of venous stasis, alterations of endothelial cells, effects of metabolic factors or drug therapy on thrombotic risk cannot be completely mirrored *in vitro*.

CONCLUDING REMARKS

Glucocorticoid excess is associated with significant morbidity and mortality rates, especially from cardiovascular causes. Clustering of cardiovascular risk factors like obesity, diabetes, hypertension and dyslipidemia which may *per se* induce hypercoagulability is common in CS and may be potentiated by hypercortisolism. Irreversible vascular, metabolic and body composition changes may persist years after cure, predisposing these patients to future vascular complications. Hemostatic abnormalities and hypercoagulability also seem to persist after surgical treatment in these patients. Despite results of previous studies, which investigated coagulation alterations in CS, it is unlikely that determination

of one or several hemostatic parameters will enable accurate assessment of thrombotic risk. Application of global hemostatic tests such as ETP, which reflect integrated activity of the coagulation network, could provide better insight into hemostatic balance in these patients, but prospective studies are needed. Until then, assessment of thrombotic risk and decision regarding antithrombotic prophylaxis in CS patients should be based both on careful history taking to cover all clinical risk factors (age, obesity, hypertension, diabetes/impaired glucose tolerance, dyslipidemia, smoking, previous vascular complications, immobility, malignancy, pregnancy and drugs) and results of coagulation testing (PT, aPTT, fibrinogen, D-dimer, FVIII) (Table 2). In the future, more randomized controlled trials are needed to investigate the efficacy of anticoagulant treatment for prevention of postoperative VTE in patients with CS.

Table 2. Individual vascular risk assessment

Clinical risk factors (see text above)
Severity of hypercorticism (clinical and biochemical evaluation)
Clinical examination of the cardiovascular system
Hematology and coagulation parameters (full blood count, aPTT, PT, fibrinogen, FVIII and D-dimer)
ECG
Echocardiography and Doppler studies

Key issues

1. CS is associated with increased cardiovascular morbidity and mortality due to clustering of cardiovascular risk factors including hypercoagulability. If present, vascular structural changes, due to glucocorticoid excess, may be irreversible predisposing to persistently increased cardiovascular risk in these patients.
2. The mechanisms of blood hypercoagulability in CS include high levels of factor VIII and von Willebrand factor, evidence of enhanced thrombin generation and decreased fibrinolytic activity. These changes may persist for months after curative surgery.
3. Risk of unprovoked and post-operative VTE is increased in CS, especially in CD. Guidelines for thromboprophylaxis in patients undergoing curative surgery are as yet lacking.
4. Further studies are needed to establish protocols for cardiovascular risk evaluation and protection in these patients.

ACKNOWLEDGMENTS

This work was supported by the Serbian Ministry of Science (project number 175033).

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