

HORMONAL MECHANISMS IN THROMBOSIS

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Abstract

A great number of studies confirm the fact that haemostatic anomalies occur rather often in endocrine diseases. Multiple endocrine and metabolic disorders can alter the haemostatic balance and favour thrombotic risk, with stroke being the most frequent and feared clinical manifestation. Hormonal factors are important, especially in arterial thrombosis, and, to a lesser extent, in venous thrombosis. This process causes ischaemic lesions, with the most severe clinical manifestations being stroke and myocardial ischaemia.

Keywords: thrombotic risk, ischaemic stroke, sex differences, endocrine diseases

Abstract

O multitudine de studii confirmă faptul că anomaliile hemostatice nu sunt rare în bolile endocrine. Numeroase tulburări endocrine și metabolice pot altera echilibrul hemostatic și favorizează riscul trombotic, cu cea mai frecventă și de temut manifestare clinică și anume accidentul vascular cerebral. Factorii hormonalți sunt importanți mai ales în tromboza arterială și, în mai mică măsură, în tromboza venoasă. Acest proces produce leziuni ischemice, iar manifestările clinice cele mai severe sunt accidentul vascular cerebral și ischemia cardiacă.

Cuvinte cheie: risc trombotic, accident vascular cerebral ischemic, diferențe de sex, boli endocrine.



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The knowledge of many risk factors of thrombosis has currently led to a concept according to which thrombosis is a multigenic and multicausal disease, which develops in the presence of the interaction of several causal factors.

Thrombus formation occurs when the normal balance between thrombogenesis and thrombolysis is disrupted, with the involvement of several vascular and haemostatic mechanisms.

Arterial thrombosis usually occurs after the erosion or rupture of an atherosclerotic plaque, platelets contributing to the thrombus formation.

This process causes ischaemic lesions, with the most severe clinical manifestations being myocardial ischaemia and stroke. Ischaemia can have a slow onset, through the progression of the atherosclerotic disease (stable angina, claudication), or acute, in the case of vascular (atherosclerotic plaque rupture) or intracardiac (atrial fibrillation, mechanical valvular prostheses) thrombus embolization.

Venous thromboembolism (VTE) is the most frequent vascular disease after myocardial infarction and stroke. VTE can manifest clinically through deep venous thrombosis and pulmonary embolism, which often constitutes a clinical presentation in which pulmonary embolism occurs after deep vein thrombosis. Pathogenic mechanisms

involved in VTE are only partly known, but it is unanimously agreed that stasis and hypercoagulability are key VTE causal factors, compared to the essential role of the endothelial injury in atherothrombosis. Venous thrombi are formed especially of fibrin and red blood cells, and less of platelets. However, platelets have an important role in primary haemostasis, endothelial injury, and development of atherosclerosis; likewise, inflammation, lipids, smoking, diabetes, hormonal factors and the immune system are major factors in arterial thrombosis, and, to a lesser extent, in venous thrombosis; pregnancy, oral contraceptives and hormone therapy, metabolic syndrome, surgery, and cancer increase the risk of venous thrombosis.

Multiple endocrine and metabolic disorders can alter the haemostatic balance and favour either thrombotic risk or bleeding in patients. There is available evidence on the influence of pituitary, adrenal and parathyroid hormones on the coagulation and the fibrinolytic system. Also, there is data regarding the possible procoagulant effect in hyperprolactinemia, growth hormones excess or deficiency, pheochromocytoma, hyperparathyroidism, hyperaldosteronism^(1,2).

Hypercortisolism is associated with a state of hypercoagulability, increasing the risk of thromboembolic events, manifested by the shortening of the activated partial

thromboplastin time. A great number of studies show the obvious clinical consequences of the imbalance between coagulation and fibrinolysis, accompanied by thrombotic events in endogenous Cushing syndrome, or in exogenous hypercortisolism. On the other hand, adrenal insufficiency is recognized as the most frequent endocrine disorder associated with antiphospholipid syndrome (APS); hypopituitarism is also associated with APS.

Fibrinogen production increases under the influence of glucocorticoids, both *in vitro* and *in vivo*. There is also an elevation in homocysteine levels. The fibrinolytic system is affected under the action of corticosteroids, by blocking the plasminogen tissuer activator (PAI) production, and the lowering of antithrombin III activity. Furthermore, glucocorticoids impact the thrombosis-fibrinolysis balance by limiting the amount of arachidonic acid available for prostacyclin synthesis in the vascular wall. They inhibit A2 phospholipase under the action of lipocortin, a protein produced through DNA transcription. Thus, the formation of cyclooxygenase and lipooxygenase system products, such as prostaglandins, prostacyclins, leukotrienes, and thromboxanes, is inhibited. At therapeutic doses, glucocorticoids inhibit the synthesis of prostacyclin more than that of thromboxane: the relative deficit of prostacyclin allows the dominant effect of platelet thromboxanes in the vascular endothelium, favouring vasoconstriction, thrombus formation, platelet growth factors release, which stimulate the cellular proliferation and atheroma formation. Association of anti-platelet agents (e.g., aspirin) could compete with glucocorticoids to restore the balance.

Type 1 diabetes mellitus has adverse effects on coagulation and fibrinolysis. Current data indicate that type 1 diabetes

mellitus is associated with a pro-inflammatory and procoagulant status which results from the increase in platelet adhesion, the activation of the coagulation system, and the decrease in the plasmatic fibrinolytic capacity.

An increase in aldosterone level correlates with an elevated risk for thrombotic cardiovascular events. There are studies showing the acute effects of aldosterone on platelets, coagulation, and fibrinolysis, increasing the risk of thrombosis. Acute administration of aldosterone in mice increases the density of fibrin in thrombi, accumulation of platelets at the site of experimental endothelial injury, platelet aggregation and reduces fibrinolysis. These effects are partially reduced by eplerenone. Experimentally, aldosterone increases the risk of thrombosis in the adrenalectomized animal, and this effect is enhanced in the diabetic animal; aldosterone increases the plasma level of the tissuer factor (TF) and of the plasminogen activator inhibitor (PAI), lowers the plasma level of nitric oxide metabolites and increases the oxidative stress. TF is an essential initiator of aldosterone-induced thrombogenesis. On the other hand, arterial thrombosis favoured by aldosterone was correlated to a decrease in fibrinolysis, expressed by the increasing PAI-1 plasma levels, such levels being reduced after blocking MR and GR. These effects are amplified when diabetes is associated, and current experimental data support the synergic action of aldosterone with endothelial injury and oxidative stress observed in diabetes, leading to an enhanced prothrombotic effect.

Many clinical data indicate increased coagulation and risk of thrombosis in **hyperthyroidism**. The procoagulant effect of T₄ implies platelet activation and favouring



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platelet aggregation, cytokine and chemokine cell production with increasing platelet aggregation and adhesion, endothelial dysfunction, and activated platelet interaction with the endothelial cells involved in the early stages of local thrombus production^(3,4,5,6,7).

Hypothyroidism can increase the risk of thromboembolism or create a bleeding tendency, and even severe haemorrhagic disorders^(9,10,11,12). Hypercoagulation tendency and the risk of thromboembolism have been reported not only in manifest thyroid dysfunction, but in subclinical forms as well. Subclinical hypothyroidism has been associated with the risk of thrombosis in recent studies in patients with acute coronary syndrome, or with deep vein thrombosis. In these cases, the participation of the interaction between platelets and endothelial cells comes into the discussion (endothelial dysfunction with alteration of endothelial nitric oxide synthesis).

Thromboses associated with neoplastic disease are being intensely studied. Numerous observations show the contribution of platelets in these cases and the relationship between platelet function and the level of thyroid hormones. Some studies support even the contribution of thyroid hormones in the pathogenesis of thromboses in cancer patients. Thyroid hormones are also being studied in relation to

the risk of cerebral thrombosis in general and in cerebral tumours (especially glioblastoma) in particular. Therefore, it has been proposed to evaluate the patients with neoplastic disease for the elevated risk of coagulation; this protocol should include measuring of free-T₄, aPTT and the production of D-dimers or other coagulation risk elevation indicators^(13,14,15,16,17,18).

Sex differences in thromboses

Female hormones have several side-effects, of which thromboses are the most frequent and the most important. Sex differences in terms of risk of thrombosis are obvious when analysing the risk of stroke, while the role of oestrogen in acute neuroprotection against thrombosis has been studied in experimental stroke models.

Current data are contradictory and show the prothrombotic effect of oestrogens; thus, premenopausal woman with an increased level of oestrogens has a reduced incidence of stroke compared to postmenopausal woman. Premenopausal women seem to be protected against stroke, compared to men, who have a reduced protection against stroke. This difference is reduced or even reversed in postmenopausal women, when the levels of sex hormones, especially oestrogens, are lower. Many studies show that a low level of testosterone in older men is



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a risk factor for atherosclerosis and cardiovascular disease, but it has not been proven that the administration of androgens would have an effect on coagulation or cardiovascular disease complication prophylaxis⁽⁸⁾.

Nonetheless, differences in stroke caused by sexual dimorphism are not completely explained, due to the complex relationship between age and sex. It is not clear whether sex differences in stroke are due to only biological and hormonal particularities.

These particularities have been correlated with the dominant oestrogen form, namely: the predominant oestrogen form before menopause is 17 β -estradiol (E2), which has a high binding affinity to oestrogen receptors compared with other oestrogens. However, E2 level is significantly reduced after menopause, while the incidence of ischaemic stroke increases.

Additionally, the role of oestrogens in haemostasis is closely related to platelets and is mediated by the α - and β -oestrogen receptors, with effects on platelet aggregation. Sex differences have also been demonstrated in the mechanisms of thrombogenesis. The procoagulant effect of oestrogen treatment is being studied, which is associated with increasing levels of factors II, VII, VIII, X, and fibrinogen, and decreasing levels of factor V, as well as the plasma level of endogenous inhibitors of thrombosis, such

as tPA and antithrombin. Recent studies suggest that oestrogen contributes to the procoagulant status via the activation of the platelet surface receptor through the vWF pathway.

Von Willebrand factor (vWF) has an important role in platelet adhesion and activation, and this factor is regulated by oestrogens, together with other hormones. Through its action on platelets, vWF contributes to the amplification and propagation of thrombosis. Oestrogens are associated with elevated vWF levels, and thus, hormone therapy would favour pathologic thrombosis and risk of ischaemic stroke. Current studies are related to the role of vWF in blood clot formation and the ability to target this pathway as a therapeutic alternative to therapeutic thrombolysis with alteplase (a recombinant tissue plasminogen activator, rtPA), currently approved. Therefore, additional studies are needed to clarify the relationship between age, female sex steroids, and thrombosis^(19,20,21,22).

Hormone therapy and risk of venous thromboembolism

The most well-known clinical manifestation of thrombotic risk of hormonal cause is that related to oestrogenic hormone therapy. Hormone therapy (HT) includes hormonal contraception (HC), post-menopause hormone therapy (PHT), and selective

oestrogen receptor modulators (SERMs), or oestrogenic agonists and oestrogenic antagonists. Retrospective and prospective studies showed a two- to four-fold increase of relative risk for VTE (venous thromboembolism) when using HC, PHT, or SERM.

Hormone therapy produces changes in the coagulation system, as well as in inflammation markers or lipids. The most important effects are elevation of procoagulant factors FVII, FVIII, FIX, FX, FXII, FXIII, fibrinogen, tPA, von Willebrand factor (vWF) with an important role in platelet adhesion and activation. Additionally, hormone therapy reduces the level of anticoagulant factors - protein S and antithrombin. These changes cause a haemostatic imbalance and a pro-thrombotic status.

Hundreds of millions of women worldwide use oral contraceptives or hormone replacement therapy. The use of oral contraceptives is associated with an increased risk of venous thrombosis, myocardial infarction, stroke, and peripheral artery disease, the risk being higher in the first year of use. The risk of venous thrombosis is increased in women with coagulation abnormalities, obesity, a history of venous thrombosis, current pregnancy; arterial thrombosis is especially favoured by atherosclerotic lesions of the arterial wall, high blood pressure, diabetes mellitus, hypercholesterolemia; older age and smoking increase the risk for both types of thrombosis. The risk is also increased in women with coagulation abnormalities, a history of thrombosis, obesity, and depending on the type of product used; products containing third-generation progestins (desogestrel, gestodene) carry a higher risk of thrombosis compared to those containing second-generation progestins (levonorgestrel)^(24,25,26). Therefore, the indication for HC depends on the benefit/risk ratio of administration, as well

as on the associated risk factors that include the differences in the thrombotic potential of various products, the oral or transdermic method of administration, the personal or family history of VTE. Additionally, the presence of factors such as obesity, surgery, anaesthesia, immobility, smoking, and varicose vein disease, increases the risk of VTE.

Post-menopause hormone therapy (PHT) generally contains an oestrogen and progestin, or oestrogen only, and is given to women who have undergone hysterectomy. Numerous studies show a significant increase in the risk of VTE in the case of PHT use, such as an increase in risk depending on the method of administration (especially oral), duration of treatment, the association of obesity, or of genetic disorders causing hypercoagulation (factor V Leiden mutation).

Thrombotic risk mechanisms in PHT are similar to those in HC, namely, the elevation of procoagulant factors fibrinogen, VII, X, XII, and XIII, and the decrease of anticoagulant factors, such as protein S and antithrombin, resulting in a procoagulant status unbalanced by the fibrinolytic activity^(27,28,29,30).

SERM (oestrogen agonists-oestrogen antagonists): tamoxifen (used in the treatment and prevention of breast cancer), raloxifene (used in the prevention and treatment of osteoporosis), and others, have also the effects on coagulation previously known for oestrogens, with a risk of VTE, as it is the case for PHT.

RAAS and the fibrinolytic balance

Vascular toxicity mechanisms associated with RAAS activation are linked especially to the effects of angiotensin II (At-II) and aldosterone on the balance of coagulation and fibrinolysis.



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Both angiotensin II and aldosterone increase the PAI-1 (plasminogen activator inhibitor-1) expression, the major physiologic inhibitor of fibrinolysis *in vivo*. Aldosterone increases the PAI-1 expression in the vascular smooth muscle cells, endothelial cells, and monocytes, while MR receptor antagonists influence the concentration of PAI-1 and enhance the fibrinolytic balance.

Furthermore, the activation of RAAS in humans, caused by Na depletion or diuretics (hydrochlorothiazide), contributes to the increase in plasma concentration of PAI-1^(31,32,33,34).

Pro-thrombotic effects of aldosterone

Pro-thrombotic effects of aldosterone have been proved in both its long-term, chronic increases, and in short-term increases, and are correlated with changes in haemostasis linked to changes in endothelial activity, increasing platelet aggregation, reduction of fibrinolysis through the increase of PAI-1 (plasminogen activator inhibitor), and of TAFI (thrombin activatable fibrinolysis inhibitor), and the decrease of t-PA (tissue plasminogen activator); additionally, acute administration of aldosterone in mice increases the fibrin density in thrombi, accumulation of platelets at the experimental endothelial injury site, platelet aggregation, and reduces fibrinolysis. Some of these effects are

mediated by ANG-II and are mitigated by a blocker of AT₁ receptors and eplerenone.

The increase of plasma aldosterone during venous stasis could have a role in haemostasis disorders encountered in prolonged immobilisation, congestive heart failure, obesity. The plasma level of aldosterone also increases significantly during and after surgeries, in relation to the electrolytic imbalances and RAAS changes. This is the reason for suggesting that thrombotic events occurring in relation to surgeries would be a consequence of "operatory hyperaldosteronism". On the other hand, aldosterone secretion has a circadian rhythm, with a major peak in the morning; the morning increase in aldosterone correlates with both morning BP peaks and haemostatic changes. Therefore, the existence of a correlation between the circadian variations of aldosterone, the variations in thrombotic disorders and the acute cardiovascular events, has been suggested^(35,36,37).

Conclusions

Numerous endocrine dysfunctions are associated with haemostatic imbalances, especially with procoagulant effects and thrombogenic risk. A great number of studies show the obvious clinical consequences of the imbalance between coagulation and fibrinolysis, accompanied by thrombotic

events in endogenous Cushing syndrome, or in exogenous hypercortisolism, hyper- and hypothyroidism. There is also data regarding the possible procoagulant effect in hyperprolactinemia, growth hormones excess or deficiency, pheochromocytoma, hyperparathyroidism, hyperaldosteronism. However, experimental data suggests the favourable effect of MR antagonists in the decrease of thrombotic complications studied in diabetes, heart failure. Therefore, endocrinologists should be advised about the possibility that their patients could exhibit haemorrhagic or thrombotic complications at the time of the diagnosis or during the course of the disease. On the other hand, some presentations with haemorrhagic or thrombotic complications in other specialities could be associated with endocrine disorders. Sex differences in thrombosis are an intensely studied reality, especially linked to stroke, one of the most frequent clinical manifestations, the differentiated response to treatment being studied in parallel. Oestrogen medication is associated with haemostatic balance changes and contributes to an increase in the risk of developing venous thromboembolism. In these cases, the risk is dependent on the dose and the type of medication, increases with age, the genetic or acquired predisposition to thrombosis and the method of administration. Oral contraception is contraindicated not only in women with any kind of thrombophilia, but also in those with a history of thrombotic events, history family of venous thromboembolism or thrombophilia. Hormone replacement therapy has limited indications, as the risk of venous thromboembolism, cardiovascular disease or breast cancer counterbalances the benefits of the reduction of osteoporosis and the prevention of colorectal cancer. Therefore, prescribing any kind of hormone therapy depends on the

benefit of the treatment for the patient, after any additional risk factor has been considered. Risk factors include the differences in thrombotic potential for various products, the oral or transdermal method of administration, personal and family history regarding venous thromboembolism. The presence of additional risk factors, such as immobilisation, obesity, surgery, anaesthesia, varicose veins, all increase the risk of venous thromboembolism. Renin-angiotensin-aldosterone system (RAAS) activation is associated with an increased cardiovascular risk, independent of the BP, while blocking the RAAS through ACE inhibitors improves the fibrinolytic balance and reduces cardiovascular mortality.

In perspective, additional studies are needed to clarify the relationship between age, female sex steroids, and thrombosis. A better understanding of sex-related thrombogenesis has led to the study of new compounds that are efficient in thrombolysis. Therefore, there are studies regarding compounds that interfere with vWF in thrombus formation and the possibility of alternative ways for thrombolysis. Subsequent studies are needed to explore new strategies for the treatment of patients at high risk for cardiovascular events or thrombotic disorders.

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