



Immature Platelet Fraction and Thrombin Generation: Preeclampsia Biomarkers

Fração de plaquetas imaturas e geração de trombina: Biomarcadores da pré-eclâmpsia

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Abstract

Preeclampsia, a human pregnancy syndrome, is characterized by elevated blood pressure and proteinuria after the 20th week of gestation. Its etiology remains unknown, and its pathophysiological mechanisms are related to placental hypoperfusion, endothelial dysfunction, inflammation, and coagulation cascade activation. Recently, the role of the complement system has been considered. This syndrome is one of the main causes of maternal and fetal mortality and morbidity. This article discusses the hypothesis of preeclampsia being triggered by the occurrence of inadequate implantation of the syncytiotrophoblast, associated with bleeding during the first stage of pregnancy and with augmented thrombin generation. Thrombin activates platelets, increasing the release of antiangiogenic factors and activating the complement system, inducing the membrane attack complex (C5b9). Immature platelet fraction and thrombin generation may be possible blood biomarkers to help the early diagnosis of preeclampsia.

Keywords

- ▶ platelets
- ▶ platelet activation
- ▶ hypertension pregnancy-induced
- ▶ blood coagulation
- ▶ complement system proteins

Resumo

A pré-eclâmpsia, uma síndrome da gestação humana, é caracterizada por elevação da pressão arterial e proteinúria patológica após a 20ª semana de gestação. Sua etiologia permanece desconhecida, e seus mecanismos fisiopatológicos estão relacionados à hipoperfusão placentária, disfunção endotelial, inflamação, e ativação da cascata de coagulação. Recentemente, o papel do sistema do complemento foi considerado. Essa síndrome é uma das principais causas de morbidade e mortalidade materna e fetal. Este artigo discute a hipótese de a pré-eclâmpsia ser desencadeada pela ocorrência da implantação inadequada do sincitiotrofoblasto, associada ao sangramento durante o primeiro trimestre da gravidez com aumento da geração de trombina. A trombina ativa plaquetas, aumentando a liberação de fatores antiangiogênicos na circulação e ativando o sistema do complemento, especialmente o complexo de ataque de membrana (C5b9). Portanto, a fração de plaquetas imaturas e a geração de trombina podem ser possíveis biomarcadores sanguíneos para auxílio no diagnóstico precoce da pré-eclâmpsia.

Palavras-chave

- ▶ plaquetas
- ▶ ativação plaquetária
- ▶ hipertensão induzida pela gravidez
- ▶ coagulação sanguínea
- ▶ proteínas do sistema do complemento

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Introduction

Hypertensive disorders are very frequent complications in pregnancy. It is one of the main causes of maternal and fetal morbidity and mortality.^{1,2} Preeclampsia (PE) is characterized by elevated blood pressure and pathological proteinuria after the 20th week of pregnancy. The incidence varies depending on where the study is being performed, but it is estimated to compromise from 2 to 8% of pregnancies.^{1,3,4} The etiology is unknown, and its pathophysiological mechanisms are related to placental hypoperfusion, endothelial dysfunction, oxidative stress, inflammation, and coagulation changes.⁵⁻¹⁴

Theory

Defective implantation of the syncytiotrophoblast and bleeding in the first trimester of pregnancy contribute to increased thrombin generation, causing increased platelet activation and release of antiangiogenic factors in the maternal circulation (such as sFLT-1). The activation of platelets also triggers the complement system, membrane attack complex (C5b9) (→ Fig. 1).

Discussion

Platelets and Preeclampsia

Hemostatic changes occur during pregnancy, shifting the balance in favor of hypercoagulability with an increased thrombosis risk.¹⁵ These changes are aggravated in PE, as there is an abnormal activation of the hemostatic and immune system, which are responsible for most complications of the disease. In pregnant women with hypertensive disorder, especially PE, the total number of platelets and platelet parameters modifications, including the mean platelet volume (MPV) and the immature platelet fraction (IPF),

platelet activation markers, and the complement system. Mean platelet volume, platelet distribution width (PDW), and IPF values are proportionally increased in relation to the severity of preeclampsia when compared with pregnant women without PE.¹⁶⁻²¹ Thrombocytopenia results from increased platelet activation, aggregation, and consumption, and it maybe considered a platelet activation marker.^{22,23}

Platelets play an important role in the pathophysiology of PE, being responsible for coagulation and participating as an important inflammatory mediator. There is evidence of PE with platelet activation and increased platelet surface markers (CD62P) when comparing women with PE to healthy women.²⁴⁻²⁶ In addition, there is an increase in CD41 expression in pregnant women with PE, evidencing platelet activation.²⁵

Platelet and Thrombin Generation

Platelet activation may be due to increased thrombin generation. Thrombin is a multifunctional protease, responsible for coagulation cascade and one of the most potent platelet activators. Activation through thrombin generation causes degranulation and platelet activation, which displaces adhesion receptors to the cell surface and releases hemostatic and inflammatory mediators in the bloodstream, facilitating cell adhesion.²⁷⁻³⁰ It is known that uterine bleeding or bruising at the moment of the syncytiotrophoblast implantation are associated with the development of PE and generates excess thrombin.³¹ Bleeding in the first 20 weeks of pregnancy is a common complication, affecting about 1 in 5 pregnant women.³² It has clinical relevance, as these patients develop an increased risk for unfavorable outcomes, mainly placental abruption, low birth weight, and premature birth.^{33,34} As bleeding in pregnant women can be used as an early marker of placental dysfunction, there are studies associating bleeding with the development of PE.²⁵ However, findings remain conflicting. On the one hand, some authors disclosed a 35 to

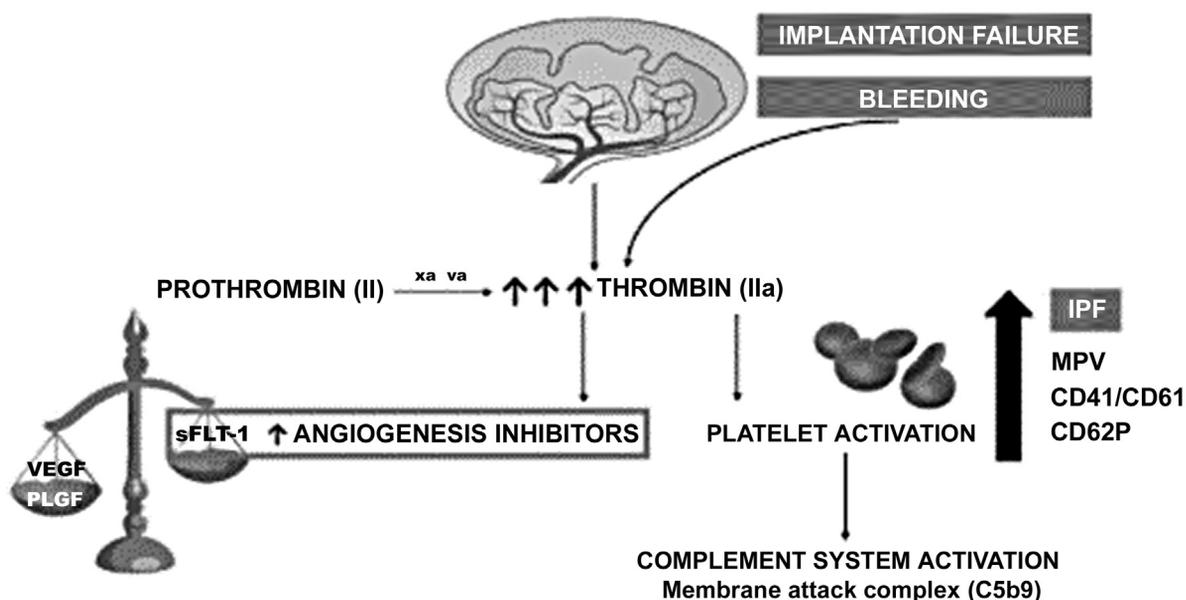


Fig. 1 Scheme of the theory.

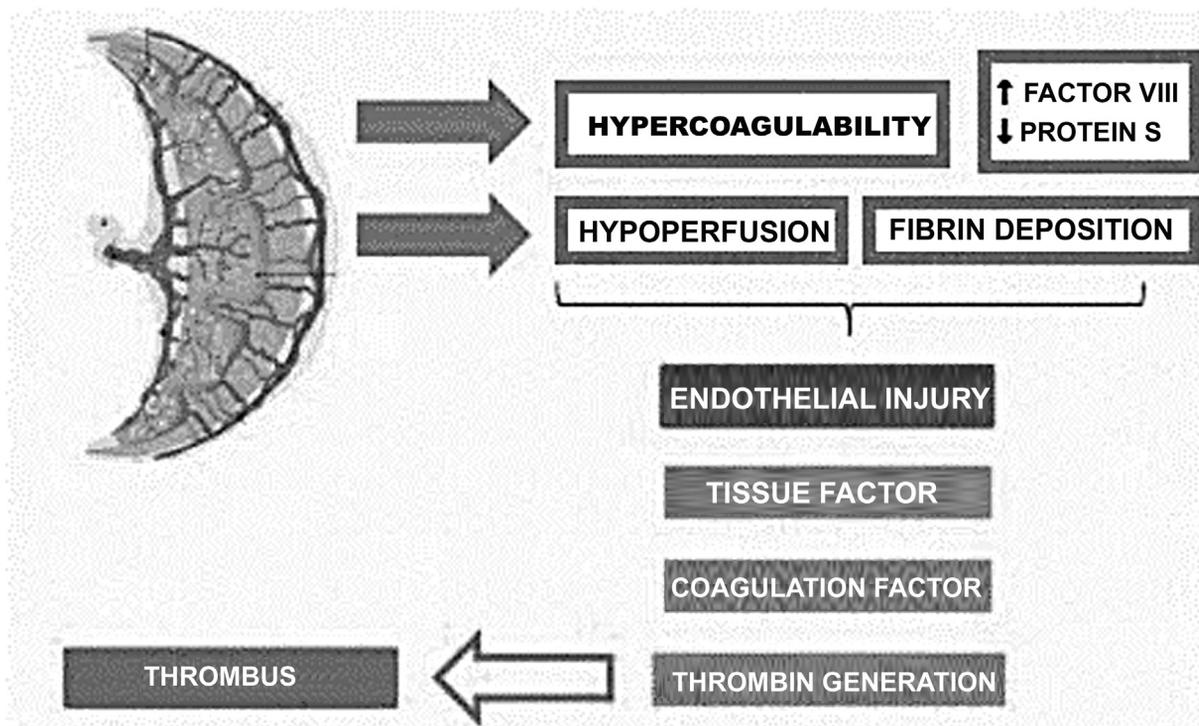


Fig. 2 Factors involved in thrombus generation in preeclampsia.

40% increase in the risk of developing PE in patients with mild bleeding in the first stage of pregnancy when compared with pregnant women who did not present bleeding.^{35,36} On the other hand, Smits et al.³¹ found no association between bleeding (mild or severe) and the development of PE in primiparous women at low risk. However, among women with bleeding disorders, the results indicated that the analysis of intensity, pattern, and frequency of bleeding may indicate the risk of subsequent development of PE.

There are some studies showing association between thrombin generation increase and the pathogenesis of PE.^{37,38} The excess of thrombin generated due to hemorrhage during placental development increases the expression of soluble feline McDonough sarcoma-like tyrosine kinase-1 (sFlt-1) by the trophoblast through the activation of the PAR-1/NADPH oxidase/ROS signaling pathway (specific receptors activated by proteinase).³⁹

There is evidence shown by the increased generation of thrombin in pregnant women with PE.^{40,41} This activation induces neutrophil recruitment, activation, and oxidation. The excess of tissue factor binds to platelets, causing ADP release. This release increases thrombin generation, which has a high affinity for PAR-1 in the syncytiotrophoblast, platelets, and neutrophils, thus causing cell activation.³⁹

Thus, thrombin increases the secretion of sFlt-1. Soluble feline McDonough sarcoma-like tyrosine kinase-1 is a receptor protein produced by syncytiotrophoblast, and its concentration in normal pregnancies is only a few times higher than that of placental growth factor (PlGF). It is related to the maternal endothelial dysfunction, a PE feature.^{5,42} In hypoxia or inadequate perfusion of the placenta, the trophoblast produces a large quantity of sFlt-1, and its concentration in

the maternal bloodstream is, at least, 12 times higher than the concentration of PlGF.⁴³

Increased sFlt-1 in maternal circulation is one of the elements that determines the PE maternal multisystemic syndrome. These changes in sFlt-1 concentration precede the onset of clinical and laboratory symptoms in preeclamptic women by ~ 5 to 6 weeks.⁴⁴ Therefore, laboratory tests to measure platelet activation and thrombin generation along with the sFlt-1 measurement could contribute to an early diagnosis of PE syndrome (► Fig. 2).

Platelets and Complement System Activation

In addition, evidence brought by few studies shows the involvement of the excessive increase in tissue factor (TF) with the activation of proteins C3 and C5 of the complement system. This activation is probably due to the stress generated by the syncytiotrophoblast. Although TF is important for placental development, its increase during trophoblast implantation and tissue hemorrhage exacerbates the activation of coagulation cascade, which has been the first hypothesis of abnormal implantation of trophoblast.⁴⁵

The activated platelets trigger the alternative complement pathway, especially the membrane attack complex (C5b9). And the activation of complement proteins may help to trigger PE and hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome. Burwick et al.⁴⁶ showed an increase in plasma concentration of C5b9 complement proteins in patients with gestational hypertension. The activation of the membrane attack complex in hypertensive disorders reflects endothelial dysfunction and systemic inflammation (► Fig. 3).

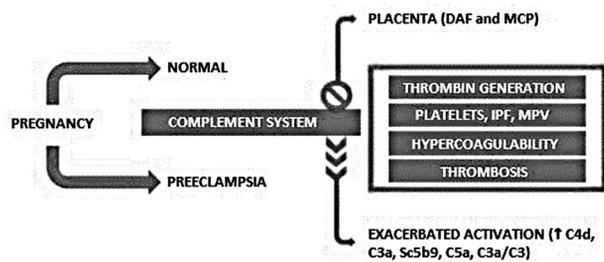


Fig. 3 Complement cascade in normal pregnancy and preeclampsia.

Conclusion

Platelets play a fundamental role in the pathophysiology of PE. We suggest that platelet activation in preeclamptic pregnancy is caused by the excess generation of thrombin associated with bleeding in the first trimester, increasing the release of antiangiogenic factors and activating the complement system before the onset of the clinical symptoms of the syndrome. Although platelet activation and increased IPF is confirmed in pregnant women who develop PE, these tests are not routinely performed for diagnosis. Laboratory essays for measuring IPF and thrombin generation are simple and easily accessible in laboratories that use advanced technology to perform them. They may be useful for the early diagnosis of this syndrome and the management of patients. Therefore, we believe further studies focused on these laboratory tests are required to enable early diagnosis and treatment of the disease.

Conflicts of Interests

The authors have no conflict of interests to declare.

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References

- 1 Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Preeclampsia. *Lancet*. 2010;376(9741):631–644. Doi: 10.1016/S0140-6736(10)60279-6
- 2 Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. *Eur J Obstet Gynecol Reprod Biol*. 2013;170(01):1–7. Doi: 10.1016/j.ejogrb.2013.05.005
- 3 Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol*. 2009;33(03):130–137. Doi: 10.1053/j.semperi.2009.02.010
- 4 Khan KS, Wojdyla D, Say L, Gülmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. *Lancet*. 2006;367(9516):1066–1074. Doi: 10.1016/S0140-6736(06)68397-9
- 5 Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003;111(05):649–658. Doi: 10.1172/JCI17189
- 6 Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. *Placenta*. 2009;30(Suppl A, Suppl A) S32–S37. Doi: 10.1016/j.placenta.2008.11.009
- 7 Quinn MJ. Pre-eclampsia - The "uterine reinnervation" view. *Med Hypotheses*. 2014;83(05):575–579. Doi: 10.1016/j.mehy.2014.08.020
- 8 Abou El Hassan M, Diamandis EP, Karumanchi SA, Shennan AH, Taylor RN. Preeclampsia: an old disease with new tools for better diagnosis and risk management. *Clin Chem*. 2015;61(05):694–698. Doi: 10.1373/clinchem.2014.230565
- 9 Tanrikulu L, Naraghi R, Ernst V, Voigt F, Hastreiter P, Doerfler A, et al. Neurovascular compression of medulla oblongata - Association for gestation-induced hypertension. *Med Hypotheses*. 2015;84(06):605–610. Doi: 10.1016/j.mehy.2015.03.024
- 10 Gathiram P, Moodley J. Pre-eclampsia: its pathogenesis and pathophysiology. *Cardiovasc J Afr*. 2016;27(02):71–78. Doi: 10.5830/CVJA-2016-009
- 11 Brew O, Sullivan MH, Woodman A. Comparison of normal and pre-eclamptic placental gene expression: a systematic review with meta-analysis. *PLoS One*. 2016;11(08):e0161504. Doi: 10.1371/journal.pone.0161504
- 12 Cunningham GF, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL, et al. *Williams obstetrics*. 24th ed. New York: McGraw-Hill Education; 2014
- 13 Ngene NC, Moodley J. Role of angiogenic factors in the pathogenesis and management of pre-eclampsia. *Int J Gynaecol Obstet*. 2018;141(01):5–13. Doi: 10.1002/ijgo.12424
- 14 Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science*. 2005;308(5728):1592–1594. Doi: 10.1126/science.1111726
- 15 Brenner B, Grabowski EF, Hellgren M, Kenet G, Massicotte P, Manco-Johnson M, et al. Thrombophilia and pregnancy complications. *Thromb Haemost*. 2004;92(04):678–681. Doi: 10.1160/TH04-02-0096
- 16 Järemo P, Lindahl TL, Lennmarken C, Forsgren H. The use of platelet density and volume measurements to estimate the severity of pre-eclampsia. *Eur J Clin Invest*. 2000;30(12):1113–1118. Doi: 10.1046/j.1365-2362.2000.00753.x
- 17 Annam V, Srinivasa K, Yatnatti SK, Suresh DR. Evaluation of platelet indices and platelet counts and their significance in pre-eclampsia and eclampsia. *Int J Biol Med Res*. 2011;2(01):425–428
- 18 Moraes D, Munhoz TP, Pinheiro da Costa BE, Hentschke MR, Sontag F, Lucas LS, et al. Immature platelet fraction in hypertensive pregnancy. *Platelets*. 2016;27(04):333–337. Doi: 10.3109/09537104.2015.1101060
- 19 Bernstein U, Kaiser T, Stepan H, Jank A. The immature platelet fraction in hypertensive disease during pregnancy. *Arch Gynecol Obstet*. 2019;299(06):1537–1543. Doi: 10.1007/s00404-019-05102-2
- 20 Mannaerts D, Heyvaert S, De Cordt C, Macken C, Loos C, Jacquemyn Y. Are neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and/or mean platelet volume (MPV) clinically useful as predictive parameters for preeclampsia? *J Matern Fetal Neonatal Med*. 2019;32(09):1412–1419. Doi: 10.1080/14767058.2017.1410701
- 21 Gogoi P, Sinha P, Gupta B, Firmal P, Rajaram S. Neutrophil-to-lymphocyte ratio and platelet indices in pre-eclampsia. *Int J Gynaecol Obstet*. 2019;144(01):16–20. Doi: 10.1002/ijgo.12701
- 22 Burrows RF, Kelton JG. Thrombocytopenia at delivery: a prospective survey of 6715 deliveries. *Am J Obstet Gynecol*. 1990;162(03):731–734. Doi: 10.1016/0002-9378(90)90996-k
- 23 Giles C, Inglis TC. Thrombocytopenia and macrothrombocytosis in gestational hypertension. *Br J Obstet Gynaecol*. 1981;88(11):1115–1119. Doi: 10.1111/j.1471-0528.1981.tb01764.x
- 24 Konijnenberg A, Stokkers EW, van der Post JA, Schaap MC, Boer K, Bleker OP, et al. Extensive platelet activation in preeclampsia compared with normal pregnancy: enhanced expression of cell

- adhesion molecules. *Am J Obstet Gynecol.* 1997;176(02): 461–469. Doi: 10.1016/s0002-9378(97)70516-7
- 25 Holthe MR, Staff AC, Berge LN, Lyberg T. Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. *Am J Obstet Gynecol.* 2004;190(04):1128–1134. Doi: 10.1016/j.ajog.2003.10.699
- 26 Macey MG, Bevan S, Alam S, Verghese L, Agrawal S, Beski S, et al. Platelet activation and endogenous thrombin potential in preeclampsia. *Thromb Res.* 2010;125(03):e76–e81. Doi: 10.1016/j.thromres.2009.09.013
- 27 Miao D, Li DY, Chen M, Zhao MH. Platelets are activated in ANCA-associated vasculitis via thrombin-PARs pathway and can activate the alternative complement pathway. *Arthritis Res Ther.* 2017;19(01):252. Doi: 10.1186/s13075-017-1458-y
- 28 Davey MG, Lüscher EF. Actions of thrombin and other coagulant and proteolytic enzymes on blood platelets. *Nature.* 1967;216(5118):857–858. Doi: 10.1038/216857a0
- 29 Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin SR. Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J Clin Invest.* 1999;103(06): 879–887. Doi: 10.1172/JCI6042
- 30 Linden MD. Platelet physiology. *Methods Mol Biol.* 2013; 992:13–30. Doi: 10.1007/978-1-62703-339-8_2
- 31 Smits LJ, North RA, Kenny LC, Myers J, Dekker GA, McCowan LM. Patterns of vaginal bleeding during the first 20 weeks of pregnancy and risk of pre-eclampsia in nulliparous women: results from the SCOPE study. *Acta Obstet Gynecol Scand.* 2012;91(11): 1331–1338. Doi: 10.1111/j.1600-0412.2012.01496.x
- 32 Everett C. Incidence and outcome of bleeding before the 20th week of pregnancy: prospective study from general practice. *BMJ.* 1997;315(7099):32–34. Doi: 10.1136/bmj.315.7099.32
- 33 Mulik V, Bethel J, Bhal K. A retrospective population-based study of primigravid women on the potential effect of threatened miscarriage on obstetric outcome. *J Obstet Gynaecol.* 2004;24(03):249–253. Doi: 10.1080/01443610410001660724
- 34 Hossain R, Harris T, Lohsoonthorn V, Williams MA. Risk of preterm delivery in relation to vaginal bleeding in early pregnancy. *Eur J Obstet Gynecol Reprod Biol.* 2007;135(02):158–163. Doi: 10.1016/j.ejogrb.2006.12.003
- 35 Weiss JL, Malone FD, Vidaver J, Ball RH, Nyberg DA, Comstock CH, et al; FASTER Consortium. Threatened abortion: A risk factor for poor pregnancy outcome, a population-based screening study. *Am J Obstet Gynecol.* 2004;190(03):745–750. Doi: 10.1016/j.ajog.2003.09.023
- 36 Dadkhah F, Kashanian M, Eliasi G. A comparison between the pregnancy outcome in women both with or without threatened abortion. *Early Hum Dev.* 2010;86(03):193–196. Doi: 10.1016/j.earlhumdev.2010.02.005
- 37 Xin H, Zhang Y, Wang H, Sun S. Alterations of profibrinolytic receptor annexin A2 in pre-eclampsia: a possible role in placental thrombin formation. *Thromb Res.* 2012;129(05):563–567. Doi: 10.1016/j.thromres.2011.07.039
- 38 Yin SM, Li YQ, Xie SF, Ma LP, Wu YD, Nie DN, et al. [Study on the variation of platelet function in pregnancy induced hypertension and gestational diabetes mellitus]. *Zhonghua Fu Chan Ke Za Zhi.* 2005;40(01):25–28 Chinese.
- 39 Gardiner C, Vatish M. Impact of haemostatic mechanisms on pathophysiology of preeclampsia. *Thromb Res.* 2017;151(Suppl 1):S48–S52. Doi: 10.1016/S0049-3848(17)30067-1
- 40 de Boer K, ten Cate JW, Sturk A, Borm JJ, Treffers PE. Enhanced thrombin generation in normal and hypertensive pregnancy. *Am J Obstet Gynecol.* 1989;160(01):95–100. Doi: 10.1016/0002-9378(89)90096-3
- 41 Halligan A, Bonnar J, Sheppard B, Darling M, Walshe J. Haemostatic, fibrinolytic and endothelial variables in normal pregnancies and pre-eclampsia. *Br J Obstet Gynaecol.* 1994;101(06): 488–492. Doi: 10.1111/j.1471-0528.1994.tb13147.x
- 42 Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med.* 2004;350(07):672–683. Doi: 10.1056/NEJMoa031884
- 43 Jardim LL, Rios DR, Perucci LO, de Sousa LP, Gomes KB, Dusse LM. Is the imbalance between pro-angiogenic and anti-angiogenic factors associated with preeclampsia? *Clin Chim Acta.* 2015; 447:34–38. Doi: 10.1016/j.cca.2015.05.004
- 44 Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Kim MR, Espinoza J, et al. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. *J Matern Fetal Neonatal Med.* 2005;17(01):3–18. Doi: 10.1080/14767050400028816
- 45 Lockwood CJ, Huang SJ, Krikun G, Caze R, Rahman M, Buchwalder LF, et al. Decidual hemostasis, inflammation, and angiogenesis in pre-eclampsia. *Semin Thromb Hemost.* 2011;37(02):158–164. Doi: 10.1055/s-0030-1270344
- 46 Burwick RM, Velásquez JA, Valencia CM, Gutiérrez-Marín J, Edna-Estrada F, Silva JL, et al. Terminal complement activation in preeclampsia. *Obstet Gynecol.* 2018;132(06):1477–1485. Doi: 10.1097/AOG.0000000000002980