Kosmalska Martyna, Znajewska-Szulc Karolina, Bronowski Adrian. Hemostasis – compendium for students. Journal of Education, Health and Sport. 2020;10(12):214-227. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2020.10.12.021
https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.12.021
https://zenodo.org/record/4405129

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.
© The Authors 2020;
This article is published with open access at Licensee Open Journal Systems of Nicolaus Copernicus University in Torun, Poland
Open Access. This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author (s) and source are credited. This is an open access article licensed under the terms of the Creative Commons Attribution Non commercial license Share alike. (http://creativecommons.org/licenses/by-nc-sa/4.0/) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.
The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 20.12.2020. Revised: 29.12.2020. Accepted: 31.12.2020.

Hemostasis – compendium for students

Martyna Kosmalska[1], Karolina Znajewska-Szulc[2], Adrian Bronowski[1]

1 – Students' Scientific Circle of Laboratory Hematology operating at the Department of Pathophysiology, Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland
Supervisor: Associate Professor Artur Słomka, PhD, DSc
2 - The Franciszek Lukaszczyk Oncology Centre in Bydgoszcz, Poland

Summary

Hemostasis is one of many well-known systems that ensure a constant conditions of the organism's internal environment. This process is usually divided into two main stages: coagulation and fibrinolysis. Both of them occur simultaneously and under physiological conditions they are in dynamic equilibrium. There are primary and secondary hemostasis. The primary hemostasis leads to the formation a platelet clot that forms almost directly after a blood vessel has been ruptured. In the other hand, the secondary hemostasis is the activation of coagulation, which leads to the strengthening of the platelet plug through the fibrin network formation as a result of the polymerization of the soluble plasma protein fibrinogen. The key role in hemostasis plays the interaction of platelets – which are the smallest non nucleated blood cells that are formed from the cytoplasm of megakaryocytes, and plasma coagulation factors with vascular endothelial cells and subendothelial tissues. In this report it is decribed the process of physiological hemostasis, especially the newer concept of blood coagulation.

Key words: hemostasis; platelets; blood coagulation; blood coagulation factors; endothelium

Introduction

Hemostasis is a set of mechanisms that are responsible for keeping blood in a liquid state in the blood vessels, keep them tight and also prevent bleeding at the site of damage to the wall of blood vessel. This happens by forming a platelet clot and then a fibrin clot. [1] This is one of many well-known systems that ensure a constant conditions of the organism's internal environment, i.e. homeostasis. Hemostasis is usually divided into two main stages: coagulation and fibrinolysis. Both of these process occur simultaneously and under physiological conditions they are in dynamic equilibrium. The dominance any of them, which is observed in many pathological conditions, leads to either blood clots or bleeding. [2] There are primary and secondary hemostasis. The primary hemostasis leads to the formation of a platelet clot that forms almost directly after a blood vessel has been ruptured. That is a result of platelet adhesion to damaged endothelium, their aggregation and also vasoconstriction. In contrast, secondary hemostasis is the activation of coagulation, which leads to the strengthening of the platelet plug through the fibrin network formation as a result of the polymerization of the soluble plasma protein fibrinogen. Its growth, and thus clot formation, is prevented by endogenous inhibitors of blood coagualation. The fibrin that builds the resulting hemostatic plug is disolved by the activity of the fibrinolytic system and its other components are removed by the feeding cells. The key role in hemostasis plays the interaction of platelets and plasma coagulation factors with vascular endothelial cells and subendothelial tissues. [1] In this report it is decribed the process of physiological hemostasis, especially the newer concept of blood coagulation.

Vascular hemostasis

The wall of a blood vessels consists of three layers: the inner layer, also known as the intima, the middle layer and the outer layer, called adventita. Primary haemostasis is mainly attended by the inner membrane, which consists of a single layer of endothelial cells (endothelium) and subendothelial connective tissue. The role of the endothelium, which is in the constant contact

with the blood flowing in the blood vessels, is to maintain its in a liquid state by inhibiting not only the aggregation of platelets, but also the coagulation cascade reaction and also by enhancing the fibrinolysis process. [5] What is more, the endothelium modulates the permeability of the vascular wall, maintains a balance in the production of inhibitors and activators of fibrinolysis and creates a physiological barrier between the circulating blood and the subendothelial matrix, rich in proteins with strong adhesive properties. [6] A different role plays the subendothelial matrix, which includes adhesins produced and released by endothelial cells, such as collagen, laminin, fibronectin, vitronectin and von Willebrand factor (vWF - von Willebrand factor). To these components a platelets adhesion is observed when the blood vessel wall has been injured. Moreover, tissue factor (TF) that initiates blood coagulationis is present in the subendothelial layer. [7] The endothelium appears a function also as a biologically active tissue capable of synthesizing compounds that promote and control hemostatic function. The luminal surface of all vascular endothelial cells is covered by the glycocalyx, which comprises membrane-bound negatively charged glycolipids and glycosaminoglycans. It has anticoagulant properties - reduces the activity of antithrombin (AT) and reduces the amount of active factor X and thrombin in the bloodstream. Endothelial cells produce thrombomodulin (TM) and the endothelial protein C receptor (EPCR) which are part of protein C anticoagulant system. Endothelium also has a significant impact on the functioning of the fibrinolysis system, primarily through the synthesis and release into the blood of both activators and inhibitors of plasminogen. Many factors that effectively influence the adhesion, activation and aggregation of platelets, which are also having a vasomotor effect, come from endothelial cells. The most important of them are: prostacyclin (PGI_2), endothelin, nitric oxide (NO) and platelet activating factor (PAF). [8] **Figure 1**. A disturbed structure or impaired function of the vascular wall may cause an excessive bleeding. This is known as a vascular hemorrhagic diathesis. On the other hand, the loss of the anticoagulant properties of the endothelium can lead to the formation of blood clots. [7]

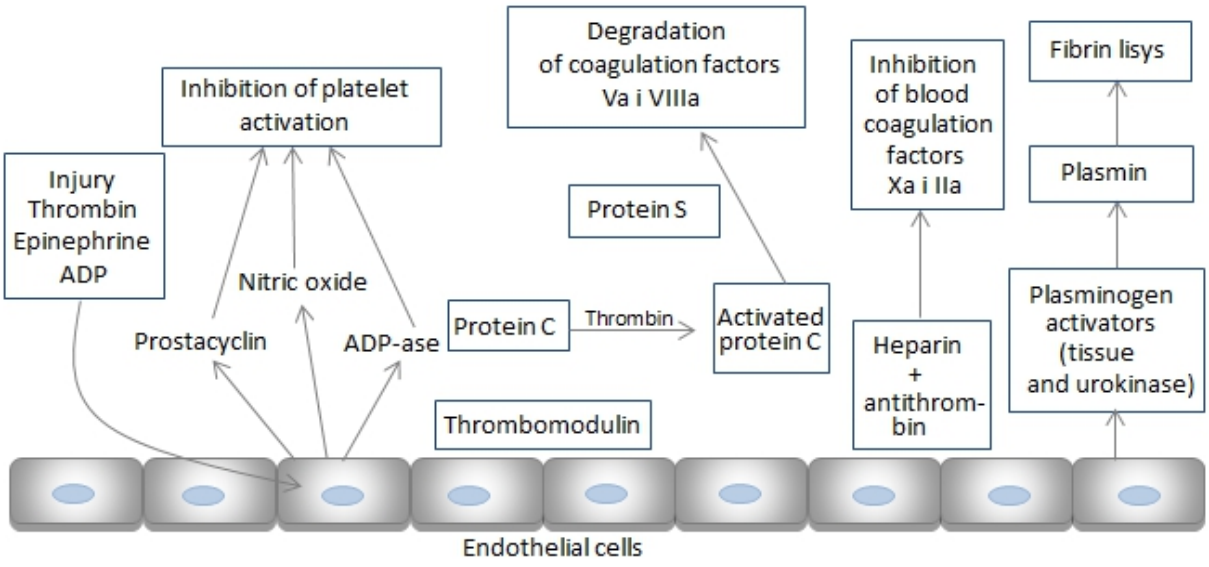

Figure 1. Participation of endothelial cells in hemostasis

Platelets

Platelets are the smallest non nucleated blood cells that are formed from the cytoplasm of megakaryocytes, their precursor cells, which reside in the bone marrow. [9] They have a discoidal shape with a diameter of 3-4 μm and a volume of 5-10 μm3. The normal range of platelet counts are between 140-440 G/L in healthy people. About 30% of them is in the spleen (so-called „splenic pool"), the rest of the platelets circulate in the blood vessels. Their survival time is 5-9 days, after which they are removed by cells of the reticuloendothelial system. [10] The platelet is surrounded by a cell membrane formed by two layers of lipids (internal and external) in which proteins are distributed. [11] Many of them pass through both lipid layers in such a way that their ends are present both on the inside and outside. The outer ends are binding to oligosaccharides and form glycoproteins (GPs), which are receptors for many factors that stimulate or inhibit platelet activity. The most abundant membrane lipids are the phospholipids. [7] There are several types (at least three) of granules in platelet cytoplasm. (**Table 1**) The largest group is an α granules, which consist of adhesive proteins such as fibrinogen, fibronectin, vWF, growth factors such as platelet factor 4 (PF4 - platelet-derived growth factor) and thrombospondin as well as coagulation and fibrinolysis factors, i.e. factor

V, factor XI, high molecular-weight kininogen (HMWK), protein S and plasminogen activator inhibitor-1 (PAI-1). [12] The content of α granules undergoes rapid exocytosis during platelet activation, and releases mediators which gradually enhance hemostasis. [13] In turn, adenine nucleotides (ADP and ATP), catecholamines, magnesium and calcium ions, serotonin and pyrophosphates are stored in dense granules, also called δ granules, which are smaller than α-granules. In addition, the acid hydrolases are present in the few lysosomes and the catalase is a component of the most rarely peroxisomes. [14] As a result of platelet activation, the content of granules α and δ are released into the external environment. Activated platelets express negative phospholipids and P-selectin on the exterior surface of their cells and then they are associated with coagulation factors involved in blood clotting. [15] On the surface of platelets, in the plasma membrane and within the granularity of α there are glycoprotein platelet receptors. The most important role is played by GP IIb/IIIa and GP Ib/IX/V. Fibrinogen is the main ligand of GP IIb/IIIa, so that is the most important mediator of platelet aggregation. This complex, belongs to the integrin receptor family, has a relatively low affinity for fibrinogen on the resting plate. Only as a result of platelet activation, and thus conformational changes of GP IIb/IIIa, the glycoprotein's ability to bind fibrinogen increase. It is necessary to form fibrinogen bridges between platelets to form their aggregate. [16] In turn, GP Ib/IX/V is a key receptor for von Willebrand factor. This complex is mainly responsible for the course of the plate adhesion process to the endothelial matrix. Its binding to vWF also contributes to platelet aggregation both directly and by GP IIb/IIIa activation. [17]

Table 1. The content of platelet granules

Platelet granule contents, surface molecules and platelet-derived mediators involved in inflammation and immunity	
Granules	Adhesive glycoproteins P-selectin, fibrinogen, vWF, fibronectin, thrombospondin Coagulation factors Factor V, protein S, factor XI, factor XIII Mitogenic factors PDGF, TGF-, EGF Angiogenic factors VEGF, PF4 inhibitor Fibrinolytic inhibitors 2-plasmin inhibitor, PAI–1 Immunoglobulins Granule membrane- specific proteins P-selectin, CD 63, GMP 33 Chemokines CXCL7, CXCL4 (PF4), CXCL1 (GRO), CXCL5, CCL5 (RANTES), CCL3 (MIP1)
Dense granules	Amines Serotonin (5-HT), histamine Bivalent cations Ca^{2+}, Mg^{2+} Nucleotides ATP, ADP, GTP, GDP
Lysosome granules	Acid proteases Carboxypeptidases (A, B), cathepsins D,E, Acid phosphatase, collagenase Glycohydrolases Heparinase, -N-acetyl-glucosaminidase, -glucoronidase, -glycerophosphatase, -galactosidase, -D-glucosidase, -L-fucosidase, -D-fucosidase
Other soluble mediators	Other molecules with immune functions: CCL7 (MCP3), IL1 , HMGB1, Defensins, thromboxane A2, PAF, sCD40L
Plasma membrane	TLR1, TLR2, TLR5, TLR4, TLR6, CD40 CD40L, TREM-1 ligand

VWF - von Willebrand factor; PDGF - platelet-derived growth factor; TGF - transforming growth factor, EGF - epidermalgrowth factor; PAI-1 - plasminogen activator inhibitor 1; PAF - platelet activating factor; TLR - Toll-like receptor.

In addition to the glycoprotein complexes described above, there are some other receptors on the platelet surface, mainly interacting directly with collagen. These are GP IV (collagen II and thrombospondin receptor), GP VI (collagen receptor), GP Ia/IIa (collagen receptor), CD9

(fibronectin receptor) and associated with platelet cytoskeleton platelet-endothelial cell adhesion molecule-1 (PECAM-1). Collagen receptors are used to capture the platelets moving with the blood and bind them at the site of damage. [18] Moreover, the GP VI receptor plays the most important role in the process of initiating the activation of platelets. [19] The effect of its stimulation is the local release of platelet activating factors, such as ADP or thromboxane (TxA2). [20] These biologically active substances recruit more platelets, thus enhancing their aggregation.

The agonists of platelet receptors located inside the granules also include serotonin. Collagen and thrombin are strong activators of platelets, which are not found in intra-platelet granules. Thrombin is considered to be the strongest physiological activator of platelets. [21] It is formed in the plasma under the influence of the tissue factor (TF) which is released upon tissue injury. [22] Thrombin catalyzes the conversion of fibrinogen into fibrin, and is therefore a common element of primary and secondary hemostasis. [23,24]

The role of platelets in hemostasis

Platelets are considered to be important players in primary haemostasis. We can distinguish the several-stage participation of platelets in the blood coagulation process: adhesion, activation and aggregation. [18,25] At the site of damage to the vascular wall, the collagen-rich subendothelial layer of the vessel is exposed. Then the platelet glycoproteins GP VI and GP Ia/IIa are bound to it directly or GP Ib/IX/V and GP IIb/IIIa indirectly by vWF. On the one hand, the von Willebrand factor interacts with a specific receptor located in the subendothelium, and on the other hand, with the platelet receptor, in particular glycoprotein Ib. This leads to the accumulation of platelets at the site of damage as a single layer of cells. [25] It is the first step in primary hemostasis, and this is known as adhesion. This process depends to a large extent on the rheological conditions. In small vessels where the flowing blood generates a high shear force, the binding of platelets to the subendothelial matrix is mediated by vWF. In larger vessels, where low shear force is generated by blood flow, platelets interact with collagen without other proteins. As a consequence of binding to collagen, intracellular activation systems are activated, which results in the transition of platelets from a resting to an active state. [7] Then it is observed a change in the shape of the platelets from discoidal to spherical. [10] As a result of changes in metabolism and the reorganization of the cytoskeleton of the platelets, they become irregular cells with numerous protrusions, called pseudopodia. [7,26] Various receptors are also expressed on their surface, including receptors for fibrinogen.

At the same time, it takes place a calcium ion-dependent degranulation process, consisting in the release of biologically active substances stored in the granules, which further intensify the activation and aggregation of subsequent platelets. The above described phenomenon is called activation. There are probably two pathways for platelet activation but the metabolic integration processes required for platelet activation are not fully clear and understood. The first is the degradation of phosphatidylinositol 4,5-bisphosphate (PIP_2). This pathway probably plays an important role in the initial activation of GP IIb/IIIa as well as the subsequent stabilization interactions of GP IIb/IIIa-fibrinogen, which ultimately leads to irreversible aggregation of platelets. [27] The second metabolic pathway is the arachidonic acid cascade, leading to the formation in platelets of the highly reactive arachidonic acid metabolite of the cyclooxygenase pathway - thromboxane A2. [28]

Similar processes taking place in endothelial cells lead to the synthesis of prostacyclin (PGI_2), which in turn inhibits platelet aggregation and has vasodilating properties. The GP IIb/IIIa, which is a receptor for fibrinogen, plays a key role in the process of platelet aggregation. The platelet acquires the ability to bind fibrinogen only after agonist-induced GP IIb/IIIa conformation change. [29] Moreover, the activation of platelets is accompanied by the exposure of many more molecules of this complex to the surface of the platelets, as a result of their movement from the intraplatelet pool. Due to the fact that fibrinogen has a dimeric structure, one of its molecules is able to bind a two adjacent platelets. The fibrinogen bridges formed in this way between the platelets are essential for the platelet aggregation process, regardless of the type of activating agent. The end result is that a hemostatic platelet plug closes up the damaged site of the blood vessel and that controls the bleeding. [30] Subsequently, it is strengthened by the fibrin network and at the same time transformed into a clot.

Activated platelets also participate in the next stages of the blood clotting process, and their role is to make available negatively charged phospholipids, primarily phosphatidylserine (PS) and phosphatidylethanolamine (PE), on which enzyme-cofactor-substrate complexes are formed by coagulation factors. [31] Under the influence of activating factors, phospholipids which have procoagulant properties are exposed on the surface of the platelets' membrane. Factor IXa binds to factor VIIIa on anionic platelet surfaces to form the intrinsic tenase complex which activates factor X. In the same way, the prothrombinase complex is formed on the platelet surface by the interaction of factor Xa, Va and platelet phospholipoproteins. It is responsible for the conversion of prothrombin to thrombin (IIa). Both reactions require calcium ions. The extremely high speed and efficiency of the clotting reaction is made

possible by PS and PE. At the same time, it is limited only to the site of damage to the vascular wall, thus preventing the transformation of a physiological clot into a pathological clot.

Secondary hemostasis

The transformation of fibrinogen, which is a soluble plasma protein, into a spatial network of fibrin, which strengthens the hemostatic platelet plug under the influence of thrombin, is crucial in the blood coagulation process. It takes place with the participation of a several different factors (**Table 2**), including 12 coagulation factors, which are plasma proteins, tissue factor, that is an integral protein of cell membranes, phospholipids of cell membranes and calcium ions. [7] The clotting factors can be divided into three groups:

1) The factors included in the prothrombinase complex – II, VII, IX i X
2) The factors affected by thrombin – I, V, VIII i XIII
3) The contact factors of hemostasis – XI, XII, prekalikrein and high molecular weight kininogen (HMWK)

Some of the clotting factors are present in the plasma in the form of inactive proenzymes, also known as the zymogens. These are serine proteases including FII (Blood-coagulation factor II, prothrombin), FVII (Blood-coagulation factor VII, proconvertin), FIX (Blood-coagulation factor IX), FX (Blood-coagulation factor, Stuart-Prower factor), FXI (Blood -coagulation factor XI), FXII (Blood-coagulation factor XII) and prekalikrein. Their transformation into active forms is accomplished by limited proteolysis.

Blood clotting occurs in a multi-step process known as the coagulation cascade. The cascade is a chain reaction in which one step leads to the next. In general, each step produces a new protein which acts as an enzyme, or catalyst, for the next step. Moreover, in the following reactions, we are dealing with a multiplication of the number of active substrate molecules. This is because a small amount of the enzyme hydrolyzes more substrate molecules. [7] In the blood coagulation cascade, the enzyme-cofactor complex is taken as the functional unit.

Table 2. Blood coagulation factors

Factor	Synonym	Role in blood coagulation
I	Fibrinogen	Fibrin precursor
II	Prothrombin	Proenzyme
III	Tissue factor	Cofactor
IV	Calcium ions	Cofactor
V	Proaccelerin	Cofactor
VI	Accelerin	-
VII	Convertin	Proenzyme
VIII	Antihemophilic factor A	Cofactor
IX	Antihemophilic factor B	Proenzyme
X	Stuart factor	Proenzyme
XI	Antihemophilic factor C	Proenzyme
XII	Hageman factor	Proenzyme
XIII	Fibrin stabilizing factor	Proenzyme
Prekallikrein	Flether factor	Proenzyme
High molecular kininogen	Fitzgerald factor	Cofactor

To summarizing, the whole process is based on the formation of three enzyme complexes with a homologous structure and function. They consist of a vitamin K-dependent serine protease, a protein cofactor and a substrate. The place of their formation is the negatively charged surface of phospholipids provided mainly by active platelets. There are approximately 2,800 binding sites on each of them. Moreover, in addition to platelets, lymphocytes, monocytes, and activated endothelial cells can also be source of negatively charged phospholipids.

There are two pathways to formation of the prothrombinase complex: (**Figure 2**)

– the intrinsic pathway that FXII, HMWK and prekalikrein, called contact factors, are activated by contact with negatively charged surfaces;

– the extrinsic pathway that the clotting initiator is a tissue factor. [7]

Thanks to the separation of two pathways, the interpretation of blood coagulation tests in vitro

has become easier. The ignition factor for the intrinsic pathway is the activation of factor XII as a result of its contact with the negatively charged collagen surface, exposed as a result of damage to the vascular wall, as well as a result of contact with sulfonated glycolipids or acid mucopolysaccharides. The simultaneous absorption of prekalikrein and HMWK enables faster and more efficient activation of FXII. Under the influence of FXIIa (Activated blood-coagulation factor XII), FXI is activated on negatively charged surfaces, which in turn activates FIX. Next, FIXa (Activated blood-coagulation factor IX) in the presence of FVIIIa (Activated blood-coagulation factor VIII), phospholipids and calcium ions activates factor X. This leads to the degradation of prothrombin (FII) into thrombin (FIIa - activated blood-coagulation factor II) [15, 32, 33] It is a common step to both ways of clotting activation.

A key role in the process of coagulation initiation in vivo plays the tissue factor-dependent pathway. [34] As a result of vessel damage, the tissue factor is exposed to the surface of fibroblasts or monocytes. Then, on the surface of these cells, in the presence of calcium ions, FVII forms an active TF-VIIa complex with TF. The FVII activation can occur under the influence of thrombin, FXIa (Activated blood-coagulation factor XI), FXIIa, FXa (Activated blood-coagulation factor X) and plasmin. As a result, a much more of FVIIa is formed in the circulating blood than other active clotting factors. The main task of the cell surface-bound TF-VIIa complex is the activation of FX to FXa [35,34] The active factor X can initiate the degradation of prothrombin into thrombin without the participation of its cofactor, FVa (Activated blood-coagulation factor V). Then it is formed a small amount of thrombin, and a specific inhibitor of the extrinsic pathway of coagulation (TFPI - tissue factor pathway inhibitor) quickly inactivates the Xa-VIIa-TF complex. [36] This amount of thrombin is too small to form a stable fibrin. However, it is sufficient to activate platelets, separate FVIII (Blood-coagulation factor VIII) from vWF and convert FV (Blood-coagulation factor V), FVII and FXI to their active forms. This in turn leads to the formation of much more thrombin, which is known as a thrombin burst. Thus a large amount of thrombin, that causes the conversion of fibrinogen to fibrin, is produced on the intrinsic pathway. According to the new concept of the coagulation cascade, the physiological activator of FXI is not only FXIIa as previously thought, but also thrombin formed on the extrinsic pathway.

The conversion of fibrinogen (FI - Blood-coagulation factor I) to fibrin (FIa - Activated blood-coagulation factor I) is the last stage of the blood coagulation. This happens under the influence of thrombin, which splits off two pairs of small fibrinopeptides A and B from fibrinogen. As a result, the obtained fragment, which is a fibrin monomer, under physiological conditions polymerizes and forms a fibrin network. [37] In the final step of blood coagulation,

FXIII (Blood-coagulation factor XIII) catalyse the fibrin polymer stabilization process. Under the influence of thrombin and calcium ions, FXIII acquires the properties of transglutaminase and leads to the formation of covalent cross-links between adjacent fibrin monomers. [38]

References

1. Gale AJ. Current Understanding of Hemostasis. Toxicologic Pathology. 2011;39(1);273–280.
2. Kluft C, Burggraaf J. Introduction to haemostasis from a pharmacodynamic perspective. British Journal of Clinical Pharmacology. 2011;72(4);538–546.
3. Wagner DD, Frenette PS. The vessel wall and its interactions. Blood. 2008;111(11);5271-5281.
4. Sandoo A, Veldhuijzen van Zanten JCS, Metsios GS, Carroll D, Kitas GD. The Endothelium and Its Role in Regulating Vascular Tone. The Open Cardiovascular Medicine Journal. 2010;4;302-312.
5. Verhamme P, Hoylaerts MF. The Pivotal Role of the Endothelium in Haemostasis and Thrombosis. Acta Clinica Belgica. 2006;61(5);213-219.
6. Yau WJ, Teoh H, Verma S. Endothelial cell control of thrombosis. BMC Cardiovascular Disorders. 2015;15(1);130.
7. Windyga J, Undas A: Hemostaza fizjologiczna. [Physiological haemostasis]. In: Windyga J, Pasierski T, Torbicki A. Zakrzepy i zatory. [The blood clots and embolism] Wydawnictwo Lekarskie PZWL. Warszawa. 2014;1-36.
8. Wu KK, Thiagarajan P. Role of Endothelium in Thrombosis and Hemostasis. Annual Review of Medicine. 1996;47;315–331.
9. Periayah MH, Halim SA, Saad AZM. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. International Journal of Hematology-Oncology and Stem Cell Research. 2017;11(4);319–327.
10. Ghoshal K, Bhattacharyya M. Overview of Platelet Physiology: Its Hemostatic and Nonhemostatic Role in Disease Pathogenesis. The Scientific World Journal. 2014;1-16.
11. Pleban E. Hemostaza – temat zawsze aktualny. [Hemostasis – always a topical issue]. Pediatria i Medycyna Rodzinna. 2015;11(2);166-176.
12. Jenne CN, Urrutia R, Kubes P. Platelets: bridging hemostasis, inflammation, and immunity. International Journal of Laboratory Hematology. 2013;35;254–261.
13. Korzonek-Szlacheta I, Hudzik B, Zubelewicz-Szkodzińska B, Gąsior M. Płytki krwi –

ogniwo łączące zakrzepicę ze stanem zapalny. [The platelets – the link between thrombosis and inflammation]. Folia Cardiologica. 2018;13(4);303-308.

14. Holinstat M. Normal platelet function. Cancer Metastasis Review. 2017;36(2);195-198.

15. Yun SH, Sim EH, Goh RY, Park JI, Han JY. Platelet Activation: The Mechanisms and Potential Biomarkers. BioMed Research International. 2016;1-5.

16. Sivaraman B, Latour RA. Delineating the Roles of the GPIIb/IIIa and GP-Ib-IX-V Platelet Receptors in Mediating Platelet Adhesion to Adsorbed Fibrinogen and Albumin. Biomaterials. 2011;32(23);5365-5370.

17. Li R, Emsley J. The Organizing Principle of Platelet Glycoprotein Ib-IX-V Complex. Journal of Thrombosis and Haemostasis.2013;11(4);605-614.

18. Kubica J, Koziński M, Grześk G. Mechanizmy działania leków przeciwpłytkowch. [Mechanisms of action of antiplatelet drugs]. Folia Cardiologica Excerpta. 2009;4(1);10-17.

19. Broos K, Feys HB, De Meyer SF, Vanhoorelbeke K, Deckmyn H. Platelets at work in primary hemostasis. Blood Reviews. 2011;25;155–167.

20. Thonand JN, Italiano JE. Platelets: production, morphology and ultrastructure. Handbook of Experimental Pharmacology. 2012;210;3–22.

21. Ni H, Freedman J. Platelets in hemostasis and thrombosis: role of integrins and their ligands. Transfusion and Apheresis Science. 2003;28;257–264.

22. Drozdowska J. Czynnik tkankowy w komórkach śródbłonka — budowa i funkcja w świetle wyników najnowszych badań. [Tissue factor in endothelial cells – its structure and function according to the current literatutre]. Postępy Biochemii. 2012;58(3);273-280.

23. Van der Meijden PEJ, Heemskerk JWM. Platelet biology and functions: new concepts and clinical perspectives. Nature Reviews Cardiology. 2019;16(3);166-179.

24. Tillman BF, Gruber A, McCarty OJT, Gailani D. Plasma contact factors as therapeutic targets. Blood Reviews. 2018;32(6);433-448.

25. Feghhi S, Munday AD, Tooley WW, Rajsekar S, Fura AM, Kulman JD, Lo pez JA, Sniadecki N.J. Glycoprotein Ib-IX-V Complex Transmits Cytoskeletal Forces That Enhance Platelet Adhesion. Biophysical Journal. 2016;111; 601–608.

26. Nowak P, Olas B, Wachowicz B. Stres oksydacyjny w przebiegu hemostazy. [Oxidative stress in haemostasis]. Postępy Biochemii. 2010;56 (3);239-247.

27. Yun SH, Sim EH, Goh RY, Park JI, Han JY. Platelet Activation: The Mechanisms and Potential Biomarkers. BioMed Research International. 2016;1-5.

28. Fe le tou M, Huang Y, Vanhoutte PM. Endothelium-mediated control of vascular tone: COX-1 and COX-2 products. British Journal of Pharmacology. 2011;164(3);894– 912.

29. Schneider DJ. Anti-platelet therapy: glycoprotein IIb-IIIa antagonists. British Journal of Clinical Pharmacology. 2011;72(4);672-682.
30. Hinsbergh Victor WM. The endothelium: vascular control of haemostasis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2001;95(2);198-201.
31. Hou Y, Carrim Naadiya, Wang Y, Gallant RC, Marshall A, Ni H. Platelets in hemostasis and thrombosis: Novel mechanisms of fibrinogen-independent platelet aggregation and fibronectin-mediated protein wave of hemostasis. The Journal of Biomedical Research. 2015;29(6);437-444.
32. Wheeler AP, Gailani D. The Intrinsic Pathway of Coagulation as a Target for Antithrombotic Therapy. Hematology/Oncology Clinics of North America. 2016;30(5); 1099-1114.
33. Smith SA, Travers RJ, Morrissey JH. How it all starts: initiation of the clotting cascade. Critical Reviews in Biochemistry and Molecular Biology. 2015;50(4);326-336.
34. Mackman N, Tilley RE, Key NS. Role of the Extrinsic Pathway of Blood Coagulation in Hemostasis and Thrombosis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007;27; 1687–1693.
35. Butenas S. Tissue Factor Structure and Function. Scientifica. 2012;1-15.
36. Vadivel K, Ponnuraj SM, Kumar Y, Zaiss AK, Bunce MW, Camire RM, Wu L, Evseenko D, Herschman HR, Bajaj MS, Bajaj SP. Platelets Contain Tissue Factor Pathway Inhibitor-2 Derived from Megakaryocytes and Inhibits Fibrinolysis. Journal of Biological Chemistry. 2014;289(45);31647-31661.
37. Antoniak S. The coagulation system in host defense. Research and Practice in Thrombosis and Haemostasis. 2018;2(3);549–557.
38. Mackman N. The Role of Tissue Factor and Factor VIIa in Hemostasis. Anesthesia & Analgesia. 2009;108(5);1447-1452.