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REVIEW

HEREDITARY THROMBOPHILIA - RISK FACTOR FOR OBSTETRIC COMPLICATIONS

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Summary

Hereditary thrombophilias are abnormalities associated with a predisposition to thrombotic events, depending on the type of thrombophilia or on the presence of exogenous factors increasing the thrombotic risk. Their detection has become an important diagnosis and treatment issue due to laboratory techniques breakthroughs. Hereditary thrombophilia enhances the hypercoagulation trend due to the genetic abnormalities that induce coagulation-fibrinolysis system dysfunction by natural anticoagulants (Protein C, Protein S, Antithrombin III) deficiencies. A significant clinical sign of the thrombophilic status is the occurrence of recurrent miscarriages, and also the occurrence of other pregnancy-related complications such as preeclampsia or premature birth. Knowing the hemostatic and genetic abnormalities of this category of patients provides the theoretical grounds required to improve hereditary thrombophilia management, which conduct should aim at a fast accurate diagnosis setting and to the establishment of appropriate treatment.
Key words: Hereditary thrombophilia, Prothrombin G 20210A mutation, Protein S deficiency, Recurrent miscarriages

Résumé

Les thrombophilie héréditaire - facteur de risque pour les complications obstétricales

Les thrombophilies héréditaires sont des anomalies associées à une prédisposition à événements thrombotiques, en fonction du type de thrombophilie ou la présence de facteurs exogènes qui augmentent le risque de thrombose. Leur détection est devenue une question importante pour le diagnostic et le traitement en raison de techniques de laboratoire avancées. La thrombophilie héréditaire accroît la tendance de hypercoagulation en raison des anomalies génétiques qui induisent le dysfonctionnement du système coagulation - fibrinolyse par la déficience d' anticoagulants naturels (protéine C, protéine S , l'antithrombine III). Un signe clinique significatif de l'état thrombophilique est l'apparition d' avortements spontanés récurrents, et aussi l' apparition d' autres complications liées à la grossesse telles que la prééclampsie ou une naissance prématurée. La connaissances des anomalies hémostatiques et génétiques de cette catégorie de patients fournit les bases théoriques nécessaires pour améliorer la gestion de la thrombophilie héréditaire, une conduite qui devrait viser l'établissement rapide d'un diagnostic précis et l'institution d'un traitement approprié.
Mots clés: Thrombophilie héréditaires, Prothrombine 20210A G mutation, Déficit en protéine S, avortements récurrents.

Thrombophilias include congenital abnormalities predisposing to strong coagulation leading to arterial and venous thrombotic phenomena. (1) Congenital thrombophilia enhances the hypercoagulation trend due to the genetic abnormalities that induce coagulation-fibrinolysis system dysfunction by: natural anticoagulants deficiencies – antithrombin III (AT III), Protein C (PC), Protein S (PS) deficiency; excessive procoagulant factors (Prothrombin G20210A mutation) and anticoagulant factors (Factor V Leiden) efficiency decrease; endothelial dysfunction and complex disorders in the coagulation-fibrinolysis system (Hyperhomocysteinemia). The importance of hereditary thrombophilia in determining thrombotic risks is supported by their identification in a high number of pregnant patients suffering from intraplacental thrombotic phenomena. (2)

Methylenetetrahydrofolate reductase

Methylenetetrahydrofolate reductase is an enzyme that catalyzes 5,10-methylentetrahydrofolate reduction to 5-

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methylentetrahydrofolate, a homocysteine remethylation to methionine cofactor.(3) An enzymatic variant, called "thermolabile" due to its in vitro thermal instability, associated with a high risk of coronary disease, was described in 1998. This variant is the result of a punctiform mutation in the MTHFR (C677T) gene and is 20% less efficient than in homocysteine metabolism, which may lead to hyperhomocysteinemia, especially in individuals with folate deficiency. (3,4) An 11% incidence rate was reported in the Caucasian population of the homozygous status for this mutation associated with a high risk of hyperhomocysteinemia and pregnancy complications which include chromosomal aberrations, congenital malformations, recurrent miscarriages, placenta conditions and preeclampsia. (3,5) Moreover, in addition to the consequences on the embryo or fetus at the beginning of the pregnancy, hyperhomocysteinemia and homozygote status for C677T mutation are involved in thromboembolic phenomena occurring late in the pregnancy and even during post-partum. (5) Another rather common mutation in the general population – A1298C – was also described in the MTHFR gene. This mutation is not associated with hyperhomocysteinemia (regardless of the heterozygote or homozygote status), but the combined heterozygote status for the 2 MTHFR mutations may generate clinical signs similar to those induced by the homozygote status for C677T mutation.

Also, some studies have shown that the homozygote status for the C677T mutation causes a 2-3 times higher risk of neural tube defects, such as spina bifida and anencephaly, than in people not suffering this mutation, whereas the combined heterozygote status for C677T and A1298C is also a risk factor for neural tube defects.(4) The mechanism by which MTHFR gene mutations cause pregnancy complications has not been fully clarified. One hypothesis would be related to the associated hyperhomocysteinemia, which leads to vascular endothelium impairment, which in its turn induces procoagulant activity by tissue factor expression increase and heparan sulfate expression decrease; the hypercoagulant action of homocysteine may be accounted for by thrombin formation enhancement due to the increase of the factor XII level and factor V activity, by inhibiting factor V and thrombomodulin expression. All these mechanisms will lead to venous thromboembolism and placental insufficiency. Nonetheless, there have been reported cases of recurrence miscarriages associated with a homozygote status for C677T mutation, in which the homocysteine levels did not exceed the normal limits, which suggests the existence of an independent hyperhomocysteinemia mechanism.(3) The only study published on the results of the therapy of recurrent miscarriage and thrombophilia patients (12% with MTHFR mutation) reported a 94% success rate of term pregnancies in patients having undergone prophylactic anticoagulant therapy (small molecular weight heparin), which justifies thrombophilic defect testing of this category of patients. (6)

Factor V Leiden

Factor V Leiden described by Dahlbach et al. in 1993 occurs after adenine substitution by guanine in nucleotide 1691 of the Factor V gene followed by arginine substitution by glutamine in position 506 of the protein structure of Factor V (Gln506Arg). Factor V Leiden is involved in hemostasis by both procoagulant and anticoagulant activity. The procoagulant action is achieved by the activated Factor V (FVa) – essential cofactor of Factor Xa in thrombin generation. When Factor V Leiden is present, Arg substitution by Gln in position 506 slows 10 times the proteolytic inactivation of Factor V Leiden and mutant Factorul Va. Factor V Leiden proteolysis decrease followed by the occurrence of a small number of Factor V pieces reduce anticoagulant involvement by diminishing Factor VIIIa proteolysis, whereas the mutant Factor Va proteolytic inactivation decrease and the preservation of its properties as a FXa cofactor in thrombin generation enhance its procoagulant effects.(7) As compared to the general population, the thrombotic risk is 5-10 times higher for Factor V Leiden heterozygotes. The association between FV Leiden heterozygostism and hereditary quantitative deficiency of Factor V increases the thrombotic risk and causes higher resistance to activated protein C. The alleles HR2 of Factor V encode the linkage between several types of polymorphism of the Factor V gene, with the decrease of the cofactor activity of Factor V in the inactivation by activated protein C of Factor VIIIa. The haplotype HR2 of Factor V with an 8-10% incidence rate in the general population carries a moderate thrombotic risk when it is isolated and it increases the thrombotic risk 3 to 10 times when associated with Factor V Leiden heterozygotism. Literature data report a 30 times higher risk of myocardial infarction in young female smokers and high ischemic stroke incidence rates in the young, when Factor V Leiden heterozygotes are involved.(8,9,10) The thrombosis risk depends on the homo- and heterozygote status for this mutation. Heterozygote individuals run thrombosis risks that are 4-8 times higher than those run by people with normal factor V, whereas homozygotes run such risks that are 80-100 times higher.(11) The risk increases exponentially in people who also associate other thrombotic risk factors, such as: coexistence of other genetic defects (deficit of protein C, S, antithrombin III, prothrombin gene mutation, MTHFR gene mutation), oral contraceptives, pregnancy. Thus, deep venous thrombosis and cerebral sinus thrombosis are more common in patients taking oral contraceptives, in pregnant women or in postpartum women.(11)

As concerns pregnancy complications, the presence of factor V Leiden, of prothrombin and MTHFR gene mutations are associated with high risks of recurrent miscarriage, especially during the 2nd and 3rd terms. (12) The asymptomatic members of the families where the mutation was detected are not screened on a large scale, due to the low risk of life-threatening venous thrombosis. Nevertheless, investigation may have major advantages when

pregnancy or oral contraceptives are involved, in people with a family history of recurrent venous thrombosis occurring at the age of < 50 years. The people that ask to be tested and the people whose result is positive should be informed of the implications of this diagnosis and with the signs and symptoms requiring urgent medical care.(13)

Prothrombin gene mutation was discovered in 1996 by Poort et al. It is due to a nucleotide substitution (guanine G is replaced by adenine) in the 20210 base pair, located in the unshifted 3' region of the factor II gene (14). The mutation is associated with prothrombin levels that are 30% higher than normal, which will induce a slight state of hypercoagulability, which may predispose to venous thrombosis.(15) Mutation prevalence in the Caucasian population is 2% on the average, with the highest number of cases in Southern Europe countries. Heterozygote individuals run a risk of thrombosis which is 3 times higher than in individuals with normal factor II; in homozygotes the full extent of the risk has not been determined yet.(14,15) After factor V Leiden mutation, prothrombin mutation is the second cause of hereditary thrombophilia, its occurrence being proven in about 20% of the cases. Thus, this mutation was confirmed in patients with idiopathic thrombosis of the portal vein or of the cerebral sinuses, in venous thrombosis patients during oral contraceptive administration, as well as in pregnant women with pregnancy complications (miscarriage, intrauterine growth retardation, utero-placental apoplexy). This mutation is detected in 6-8% of the patients suffering the first episode of venous thrombosis.(15) It has also been found that the risk of deep venous thrombosis is 16.3 times higher in patients using oral contraception and carrying this mutation, than in the control group. During pregnancy and post-partum, this mutation increases the risk by 15.2 times than in the same category of patients not having this genetic defect, whereas women receiving hormone replacement therapy during their menopause run a risk which is 2-4 times higher.(14) Moreover, the risk of venous thrombosis increases in patients who also suffer from other genetic defects, especially factor V Leiden mutation. Up to 40% of the individuals with prothrombinic mutation concomitantly associate factor V Leiden mutation; studies have shown that in these cases the risk of recurrent venous thrombosis is higher after a first thrombotic episode. This supports the need to analyze both mutations in patients suspected of hereditary thrombophilia.(14,15) As concerns pregnancy complications, the occurrence of a prothrombin gene mutation is associated with a high risk of recurrent miscarriages, especially in the 2nd and 3rd terms. This investigation may have major advantages as far as pregnancy, oral contraception or prior surgical procedures are concerned, when the prophylactic anticoagulant therapy is a must.(14)

The C677T a MTHFR mutation, factor V Leiden heterozygostism and G20210A mutation of the prothrombin gene cause pregnancy-related complications in 52% of pregnant women. This pathology consists of: miscarriage, fetus growth delay, preeclampsia, placental abruption and stillbirth. (16) The risk of miscarriage has also been often reported in pregnant women with protein C, protein S or antithrombin III deficiency. (17,18)

Protein C

Protein C is a glycoprotein synthesized in the liver (by a mechanism dependent of vitamin K), which circulates in the plasma and is important for thrombosis prevention. Its anticoagulant function materializes in the inactivation of the coagulation factors V and VIII. It also acts as a profibrinolytic agent. (19) Protein C is activated by thrombin; its activation rate is accelerated by the formation of a thrombin and endothelial cell thrombomodulin complex. Activated protein C degrades factors Va and VIIIa by selective proteolytic cleavage, thus reducing the procoagulant activity of plasma. A cofactor-protein S, calcium ions and a phospholipidic membrane surface are required for the inactivation of the coagulation factors. Activated protein C is neutralized by a specific inhibitor, namely alpha 2-antiplasmin and alpha 2-macroglobulin.(20,21) The protein C deficit incidence is about 0.2% in the general population. The protein C deficit may be hereditary or acquired; as far as hereditary deficit is concerned, the severity of the clinical signs depends on the homo- or heterozygote status. Thus, the heterozygote deficit is associated with episodes of deep venous thrombosis, complicated or not by pulmonary embolism in young patients, and also by recurrent miscarriages in pregnant patients in the first two pregnancy terms. The homozygote deficit becomes obvious immediately after birth, by massive and potentially life-threatening thromboembolic phenomena (neonatal purpura fulminans).(21)

Protein S

Protein S is a glycoprotein dependent on vitamin K synthesized in the liver, endothelial cells and megakaryocytes. About 60% of the amount of circulating protein S is bound to the C4b-binding protein. The remaining amount of protein is unbound and may act as a non-enzymatic cofactor of activated protein C. Protein S intensifies factors Va and VIIIa inactivation on phospholipidic surfaces and also plays a part in the fibrinolytic effect of activated protein C. Recent studies have shown a possible involvement of the bound fraction of protein S in the anticoagulant activity independently of the activated protein C. The protein S deficit is associated with recurrent venous thrombosis, with or without pulmonary embolism, occurring in young patients (19,20); Protein S is also involved in miscarriage and therefore imposes the screening of this group of patients.

Antithrombin III

Antithrombin III is a α_2-globulin which, after fixation on endothelial heparan sulphate or on heparin molecule, inactivates thrombin, Factor Xa, XIa, IXa, XIIa and Kallikrein. AT III activity decrease causes thrombin neutralization decrease, protein C and S activity reduction with thrombin generation control diminution, procoagu-

lant consequences and venous thrombosis susceptibility. AT III deficiency may be quantitative – type I deficiency – occurring by gene deletion or by "frameshift" mutations with the synthesis of a hardly stable and undetectable "truncated" molecule in the plasma, and qualitative deficiencies – type II deficiencies – the consequence of punctiform mutations. The type II deficiency is the consequence of anomalies affecting the antithrombin to heparin molecule fixation locus. The AT III deficiency was detected in 2% of the thrombotic event patients and is part of the thrombophilia profile of patients known for recurrent miscarriages.

CONCLUSION

Knowing the hemostatic and genetic abnormalities of this category of patients provides the theoretical grounds required to improve hereditary thrombophilia management, which conduct should aim at a fast accurate diagnosis setting and to the establishment of appropriate treatment.

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