

Coagulation challenges in pregnancy: from thrombophilia involvement and management to the utility of thrombin generation monitoring

R. Mihăilă^{1,*}

¹Faculty of Medicine, "Lucian Blaga" University of Sibiu and Department of Hematology, CVASIC Laboratory, Emergency County Clinical Hospital Sibiu (Romania)

Summary

Despite numerous studies, there is no general agreement on an ideal biomarker for estimating thrombotic risk in pregnant women. However, it is accepted that the analysis of thrombin generation assesses the coagulation state more accurately compared to the classical tests used to investigate it. Increasing evidence has found that thrombin generation is correlated with overall plasma hypercoagulability, which is involved in the occurrence of major obstetrical syndromes, including preeclampsia, intrauterine growth restriction or recurrent abortions. It is difficult to investigate the thrombophilic mutations which, once discovered, are not able to quantify the thrombotic risk. The standardization of thrombin generation assay could allow a quantification of the individual thrombotic risk, which has a high variability, and would facilitate multicenter studies to establish the utility of thrombin generation monitoring for the prophylactic anticoagulant treatment in order to reduce pregnancy-related complications.

Key words: Coagulation; Pregnancy; Preeclampsia; Thrombin generation; Thrombophilia; Thrombotic risk.

Introduction

Pregnancy is characterized by hypercoagulability [1, 2], which may contribute to an increased risk of morbidity and mortality [1]. The hypercoagulable state of pregnant women is associated with anatomic and physiologic changes that occur during pregnancy [3]. The risk of venous thromboembolism is higher especially in thrombophilic pregnant patients [4] and in females with a low birth weight newborn or stillbirth [3]. The classical assays used to explore the coagulation state are inadequate for estimating the thrombotic risk. Global assays (including thrombin generation or thrombelastography) can better evaluate the thrombotic risk [5]. More and more evidence has found that overall plasma hypercoagulability is correlated with thrombin generation [4]. Plasma thrombin levels are higher during pregnancy [1]. An increase in mean endogenous thrombin potential was observed in the first trimester of pregnancy (1391 nmol/L·min), with even higher levels in the second trimester (1757 nmol/L·min) that remained stable during the third trimester, and a peak value was observed through delivery (1857 nmol/L·min). A decrease was noted after delivery and mean endogenous thrombin potential was 1293 nmol/L·min at 8 weeks post-delivery [1]. Unfortunately, the global assays are not standardized, so their application is currently impeded [5].

This paper summarizes the knowledge of the coagulation disorders during pregnancy and their consequences. Scientific articles from 2015 to 2019 were searched using

PubMed and the Web of Science databases. All searches were up to date as of October 2019. The search terms used were: "thrombin generation", "thrombophilia", and "pregnancy". Only English language papers were reviewed.

Thrombophilia in Pregnancy

The latest review on inherited thrombophilia was published in September 2019 and included papers indexed on Medline and PubMed in the last 20 years. The authors concluded that the types of thrombophilia are not the same in all geographic regions. In addition, there are variations regarding a certain type of thrombophilia between different regions. Screening for the diagnosis of thrombophilia is recommended for women with previous adverse pregnancy outcomes, due to the negative consequences that thrombophilia can have on the pregnancy [6].

The frequency of fetal loss is higher in women with high-risk inherited thrombophilia. The presence of antiphospholipid antibodies in pregnant women and their family history of obstetric complications were found to be associated with placenta-mediated pregnancy complications [7]. Antiphospholipid antibodies are anticardiolipin antibody, lupus anticoagulant and anti-beta2-glycoprotein I antibodies. IgG and IgM isotypes of autoantibodies found in lupus anticoagulant target the phospholipid-protein from the cell membrane. Lupus anticoagulant is involved in the interference and prolongation of the coagulation process - a risk factor for thrombosis with complications including acquired

thrombophilia, and pregnancy loss [8]. A higher prothrombin conversion and unchanged thrombin inactivation rates were found in the antiphospholipid syndrome, whereas increased peak thrombin and endogenous thrombin potential were observed in patients with antiphospholipid syndrome and thrombosis in their history [9]. Factor V Leiden is the main risk factor for thrombotic accidents and recurrent pregnancy loss, while the role of factor II G20210A, plasminogen activator inhibitor and methylenetetrahydrofolate reductase C677T is still controversial in pregnancy, according to a Bulgarian study. Factor V Leiden was most frequently found in patients with thrombotic incidents in their history, compared to those with recurrent miscarriage [10].

The disorders produced in the uteroplacental circulation can be investigated by measuring the uterine radial artery resistance index using a Doppler ultrasound examination [11]. It has been found that a normal resistance index of the umbilical artery correlates with protection against adverse pregnancy outcomes [12]. Circulatory changes in the umbilical artery are associated with complications in early pregnancy development, including recurrent pregnancy losses. If patients with thrombophilia and recurrent pregnancy losses in their personal history have a uterine radial artery resistance index ≥ 0.45 , they have an overall risk of 49.48 (adjusted odds ratio) for miscarriage versus those with an index below 0.45 at 8 weeks of gestation. Each increase of this index by 0.1 units is associated with an increase of 18.70 in the risk of miscarriage [11]. The mean resistance index of the umbilical artery measured between 28 and 38 weeks of gestation was significantly higher in women with inherited thrombophilia without anticoagulant prophylaxis compared to those who received low-molecular-weight heparin as prophylaxis [12]. Intrauterine fetal death or miscarriages, as well as fetal growth restriction and adverse pregnancy outcomes were more numerous in the group that did not receive anticoagulant prophylaxis, compared to that prophylactically treated with low-molecular-weight heparin [12].

Factor V Leiden and the prothrombin gene *G20210A* mutation were significantly more frequent in patients with severe preeclampsia compared to healthy pregnant women. This suggests that hypercoagulability is involved in the progression of preeclampsia [13].

The role of thrombin in uterus and placenta

Decidual hemorrhage is involved in excessive thrombin generation. Thrombin binds to decidual cell-expressed protease-activated receptors, which induce chemokines that promote superficial placentation. Bleeding which occurs later in pregnancy produces thrombin that down-regulates progesterone receptors on the decidual cells and up-regulates matrix metalloproteinases and cytokines involved in preterm birth. In addition, aberrant tissue factor-derived thrombin signaling is involved in the pathogenesis of endometriosis through the induction of inflammation, angiogenesis, and cell survival [14]. Thrombin generation is involved in the onset of inflammation and weakens fetal

membranes at the uteroplacental junction. *In vitro* administration of thrombin to cultured decidual cells increased the expression of tissue factor and the following cytokines: IFN- γ , IL-6, IL-8, and IL-10. The expression of tissue factors and inflammatory cytokines did not diminish when decidual cells were treated with thrombin and unfractionated heparin or enoxaparin [15].

In addition to altering decidualization, intrauterine bleeding and thrombin are involved in increased leukocyte extravasation, endometrial inflammation, and stromal cell differentiation. Suppression of prolactin gene expression, insulin-like growth factor-1 and insulin-like growth factor binding protein-1 both at baseline and during thrombin-induced decidualization are mediated by protease activated receptor-1- and protease activated receptor-1-independent mechanisms [16]. The thrombin protease-activated receptor-1 is not present in amniotic fluid, but only in decidua and chorion. For this reason, fetoscopy did not produce any significant increase in thrombin-antithrombin complexes in amniotic fluid. Thrombin generation produced *in vitro*, by needle trauma to cultured membranes, is correlated to the amount of plasma [17].

Thrombin generation is increased in placentas of patients with preeclampsia. Thrombin increases the production of soluble Fms-like tyrosine kinase 1 in extravillous trophoblasts via the signaling pathway that includes proteinase-activated receptor 1, nicotinamide adenine dinucleotide phosphate oxidase, and reactive oxygen species [18].

Coagulation During Pregnancy

Coagulation differs in pregnancy and the early neonatal period compared to that of normal and non-pregnant adult females. Peak thrombin generation is higher in maternal plasma compared to cord and non-pregnant adult levels. Plasma of normal women at delivery has a large intrinsic pathway reserve that can be used for thrombin generation compared to cord and non-pregnant adult plasma. The main factors involved in thrombin generation in cord plasma are antithrombin, the extrinsic pathway of coagulation and low levels of tissue factor pathway inhibitor [19].

Plasma mean StartTail time, peak thrombin generation and endogenous thrombin potential increased significantly in the first trimester of pregnancy and remained stable in the next two trimesters in a Chinese study [20]. Indeed, an increase of velocity index, peak thrombin and endogenous thrombin potential at the 32nd day of gestation [21] and at the 43rd day of gestation [22], compared to pre-pregnancy values, have been observed in two studies done in the U.K. These increased values persisted through to the 59th day of gestation [22]. Serum levels of estradiol and progesterone were also significantly higher by day 32 of gestation. This suggests that these two hormones are linked to the prothrombotic state of pregnant women [21]. Regarding the level of D-dimer and factor VIII, they increased in the 59th day of gestation compared to pre-pregnancy levels [3].

Not only Factor VIII but also fibrinogen and von Willebrand factor are higher in the plasma of gestational women. The increased levels of these three coagulation factors produce resistance to dilutional coagulopathy and activated protein C *in vitro* in plasma from normal pregnant women [23].

Women with blood group A and B have a significantly higher risk of developing venous thromboembolism in the antepartum and postpartum period than those with blood group O. This is an independent risk factor for venous thromboembolism, as well as for red blood cell transfusions, especially those performed near delivery. The risk (adjusted odds ratio) is directly correlated with the number of transfused units: 2.60 for 1-2 units and 3.55 for over 5 units [24].

Hypercoagulation is present after delivery. So, venous thromboembolism may occur after cesarean section with an incidence of up to 0.6%. Thereafter, coagulation status tends toward normal during the early postpartum period. The women who received low-molecular-weight heparin prophylaxis after delivery had normal coagulation parameters more often than those without anticoagulant prophylaxis [25].

The Progression of Thrombin Generation Parameters in Pregnancy

A study made by Hunt *et al.* (2018) did not find candidate biomarkers (including thrombin generation parameters) for suspected venous thromboembolism during pregnancy and puerperium [26]. Indeed, an early increase in thrombin generation parameters was observed both in normal pregnant women and in those with high risk of venous thrombosis. The levels of thrombin generation were stabilized during the second month of pregnancy. A French report did not find any correlation between the markers of coagulation activation and those of thrombin generation. The authors concluded that the measurement of thrombin generation is not conclusive for estimating thrombotic risk in women with high risk of venous thrombosis [27]. But two parameters of thrombin generation - peak thrombin and endogenous thrombin potential - were higher in pregnant women compared to non-pregnant women, especially in those with severe thrombophilia. The peak of thrombin generation was lower in women who received low molecular weight heparin as prophylaxis, but this effect diminished with the progression of pregnancy [4].

Anti-Xa levels are not the most accurate way to evaluate coagulation inhibition. Instead, the thrombin generation assay estimates the total amount of thrombin and its formation dynamics. This assay has confirmed the presence of hypercoagulability in normal pregnancies. There was a correlation between anti-Xa levels and three parameters of thrombin generation (time to peak, peak height, and area under the curve) during pregnancy, but this group correlation masked the large variability of these biomarkers from one patient to another. Thus, some patients with anti-Xa levels considered to be 'safe' may actually be under-anticoagulated,

especially during pregnancy. A better approach to the prophylaxis of thrombosis in pregnancy may be based on the study of thrombin generation and not on anti-Xa levels or serum D-dimers [2].

Endogenous thrombin potential was higher than reference values before pregnancy in half of pregnant women in the first trimester. It exceeded the reference values before pregnancy in all pregnant women in the second trimester and remained stable during the third trimester. The median D-dimer serum level increased from 0.30 mg/L to 0.91 mg/L and to 1.45 mg/L in the first, second and third trimester of pregnancy, respectively. Serum levels of protein S decreased from early pregnancy. The activity of protein S had substantial variability [28].

Pregnant women after *in vitro* fertilization have a higher level of thrombin generation, thrombomodulin and tissue factor activity, while procoagulant phospholipid clotting time is shorter compared to healthy age-matched women. The values of thrombin generation parameters increase during the *in vitro* fertilization procedure, after the start of hormone treatment. Women with *in vitro* fertilization procedures have higher levels of tissue factor activity, D-dimers and a shorter procoagulant phospholipid clotting time [29].

The Involvement of Thrombin Generation in the Major Obstetrical Syndromes

High plasma levels of thrombin are associated with the occurrence of major obstetrical syndromes, including preeclampsia, intrauterine growth restriction and recurrent abortions [30]. Elevated thrombin generation is a pathogenic factor also involved in the occurrence of preterm labor, fetal growth restriction, premature rupture of membranes, and fetal demise [31]. Increased levels of thrombin - anti thrombin III complexes, coagulation disorders or anticoagulation factors may occur in the blood before the onset of clinical manifestations of some of these obstetrical syndromes [31]. Heparins, because of their antithrombotic and anti-inflammatory effects, could be helpful in the prevention of these obstetrical complications. They can be used for the prophylaxis of gestational complications in patients with antiphospholipid syndrome. The use of heparin for other pregnancy complications is under debate [30].

Individual Thrombophilic Conditions in Relation to Serious Obstetric Complications

A meta-analysis published in 2003 found that factor V Leiden, the prothrombin gene *G20210A* mutation, and protein C and protein S deficiency were associated with recurrent pregnancy loss [32]. Women with two or more recurrent pregnancy losses had more frequent MTHFR C677T and A1298C mutant alleles in an Iranian study [33]. In a more recent study, late pregnancy loss appears to be associated with protein S deficiency, while the heterozygous mutations of factor V Leiden and *FII 20210A* are more common in later pregnancy complications, such as preeclampsia, second trimester loss, fetal growth restriction

and placental abruption and less common in recurrent first trimester loss [34, 35]. A meta-analysis established that factor V Leiden is involved in late unexplained fetal loss and is associated with a higher risk of late second trimester loss [35, 36].

A correlation was found between recurrent pregnancy loss and the following thrombophilia-associated gene polymorphisms: heterozygous factor V Leiden H1299R, heterozygous prothrombin *G20210A*, *PAI-1 4G/5G*, and *PAI-1 4G/4G* [37]. A meta-analysis established that the factor V G1691A single-nucleotide polymorphisms (SNP) and the prothrombin *G20210A* SNP are associated with a high risk for both overall preeclampsia and severe preeclampsia [38]. A Danish study found that prothrombin *G20210A* was not associated with any adverse outcome while *MTHFR C677T* was associated with severe preeclampsia [35, 39]. No associations were present between premature or term labor and *MTHFR C677T*, *MTHFR C1298T*, prothrombin *G20210A*, factor V and ACE polymorphisms [40].

Regarding acquired thrombophilia, antiphospholipid syndrome (with anti-cardiolipin and lupus anticoagulant antibodies) is also involved in recurrent pregnancy loss. There was also some evidence linking recurrent pregnancy loss to b2 glycoprotein I antibodies [41]. In addition, the presence of anti-phospholipid antibodies is associated with the early onset of preeclampsia, especially if it is complicated by fetal growth restriction [42].

A summary of international evidence-based guidelines states that congenital thrombophilias may be associated with adverse pregnancy outcomes. But the evidence linking these thrombophilias to recurrent pregnancy loss is based on weak studies and is inconclusive. For this reason, no guidelines recommend investigating for congenital thrombophilic mutations outside of a research setting [43]. Subject to future clinical trials, the study of thrombin generation could be useful for estimating the risk of recurrent pregnancy loss.

Thrombin Generation in Women with Recurrent Pregnancy Loss

The following coagulation disorders were found in women with recurrent pregnancy loss: increased factor VIII, fibrinogen, plasminogen activator inhibitor-1 levels, and thrombin generation parameters. An endogenous thrombin potential over 1222.1 nmol/L was found to be associated with a more than two-fold higher risk of recurrent pregnancy loss [44].

Thromboprophylaxis should start as soon as possible after pregnancy diagnosis in women at clinical risk of thrombosis, as the markers of thrombin generation increase early [22].

Coagulation in Preeclampsia

Preeclampsia with early onset is a proinflammatory disease in which patients have concomitant thrombotic and bleeding risks. They have a reduced level of thrombin gen-

eration stimulated by a low dose of tissue factor and an increased activity of plasma tissue factor pathway inhibitor [45]. Plasma tissue factor activity was also at higher levels in women with preeclampsia (especially in the presence of distal villous hypoplasia) than in those with normal pregnancies or small for gestational age neonates [46].

Patients with preeclampsia have higher levels of thrombin generation than those with normal pregnancies or small for gestational age neonates, according to another analysis. Those with preeclampsia have a higher endogenous thrombin potential than normal or small for gestational age pregnancies, suggesting that the former have a longer reaction, which generates more thrombin. Patients with small for gestational age neonates have a shorter median time-to-peak thrombin concentration and a higher median velocity index than females with preeclampsia or normal pregnancies, suggesting a faster plasma thrombin generation [47].

The activation of the coagulation system in preeclampsia can be studied using multicolor flow cytometry. The presence of tissue factor (CD142) on monocytes can be demonstrated with antibodies against CD14 APC, anti CD16b FITC, anti CD45 PerCP, and anti CD142 PE and the appropriate isotype controls [48].

One hypothesis argues that maternal release of epinephrine into the adrenal medulla is involved in systemic platelet activation. These activated platelets initiate coagulation through the intrinsic pathway, which results in thrombin generation. But thrombin also promotes the onset of inflammation, which is involved in the occurrence of acute atherosclerosis in preeclampsia [49].

Coagulation in Pregnant Women with Different Diseases

Treatment with insulin in women with gestational diabetes mellitus did not influence the thrombin generation test [20]. Obese female patients have higher levels of thrombin generation and lower anti-Xa levels at post-cesarean assessment. This increased procoagulant potential could only be reduced in those who received a prophylactic dose of 60 mg/day enoxaparin instead of 40 mg/day. It became comparable to the procoagulant potential of non-obese post-cesarean patients only at the higher dose [50].

Females with factor VII deficiency have an increased risk of bleeding during pregnancy. The study of the parameters of thrombin generation in a patient during the first trimester of pregnancy treated with recombinant activated factor VII at doses less than 30 $\mu\text{g}/\text{kg}/\text{day}$ found an increase in lag time, while time to thrombin peak and peak thrombin were not modified compared to the period without treatment. A prophylactic dose of 15 $\mu\text{g}/\text{kg}$ given every other day in the second half of pregnancy was sufficient to lead to a successful childbirth in the case report published by Pfrepper *et al.* [51].

High endogenous plasma thrombin potential was found, in an Israeli study, in women with active or extra-intestinal involvement of inflammatory bowel diseases; it also in-

creased with gestational age and body mass index [52]. But there was no correlation between endogenous thrombin potential and the occurrence of adverse pregnancy outcomes in pregnant women with inflammatory bowel diseases [52].

Antiplatelet and Anti-coagulant Treatment in Pregnancy

A systematic review and meta-analysis found that aspirin given at under 11 weeks' gestation was able to decrease the risk of preterm delivery, but not the risk of gestational hypertension or any hypertension during pregnancy, preeclampsia, or fetal growth restriction [53].

Women with a venous thromboembolism in their history or a strong family history of thromboembolic events, as well as those with proven thrombophilia, have an indication for anticoagulant prophylaxis in the ante- and postpartum period [3]. Prophylactic treatment with low molecular weight heparin has a protective effect against obstetric complications, including miscarriage in patients with inherited thrombophilia [7].

Women with cerebral vein thrombosis in their history who received low-molecular weight heparin prophylaxis during pregnancy had a reduced risk of recurrent thrombosis (3.2%). But their risk of miscarriage was higher (13.5%), as was their risk of advanced pregnancy complications (19.2%), regardless of previous history of obstetrical complications or thrombophilia markers [54].

There are patients with anti-Xa levels who might be considered safe, but who may be under anticoagulated during their pregnancy. Anti-Xa activity is not the best biomarker for thrombotic risk and is not the best way to monitor low molecular weight heparin treatment. The thrombin antithrombin complexes reflect thrombin generation and their levels are efficiently decreased even when anti-Xa levels are low, suggesting that the anticoagulation effect is still significant up to a day post-low molecular weight heparin. Many pregnant patients with anti-Xa levels considered safe have significant variation in thrombin generation parameters, so that thrombin generation is insufficiently suppressed in some of them. Thrombin generation assays can improve the selection of doses of anticoagulant drugs during pregnancy [55].

Conclusions

Nowadays, it is difficult to estimate the thrombotic risk during pregnancy and postnatal period, as the classical assays used to explore coagulation are inadequate and anti-Xa levels are not the most accurate way to evaluate coagulation inhibition. Therefore, the results of the studies that used these assays should be viewed with caution. Instead, the thrombin generation assay evaluates the total amount of thrombin and its formation dynamics and may confirm the presence of hypercoagulability, but the assay has not been standardized until now.

It is accepted that pregnancy is characterized by a hypercoagulable state. The high plasma levels of thrombin

generation are involved in the occurrence of major obstetrical syndromes. Thrombin generation is also involved in the onset of inflammation and weakens fetal membranes at the uteroplacental junction.

Exploring thrombophilic markers is difficult and expensive. In addition, their identification does not allow the quantification of thrombotic risk. Although there is no consensus, studying the parameters of thrombin generation could provide a picture of the balance between pro- and anticoagulant factors, including a result of possible thrombophilic mutations, and could quantify the thrombotic risk. Assessing thrombin generation may be a better way to monitor anticoagulant therapy in pregnancy. Multicenter studies are needed to confirm this hypothesis.

Acknowledgments

I would like to express my gratitude to all those who helped me during the writing of this manuscript.

Conflict of Interest

The author declares no conflict of interest.

Submitted: January 30, 2020

Accepted: June 02, 2020

Published: December 15, 2020

References

- [1] Patel J.P., Patel R.K., Roberts L.N., Marsh M.S., Green B., Davies J.G., *et al.*: "Changes in thrombin generation and D-dimer concentrations in women injecting enoxaparin during pregnancy and the puerperium". *BMC Pregnancy Childbirth*, 2014, 14, 384.
- [2] Chowdary P., Adamidou D., Riddell A., Aghighi S., Griffioen A., Priest P., *et al.*: "Thrombin generation assay identifies individual variability in responses to low molecular weight heparin in pregnancy: implications for anticoagulant monitoring". *Br. J. Haematol.*, 2015, 168, 719-727.
- [3] Rybstein M.D., Desancho M.T.: "Risk factors for and clinical management of venous thromboembolism during pregnancy". *Clin. Adv. Hematol. Oncol.*, 2019, 17, 396-404.
- [4] Selmezi A., Roach R.E., Móré C., Batta Z., Hársfalvi J., van der Bom J.G., *et al.*: "Thrombin generation and low-molecular-weight heparin prophylaxis in pregnant women with thrombophilia". *Thromb. Haemost.*, 2015, 113, 283-289.
- [5] Lipets E.N., Ataulakhanov F.I.: "Global assays of hemostasis in the diagnostics of hypercoagulation and evaluation of thrombosis risk". *Thromb. J.*, 2015, 13, 4.
- [6] Dugalic S., Petronijevic M., Stefanovic A., Stefanovic K., Perovic M., Pantic I., *et al.*: "Perinatal complications related to inherited thrombophilia: review of evidence in different regions of the world". *J. Matern. Fetal Neonatal. Med.*, 2019, 1-10.
- [7] Clavijo M.M., Mahuad C.V., Reparaz M.L.A.V., Aizpurua M.F., Ventura A., Casali C.E.: "Risk factors and role of low molecular weight heparin in obstetric complications among women with inherited thrombophilia - a cohort study". *Hematol. Transfus. Cell Ther.*, 2019, 41, 303-309.
- [8] Rasool Z.S., Tiwari V.: "Biochemistry, lupus anticoagulant". In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; July 23, 2020.
- [9] Kremers R.M.W., Zuily S., Kelchtermans H., Peters T.C., Bloemen S., Regnault V., *et al.*: "Prothrombin conversion is accelerated in the antiphospholipid syndrome and insensitive to thrombomodulin". *Blood Adv.*, 2018, 2, 1315-1324.

- [10] Levkova M., Hachmeriyan M., Stoyanova M., Miteva V., Angelova L.: "Comparison between thrombophilic gene polymorphisms among high risk patients". *Rom. J. Intern. Med.*, 2020, 58, 20-26.
- [11] Bao S.H., Chigirin N., Hoch V., Ahmed H., Frempong S.T., Zhang M., et al.: "Uterine radial artery resistance index predicts reproductive outcome in women with recurrent pregnancy losses and thrombophilia". *Biomed. Res. Int.*, 2019, 2019, 8787010.
- [12] Dugalic S., Petronijevic M., Stefanovic A., Stefanovic K., Petronijevic S.V., Stanislavljevic D., et al.: "Comparison of 2 approaches in management of pregnant women with inherited thrombophilias: Prospective analytical cohort study". *Medicine (Baltimore)*, 2019, 98, e16883.
- [13] Elzein H.O., Saad A.A., Yousif A.A., Elamin E., Abdalhabib E.K., Elzaki S.G.: "Evaluation of factor V Leiden and prothrombin G20210A mutations in Sudanese women with severe preeclampsia". *Curr. Res. Transl. Med.*, 2020, 68, 77-80.
- [14] Schatz F., Guzeloglu-Kayisli O., Arlier S., Kayisli U.A., Lockwood C.J.: "The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding". *Hum. Reprod. Update*, 2016, 22, 497-515.
- [15] Smrtka M.P., Feng L., Murtha A.P., Grotegut C.A.: "Thrombin-induced inflammation in human decidual cells is not affected by heparin". *Reprod. Sci.*, 2017, 24, 1154-1163.
- [16] Babayev S.N., Kanchwala M., Xing C., Akgul Y., Carr B.R., Word R.A.: "Thrombin alters human endometrial stromal cell differentiation during decidualization". *Reprod. Sci.*, 2019, 26, 278-288.
- [17] Engels A.C., Bauters D., Rynkevicius R., Pranpanus S., Richter J., van Mieghem T., et al.: "Thrombin generation by fetoscopic trauma to the fetal membranes: an in vivo and in vitro study". *Fetal Diagn. Ther.*, 2016, 39, 261-268.
- [18] Huang Q.T., Chen J.H., Hang L.L., Liu S.S., Zhong M.: "Activation of PAR-1/NADPH oxidase/ROS signaling pathways is crucial for the thrombin-induced sFlt-1 production in extravillous trophoblasts: possible involvement in the pathogenesis of preeclampsia". *Cell Physiol. Biochem.*, 2015, 35, 1654-1662.
- [19] Rice N.T., Szlam F., Varner J.D., Bernstein P.S., Szlam A.D., Tanaka K.A.: "Differential contributions of intrinsic and extrinsic pathways to thrombin generation in adult, maternal and cord plasma samples". *PLoS One*, 2016, 11, e0154127.
- [20] Dong F., Wang L., Wang C.: "Thrombin generation in chinese pregnant women and the effect of insulin use on thrombin generation in patients with GDM". *Clin. Appl. Thromb. Hemost.*, 2019, 25, 1076029619863492.
- [21] Bagot C.N., Leishman E., Onyiaodike C.C., Jordan F., Gibson V.B., Freeman D.J.: "Changes in laboratory markers of thrombotic risk early in the first trimester of pregnancy may be linked to an increase in estradiol and progesterone". *Thromb. Res.*, 2019, 178, 47-53.
- [22] Bagot C.N., Leishman E., Onyiaodike C.C., Jordan F., Freeman D.J.: "Normal pregnancy is associated with an increase in thrombin generation from the very early stages of the first trimester". *Thromb. Res.*, 2017, 157, 49-54.
- [23] Tanaka K.A., Bharadwaj S., Hasan S., Judd M., Abuelkasem E., Henderson R.A., et al.: "Elevated fibrinogen, von Willebrand factor, and Factor VIII confer resistance to dilutional coagulopathy and activated protein C in normal pregnant women". *Br. J. Anaesth.*, 2019, 122, 751-759.
- [24] Wang C., Le Ray I., Lee B., Wikman A., Reilly M.: "Association of blood group and red blood cell transfusion with the incidence of antepartum, peripartum and postpartum venous thromboembolism". *Sci. Rep.*, 2019, 9, 13535.
- [25] Koltsova E.M., Balandina A.N., Grischuk K.I., Shpilyuk M.A., Seregina E.A., Dashkevich N.M., et al.: "The laboratory control of anticoagulant thromboprophylaxis during the early postpartum period after cesarean delivery". *J. Perinat. Med.*, 2018, 46, 251-260.
- [26] Hunt B.J., Parmar K., Horspool K., Shephard N., Nelson-Piercy C., Goodacre S., et al.: "The DiPEP (Diagnosis of PE in Pregnancy) biomarker study: An observational cohort study augmented with additional cases to determine the diagnostic utility of biomarkers for suspected venous thromboembolism during pregnancy and puerperium". *Br. J. Haematol.*, 2018, 180, 694-704.
- [27] Joly B.S., Sudrié-Arnaud B., Barbay V., Borg J.Y., Le Cam Duchez V.: "Thrombin generation test as a marker for high risk venous thrombosis pregnancies". *J. Thromb. Thrombolysis*, 2018, 45, 114-121.
- [28] Kovac M.K., Lalic-Cosic S.Z., Dmitrovic J.M., Djordjevic V.J., Radojkovic D.P.: "Thrombin generation, D-dimer and protein S in uncomplicated pregnancy". *Clin. Chem. Lab. Med.*, 2015, 53, 1975-1979.
- [29] Gerotziakas G.T., van Dreden P., Mathieu d'Argent E., Lefkou E., Grusse M., Comtet M., et al.: "Impact of blood hypercoagulability on in vitro fertilization outcomes: a prospective longitudinal observational study". *Thromb. J.*, 2017, 15, 9.
- [30] Mastrolia S.A., Mazor M., Holcberg G., Leron E., Beharier O., Loverro G., et al.: "The physiologic anticoagulant and anti-inflammatory role of heparins and their utility in the prevention of pregnancy complications". *Thromb. Haemost.*, 2015, 113, 1236-1246.
- [31] Mastrolia S.A., Mazor M., Loverro G., Klaitman V., Erez O.: "Placental vascular pathology and increased thrombin generation as mechanisms of disease in obstetrical syndromes". *PeerJ.*, 2014, 2, e653.
- [32] El Hachem H., Crepau V., May-Panloup P., Descamps P., Legendre G., Bouet P.E.: "Recurrent pregnancy loss: current perspectives". *Int. J. Womens Health*, 2017, 9, 331.
- [33] Ahangari N., Doosti M., Mousavifar N., Attaran M., Shahrokhzadeh S., Memarpour S., et al.: "Hereditary thrombophilia genetic variants in recurrent pregnancy loss". *Arch. Gynecol. Obstet.*, 2019, 300, 777-782.
- [34] Rodger M.A., Betancourt M.T., Clark P., Lindqvist P.G., Dizon-Townson D., Said J., et al.: "The association of factor V Leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: A systematic review and meta-analysis of prospective cohort studies". *PLoS Med.*, 2010, 7, e1000292.
- [35] Simcox L.E., Ormsher L., Tower C., Greer I.A.: "Thrombophilia and pregnancy complications". *Int. J. Mol. Sci.*, 2015, 16, 28418-28428.
- [36] Robertson L., Wu O., Langhorne P., Twaddle S., Clark P., Lowe G.D., et al.: "Thrombophilia in pregnancy: A systematic review". *Br. J. Haematol.*, 2006, 132, 171-196.
- [37] Barut M.U., Bozkurt M., Kahraman M., Yıldırım E., Imirzalioglu N., Kubar A., et al.: "Thrombophilia and recurrent pregnancy loss: the enigma continues". *Med. Sci. Monit.*, 2018, 24, 4288.
- [38] Wang X., Bai T., Liu S., Pan H., Wang B.: "Association between thrombophilia gene polymorphisms and preeclampsia: a meta-analysis". *PLoS One*, 2014, 9, e100789.
- [39] Lykke J.A., Bare L.A., Olsen J., Lagier R., Arellano A.R., Tong C., et al.: "Thrombophilias and adverse pregnancy outcomes: Results from the Danish National Birth Cohort". *J. Thromb. Haemost.*, 2012, 10, 1320-1325.
- [40] Uvuz F., Kilic S., Yilmaz N., Tuncay G., Cakar E., Yuksel B., et al.: "Relationship between preterm labor and thrombophilic gene polymorphism: A prospective sequential cohort study". *Gynecol. Obstet. Invest.*, 2009, 68, 234-238.
- [41] Hong Li Y., Marren A.: "Recurrent pregnancy loss: A summary of international evidence-based guidelines and practice". *Aust J Gen Pract.*, 2018, 47, 432-436.
- [42] Berks D., Duvetkot J.J., Basalan H., De Maat M.P., Steegers E.A., Visser W.: "Associations between phenotypes of preeclampsia and thrombophilia". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2015, 194, 199-205.
- [43] Hong Li Y., Marren A.: "Recurrent pregnancy loss: A summary of international evidence-based guidelines and practice". *Aust. J. Gen. Pract.*, 2018, 47, 432-436.
- [44] Romagnuolo I., Attanasio M., Cozzolino M., Paladino E., Castaman G., Coccia M.E., et al.: "Thrombin potential and traditional coagulation assay: are they useful in exploring recurrent pregnancy loss risk". *Blood Coagul. Fibrinolysis*, 2018, 29, 160-166.
- [45] Egan K., O'Connor H., Kevane B., Malone F., Lennan A., Al Zadjali A., et al.: "Elevated plasma TFPI activity causes attenuated TF-dependent thrombin generation in early onset preeclampsia". *Thromb. Haemost.*, 2017, 117, 1549-1557.
- [46] Erez O., Romero R., Vaisbuch E., Than N.G., Kusanovic J.P., Mazaki-Tovi S., et al.: "Tissue factor activity in women with preeclampsia or SGA: a potential explanation for the excessive thrombin generation in these syndromes". *J. Matern. Fetal. Neonatal. Med.*, 2018, 31, 1568-1577.

- [47] Erez O., Romero R., Vaisbuch E., Kusanovic J.P., Mazaki-Tovi S., Chaiworapongsa T., *et al.*: "The pattern and magnitude of "in vivo thrombin generation" differ in women with preeclampsia and in those with SGA fetuses without preeclampsia". *J. Matern. Fetal. Neonatal. Med.*, 2018, *31*, 1671-1680.
- [48] Slavik L., Novak M., Ulehlova J., Prochazka M., Prochazkova J., Lattova V., *et al.*: "Possibility of coagulation system activation determination with tissue factor in pregnancy complications". *Clin. Lab.*, 2016, *62*, 1851-1856.
- [49] Eggers A.E.: "A suggestion about the cause of inflammation in acute atherosclerosis complicating poor placentation in preeclampsia". *Med. Hypotheses.*, 2015, *85*, 718-719.
- [50] Rottenstreich A., Levin G., Elchalal U., Kleinstern G., Spectre G., Ziv E., *et al.*: "The effect on thrombin generation and anti-Xa levels of thromboprophylaxis dose adjustment in post-cesarean obese patients - A prospective cohort study". *Thromb. Res.*, 2018, *170*, 69-74.
- [51] Pfrepper C., Siegemund A., Hildebrandt S., Kronberg J., Scholz U., Niederwieser D.: "Prophylactic treatment of hereditary severe factor VII deficiency in pregnancy". *Blood Coagul. Fibrinolysis*, 2017, *28*, 490-492.
- [52] Rottenstreich A., Diminsky M., Granovsky S.G., Mishaal T., Roth B., Spectre G., *et al.*: "Assessment of the procoagulant potential and associated risk factors in pregnant patients with inflammatory bowel diseases". *Eur. J. Intern. Med.*, 2019, *65*, 63-68.
- [53] Chaemsaitong P., Cuenca-Gomez D., Plana M.N., Gil M.M., Poon L.C.: "Does low-dose aspirin initiated before 11 weeks' gestation reduce the rate of preeclampsia". *Am. J. Obstet. Gynecol.*, 2020, *222*, 437-450.
- [54] Abbattista M., Capecchi M., Martinelli I.: "Pregnancy after cerebral venous thrombosis". *Thromb. Res.*, 2019, *181*, S15-S18.
- [55] Ismail S.K., Norris L., Higgins J.R.: "Thrombin generation assays for optimizing low molecular weight heparin dosing in pregnant women at risk of thrombosis". *Br. J. Haematol.*, 2016, *172*, 643-644.

Corresponding Author:

ROMEIO-GABRIEL MIHĂILĂ, M.D., Ph.D.
 Professor of Medicine, Hematology Department,
 Emergency County Clinical Hospital Sibiu,
 str Corneliu Coposu, nr 2-4, 550245 Sibiu (Romania)
 e-mail: romeomihaila@yahoo.com.