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REVIEW

Microbial Modulation of Coagulation Disorders in Venous Thromboembolism

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Abstract: Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), is the third leading cause of cardiovascular death in the world. Important risk factors of thrombosis include bed restraint, surgery, major trauma, long journeys, inflammation, pregnancy, and oral contraceptives, previous venous thromboembolism, cancer, and bacterial infections. Sepsis increases the risk of blood clot formation 2–20 times. In this review, we discussed various mechanisms related to the role of bacteria in venous thrombosis also taking into consideration the role of the human microbiome. Many known bacteria, such as *Helicobacter pylori, Chlamydia pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus*, and *Escherichia coli*, causing infections may increase the risk of thrombotic complications through platelet activation or may lead to an inflammatory reaction involving the fibrinolytic system. Additionally, the bacteria participate in the production of factors causing or increasing the risk of cardiovascular diseases. An example can be trimethylamine N-oxide (TMAO) but also uremic toxins (indoxyl sulfate), short-chain fatty acids (SCFA) phytoestrogens, and bile acids. Finally, we presented the involvement of many bacteria in the development of venous thromboembolism and other cardiovascular diseases.

Keywords: sepsis, microbiome, inflammation, deep vein thrombosis, trimethylamine N-oxide

Introduction

According to the Virchow hypothesis, blood clot formation is affected by slow blood flow, an impaired balance between hemostasis and the coagulation process (hypercoagulation), and endothelium damage. Additionally, leukocytes, platelets, tissue factor-positive microvesicles, neutrophil extracellular traps (NETs) and factors XI and XII participate in clot formation.[1] Factors that provoke thrombosis can be long-term immobilization caused by illness or surgery (especially orthopedic), long hours of plane flight, long driving without stops, excessive coagulation, high hematocrit, and cancer. For women, the use of oral contraceptives and pregnancy are additional risk factors.[2,3]

Other risk factor is sepsis, which is a serious epidemiological and therapeutic problem, mainly at intensive care units.[4] The causes of sepsis include intra-abdominal infections, pneumonia, meningitis, and urinary tract infections. It can also occur in people with low immunity. Pathogens that promote sepsis and are involved in the development of thrombosis include both gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) and gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Chlamydia pneumoniae, Helicobacter pylori, Haemophilus influenzae*).[5]

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The result of sepsis caused by gram-negative bacteria is endotoxemia, ie the appearance of released lipopolysaccharide (LPS, endotoxin) in the host's blood. The consequence of endotoxemia is the septic shock, one key element of which is intravascular coagulation.[6] It was demonstrated that fully bacterial LPS has a direct and primary role in inducing significant changes in the structure of fibrin fibers. It was also proven that LPS binds directly to fibrinogen. It was found that a small amount of LPS significantly affects fibrinogen and the coagulation process, which may indicate the involvement of a very small number of inactive bacteria in inducing inflammation and disease progression.[7]

As the studies of Patrakka et al[8] show, streptococci participate in infective endocarditis and have thrombotic properties. An important evidence for bacteria association with these diseases was the detection of deoxyribonucleic acid (DNA) in the coronary thrombosis aspirates of myocardial infarction and cerebral aneurysms. Hence, it has been proven that chronic infections can lead to a coronary artery disease and ischemic stroke.

Although thrombosis is often associated with an infection, the mechanisms that lead to its formation are relatively poorly understood. In this paper, we discussed the effect of bacteria/sepsis on the induction of thrombosis. We presented the various mechanisms involved in the participation of bacteria in venous thrombosis. The human microbiome and the presence of bacteria in blood vessels were also described. Then, we discussed the phenomenon of thrombosis and the frequency of its occurrence. Also, we presented the mechanisms of thrombosis induced by *Staphylococcus aureus* and other bacteria directly affecting the coagulation process.[9] We further showed the possibilities of bacteria binding to platelets and their activation. Then, we described the intestinal microbiome and the participation of its various bacteria in cardiovascular diseases. Finally, we presented the involvement of many bacteria in the development of cardiovascular diseases including thrombosis.

Epidemiology and Risk Factors

The incidence of venous thromboembolism (VTE) is difficult to assess because it often has an asymptomatic course.[10] Sometimes, it is misdiagnosed and unrecognized even after the patient's death due to the lack of routine post-mortem examinations.[11] VTE is the third cardiovascular cause of death after myocardial infarction and stroke, and increases steeply with age.[12] It is estimated that almost two-thirds of VTE cases are related to deep vein thrombosis (DVT), and 80% are proximal.[13] In the European population, there are about 70–140 DVT cases per 100 000 people a year.[14]

Thrombosis or VTE in more than 90% of cases refers to the limbs, especially to the vein of the leg and concerns deep veins, because approx. 90% of venous blood flows in the deep venous system of the lower extremities. Thrombosis may also occur in superficial veins, but it may also occur in other veins, such as liver veins.[15] All cases of thrombosis require immediate medical intervention, especially pulmonary embolism (PE), which is directly life-threatening. From the clot formed, small clots can detach and can be transported to the right ventricle of the heart and from there to the lungs.[13]

VTE is a common in patients undergoing major surgeries such as hip or knee arthroplasty, and abdominal cancer surgery. It was shown that the cause of thrombosis were the implanted vascular stents.[16] Release of IL-6, IL-8 and tumor necrosis factor alfa (TNF-α) cytokines is a response to systemic inflammation which leads to an increased risk of thrombosis.[17] It is believed that systemic infections increase the risk of deep vein thrombosis/pulmonary embolism about 2–20 times.[5] An additional risk factor for VTE is the age of the patients, the presence of malignant tumors, and hereditary features.[3] It is also known that the formation of blood clots in vessels occurs during sepsis, which indicates the participation of bacteria in this process. The factors that influence the occurrence of thrombosis have been presented. However, there are also those on which we have a direct impact including: sedentary lifestyle, obesity, smoking, long journeys by car, train or bus for 4 or more hours.

Bacteria Forming the Human Microbiome in Blood Vessels

Results obtained in the Human Microbiome Project revealed a great number of bacteria, viruses, fungi, and archaea inhabiting human body.[18] According to a recent study, there are over 3.8×10^{13} microorganisms living in different body sites.[19] The nature of the host-microbial interaction is complex and has a profound impact on human health.[20] This is a challenge taken up by vast research conducted according to metagenomic and metabolomic protocols after the introduction of the next-generation sequencing (NGS) and whole metagenome shotgun (WMGS) sequencing. Dysbiosis, understood as a perturbation in microbiome composition, has been regarded

as associated with numerous diseases including arteriosclerosis and thromboembolism.[21,22] Microbial communities were found in the mouth, lung, gut, and vagina of healthy humans. Most frequently identified bacterial and fungal genera are presented in Table 1.[23]

Until recently, blood circulating in healthy human bodies has been considered as sterile. The presence of bacteria in the bloodstream was always considered as bacteremia and the concept of blood microbiome has been met with criticism. Although we should approach it with some reservations, the presence of microorganisms in the blood of healthy individuals has been confirmed by different contemporary methods. The number of studies revealing the presence of blood microbiome assessed by metagenomic sequencing increases.[24–26] However, it is still unknown whether the bacteria there are stable or transient residents. In any case, their role in thromboembolism must be carefully considered. The ways for the bacteria to enter into the circulatory system are being considered. The presence of *Proteobacteria, Actinobacteria, Firmicutes*, and *Bacteroidetes* in blood is probably a consequence of their translocation from the intestinal tract.[26] In their studies, Qiu and colleagues[27] concluded that the presence of blood type *Bacteroides* caused a reduced risk of diabetes. It is also suggested that their presence could potentially trigger other not-communicable, inflammatory diseases including metabolic and hematological disorders.[28] *Bacteroidetes* members originating from the oral microbiome like *Porphyromonas gingivalis* have been implicated in the development of atherosclerosis.[29] Other studies indicated that intraplaque hemorrhage present in the samples of carotid thrombosis is associated with increased neutrophil activation markers, nuclear factor kappa B (NF-κB) activity, and LPS – part of the outer membrane of the present periodontal bacteria: *Porhyromonas gingivalis* and *Tannerella forsythia*.[30] A functional role of the bacteria in thrombus growth in carotid artery was shown also in lipoprotein receptor-deficient mice.[31]

Table 1 Diversity of Bacteria and Fungi Inhabiting Human Body

Human Body	Bacterial Genera	Fungal Genera
Mouth	*Actinomyces* *Fusobacterium* *Gemella* *Granulicatella* *Neisseria* *Rothia* *Streptococcus* *Veillonella*	*Aspergillus* *Aureobasidium* *Candida* *Cladosporium* *Cryptococcus* *Fusarium* *Malassezia*
Lung	*Fusobacterium* *Haemophilus* *Neisseria* *Prevotella* *Streptococcus*	*Aspergillus* *Candida* *Cladosporium* *Clavispora* *Davidiellaceae*
Gut	*Bacteroides* *Bifidobacterium* *Clostridium* *Enterobacter* *Eubacterium* *Lactobacillus* *Methanobacterium* *Prevotella* *Streptococcus* *Veillonella* *Verrucomicrobia*	*Candida* *Cladosporium* *Debaryomyces* *Fusarium* *Malassezia* *Pichia* *Saccharomyces*
Vagina	*Atopobium* *Escherichia* *Gardnerella* *Lactobacillus* *Mycoplasma* *Prevotella* *Staphylococcus* *Streptococcus* *Ureaplasma*	*Candida* *Cladosporium* *Davidiellaceae* *Pichia* *Saccharomyces*

Notes: Information in the table based on the analysis of documents: Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome Medicine. 2016;8(1):51[20] and Krüger W, Vielreicher S, Kapitan M, Jacobsen ID, Niemiec MJ. Fungal-Bacterial Interactions in Health and Disease. Pathogens. 2019;8(2):70.[23]

The functioning of the coagulation system is associated with the repair of damaged blood vessels and coagulation activation process. The coagulation is a response to the damage to blood vessels consisting in forming fibrin, whose main function is to seal the vessel and stop the bleeding. This process aims to bind the bacteria in a blood clot to prevent them from spreading. On the other hand, coagulation factors can bind bacteria in clots preventing immune system's actions and support their survival in the host organisms. It is known that infection increases the risk of thrombosis, but it has also been shown that the infection itself can also lead to thrombotic events.[32–34]

There are two pathways to the cascade of proteins in plasma that have an important role in host defense and hemostasis.[35] These include the complement and coagulation systems. Complement activation on bacteria provokes their killing through the membrane attack complex (MAC); additionally, small antibacterial peptides can be released.[36] Host defense associated with complement activation on bacteria helps the cellular immune response and leads to direct killing of the bacteria. Blood clotting is also thought to be an innate immune response, as it helps trigger the

inflammatory response, generates and releases antimicrobial peptides, and immobilizes bacteria in clots.[37,38]

Additionally, microbes cause an inflammatory response in which the fibrinolytic system is involved.[39] The key role in this process is held by plasminogen, which is transformed into plasmin with the participation of host physiological activators, ie urokinase plasminogen activator (u-PA) or tissue plasminogen activator (t-PA). Plasminogen activation to plasmin may also occur with the participation of bacterial plasminogen activators such as streptokinase (SK).[40,41]

The bacterial infection leads to large changes in the balance between coagulation and fibrinolysis.[42] Some bacteria use the host's hemostatic system to bypass the immune defense. Examples are *Yersinia pestis*, streptococci from Lancefield groups A, C, G, and *Staphylococcus aureus*. Bacteria produce a specific bacterial plasminogen activator which converts plasminogen to plasmin (Figure 1).[43] This section discussed a review of the various bacteria present in the human body and their involvement in diseases and binding them through the coagulation system.

Correlation of Oxidative Stress and Infections

Many studies have shown that inflammatory, immunological, hormonal, and metabolic reactions are involved in the pathogenesis of sepsis.[44] The main factor in these processes is the imbalance between the generation of reactive oxygen species (ROS) and their removal, which in consequence leads to oxidative stress.[45] The relationship between infection and the production of ROS has been clearly shown in numerous works.[46] The generation of ROS is believed to be associated at least in part with bacterial-induced metabolic pathways, and oxidative stress is involved in organ damage and cancer development.[47] It has been shown that *Helicobacter pylori* induces the enzymes that produce ROS, for example, spermine oxidase, and regulates pro-inflammatory and pro-cancerogenic genes such as cyclooxygenase 2 (COX-2).[48]

Chemical stimulation of cells by cytokines, xenobiotics, and bacterial invasion leads to ROS generation.[49] Numerous experimental and clinical studies confirm the key role of ROS in the mechanism of platelet activation. There are many activation pathways, one of which is an increased isoprostane formation.[50] Another source is arachidonic acid metabolism and associated lipoxygenase expression, the other is NADPH oxidase (NOX).[51] On the one hand, platelet can be modified by ROS, on the other, they generate ROS. ROS are formed within activated platelets and regulate their responses to collagen-mediated thrombus formation.[50]

Recently, it has been shown that acetylcholinesterase (AChE) and catalase activity decreased in the neuromuscular junction (NMJ) of the diaphragm during sepsis. Their activity was significantly negatively correlated with the level of thiobarbituric acid reactive substances (TBARS) and the level of carbonyl compounds that appear during oxidative damage to proteins. The obtained results clearly showed that during sepsis, oxidative stress occurs, which damages the biological material.[52]

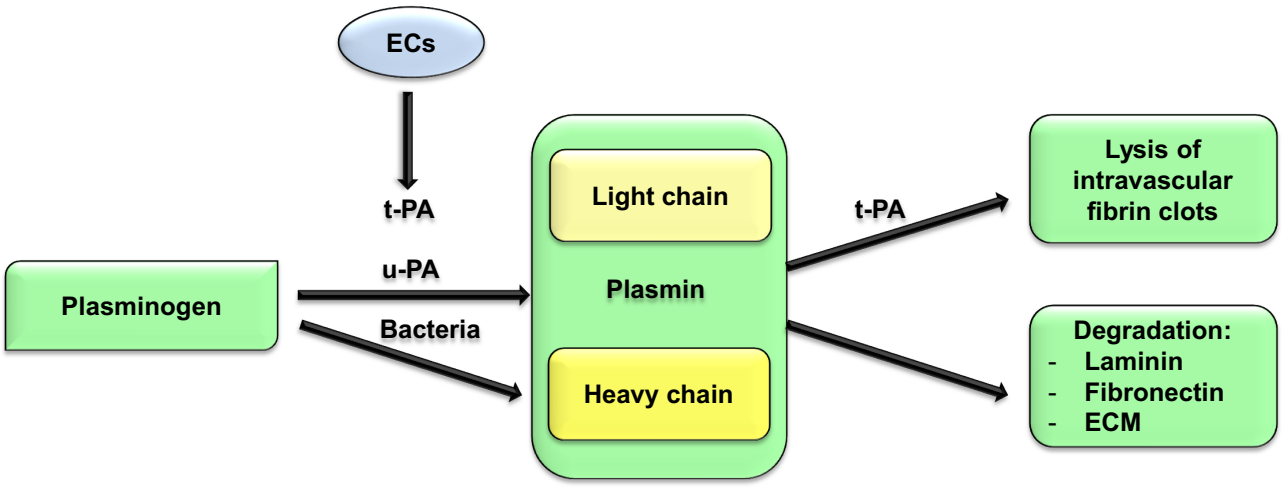

Figure 1 Use of plasminogen by bacteria for the production of plasmin, serine protease by bacterial SK or by host plasminogen activators eg u-PA and t-PA, which is released from ECs. The formed plasmin participates in the degradation of fibrin clot as well as laminin, fibronectin and type IV collagen and other components of ECM, which leads to the spread of bacteria through tissue barriers.

Abbreviations: SK, streptokinase; u-PA, urokinase plasminogen activator; t-PA, tissue plasminogen activator; ECs, endothelial cells; ECM, extracellular matrix.

Excessive release of ROS also occurs in hypoxia. Although the exact mechanism of ROS release in hypoxia is still under discussion, it seems that their generation is related to the mitochondrial electron transport chain (ETC). ROS are generated by complexes I, II and III of the endothelial cells (ECs).[53,54]

In hypoxia or low oxygen levels, the organism induces hypoxia-inducible factor (HIF)-1, which is a heterodimer and regulates the expression of over 100 genes.[55] HIF-1 is also necessary for the immune response and is an important physiological regulator of homeostasis and anaerobic metabolism. In addition, HIF-1 may interact with enzymes and other transcription factors leading to angiogenesis and tissue growth.[56,57] Hypoxia is known for deep vein thrombosis and venous thromboembolism and is a consequence of phenotypic changes in venous endothelial cells associated with activation of the elk-1/egr-1 pathway and increased expression of TF.[58] The thrombotic S protein, which is inversely regulated by HIF-1, have an important role in thrombosis. This important finding explains the mechanism of thrombosis in hypoxia.[59] *Bartonella henselae* is the only known bacterium that leads to vasculoproliferative disorders in humans. *Bartonella henselae* infection resulted in increased oxygen consumption in the host cells, causing cell hypoxia and a decrease in adenosine triphosphate (ATP) levels. This pathogen has been shown to trigger a pro-angiogenic host cell response via HIF-1, which is evidence that HIF-1 may have a role in bacterial infections.[60] Excessive release of ROS leads to damage to biological material and is a consequence of modification of metabolic pathways by bacteria.

Interaction of Platelets with Bacteria

Blood platelets are sensitive to factors from the surrounding environment due to receptors present on their surface and they interact with each other and with other cells. Platelets have a key role in cardiovascular diseases such as thrombosis, myocardial infarction, stroke.[61]

The increase in platelet activation may also be associated with a decrease in the release or/and inactivation of nitric oxide and the release of platelet agonists. Substances released from platelets, such as ROS, including nitric oxide, cytokines, growth factors, chemokines, metalloproteinases, histamine, and selectin, are involved in the immune response and inflammatory reactions.[62] Platelet may release antibacterial molecules and can induce apoptosis (phosphatidylserine exposure, caspase 3 activation, and depolarization of mitochondrial potential).[9,63]

Platelets can interact with bacteria through three mechanisms: direct binding of bacteria to a platelet receptor, indirect binding via fibrinogen, fibronectin, first complement C1q, von Willebrand factor (vWF), and binding of secreted bacterial products, mainly toxins (Figure 2).[9] Bacteria can attach to platelet via a direct interaction with GPIIb-IIIa or via an indirect interaction with GPIIb-IIIa. *Staphylococcus aureus* can bind to platelets directly by fibrinogen and/or fibronectin, which are ligands for GPIIb-IIIa and/or via a short amino acid sequence, arginyl glycyl aspartic acid.[64] Protein A exposed on the surface of bacteria *Staphylococcus aureus* can attach to the FcγRII receptor exposed on the surface of platelet membrane and can lead to the release of serotonin and platelet aggregation. Both FcγRII, required for *Staphylococcus aureus* adhesion, and aggregation induced by these bacteria depend on FcγRII, which activates GPIIb-IIIa functions.[65] In turn, *Streptococcus sanguinis* can directly affect GPIbα and vWF receptors on the platelet surface.[66] *Helicobacter pylori* can also bind to a platelet using the vWF and GPIbα receptors.[67] However, in binding to platelet, bacteria most often use an IgG molecule that interacts with the FcγRIIa platelet receptor.[64]

Binding of bacteria to platelets leads to their aggregation, as shown in the example of *Streptococcus sanguinis, Staphylococcus epidermidis*, or *Chlamydia pneumoniae*.[9] Many bacteria ie gram-positive bacteria *Staphylococcus aureus* can activate the blood clotting system and influence the individual coagulation factors, although they do not lead directly to the initiation of the coagulation cascade. Activation of the coagulation system by these bacteria protects them from being attacked by the host's immune system.[68] For example, *Staphylococcus aureus* forms aggregates with extracellular matrix (ECM) proteins, which include fibrinogen, fibronectin, and collagen.[69] The formation of platelet aggregates promotes the colonization of host tissues.[70] Many microorganisms can use blood clotting to their advantage, *Staphylococcus aureus* is one of these microorganisms.[68] On the other hand, platelets together with neutrophils form traps for bacteria, but this, in turn, leads to an increased risk of thrombosis.[9]

Further, activated platelets can also adhere to endothelial cells leading to the formation of a blood clot. EC release pro-inflammatory cytokines (IL-6, IL-8 and TNF-α) during the response to inflammation. In turn, cytokines

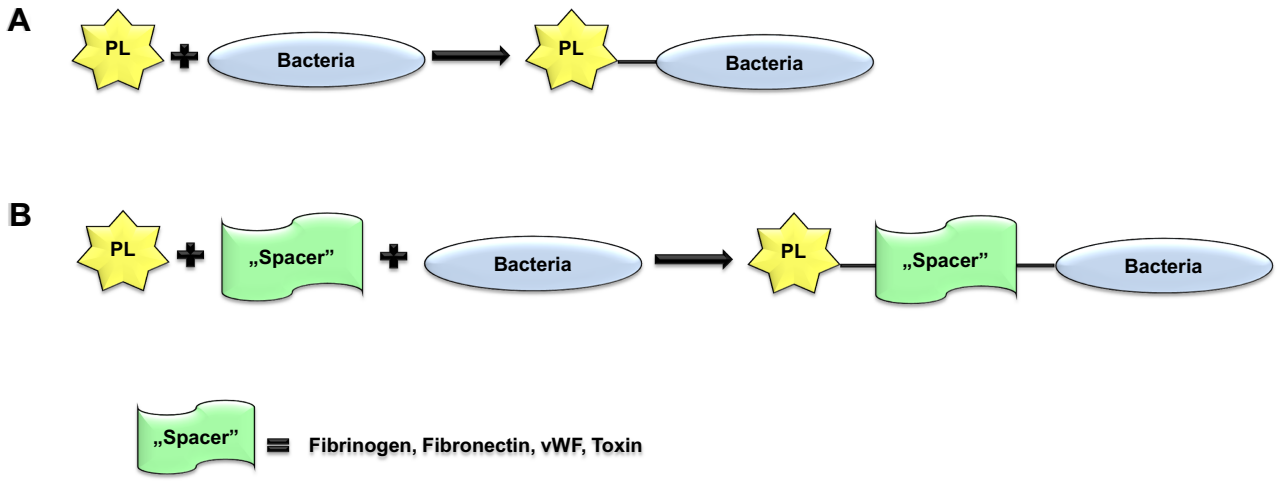

Figure 2 Direct or indirect adhesion of bacteria to platelets. (**A**) Direct biding of bacteria via GPIIb-IIIa or GPIb (α-β) exposed on platelet surface. (**B**) Indirect binding of bacteria via spacer like fibrinogen or fibronectin and GPIIb-IIIa but also GPIb (α-β) via vWF. Platelets may also bind bacteria by gC1qR or/and CD62P receptors exposed on the platelet surface. Another "spacer" that binds bacteria to platelets is IgG delivered by bacteria through the FcγRIIa receptor present on platelets.
Abbreviations: PL, platelets; GP, glycoprotein; vWF, von Willebrand factor; IgG, immunoglobulin G.

lead to a procoagulant state, mainly through the induction of tissue factor (TF) expression, which has an important role in VTE. It was shown that an inflammatory condition promotes platelets[17] and platelets aggregation.[71]

Bacterial toxins lead to an inhibition of platelet function, causing damage to protein kinase C (PKC). This impairs phosphorylation of vasodilator-stimulated phosphoprotein (VASP) and a loss of fibrinogen activity or fibrinogen damage.[9] Excessive platelet activation leads to vascular thrombosis and is associated with acute coronary syndrome and ischemic stroke.[62] *Escherichia coli* releases Shiga-like toxin (verotoxin), which increases platelet aggregation. This toxin binds to the surface of platelets via glycosphingolipid receptors.[72,73] Using the intravital imaging method, it has been shown that *Staphylococcus aureus* toxin directly attacks platelets, leading to their aggregation in the circulation.[74]

Platelets and neutrophils are necessary for the initiation and growth of thrombus. Platelets and neutrophils form NETs in thrombosis (Figure 3). The traps also contain other components such as extracellular DNA, proteins, histones, serine proteases, and others, which in total lead to platelet coagulation and aggregation. Additionally, peptidylarginine deiminase type 4 (PAD4), an enzyme that causes chromatin decondensation, but also controls NETs formation (NETosis) and thrombosis, promotes blood clot formation.[75,76] Further, NETs bacteria bind to platelets and red blood cells (RBCs) to form red thrombi. Also, it binds nucleic acids and histones that can activate coagulation. A similar effect is shown by histones, which activate platelets, increasing thrombin production, thus promoting coagulation.[77] Platelets have a key role in hemostasis, but are also important in bacterial-induced thrombosis.

Gut Microbiome and Cardiovascular Diseases

As research has shown, the number of bacteria forming the microbiome inhabiting the human digestive tract (DT) increases with an increasing pH in subsequent sections of the DT and a reduction in oxygen concentration. It is estimated that the largest number of microorganisms is in the large intestine, and the diversity of composition reaches about 2000 species there.[78] The microbiome of colon consists predominantly of *Bacteroidetes* (with predominant *Bacteroides, Prevotella*, and *Porphyromons*) and *Firmicutes* with *Clostridium, Bacillales*, and *Lactobacillales* accounting for over 90% of all bacteria in that niche. *Actinobacteria* and *Proteobacteria* are the other part of this ecosystem. In the small intestinal aspirate in healthy humans, there is 10^4–10^7 CFU/g and genera *Streptococcus, Lactobacillus*, and *Veilonella* are predominant.[79] Microbiome disorders lead to many pathological conditions ranging from infectious diseases to cardiovascular and metabolic disorders, gastrointestinal diseases, and neurological diseases.[80] A disturbance in the number and diversity of the intestinal microbiome has been associated with Crohn's disease, irritable bowel syndrome but also, cancer, malabsorption syndrome as well as neurological and psychological diseases.[81–84]

It was indicated that there is a connection between the number and composition of intestinal microbiome

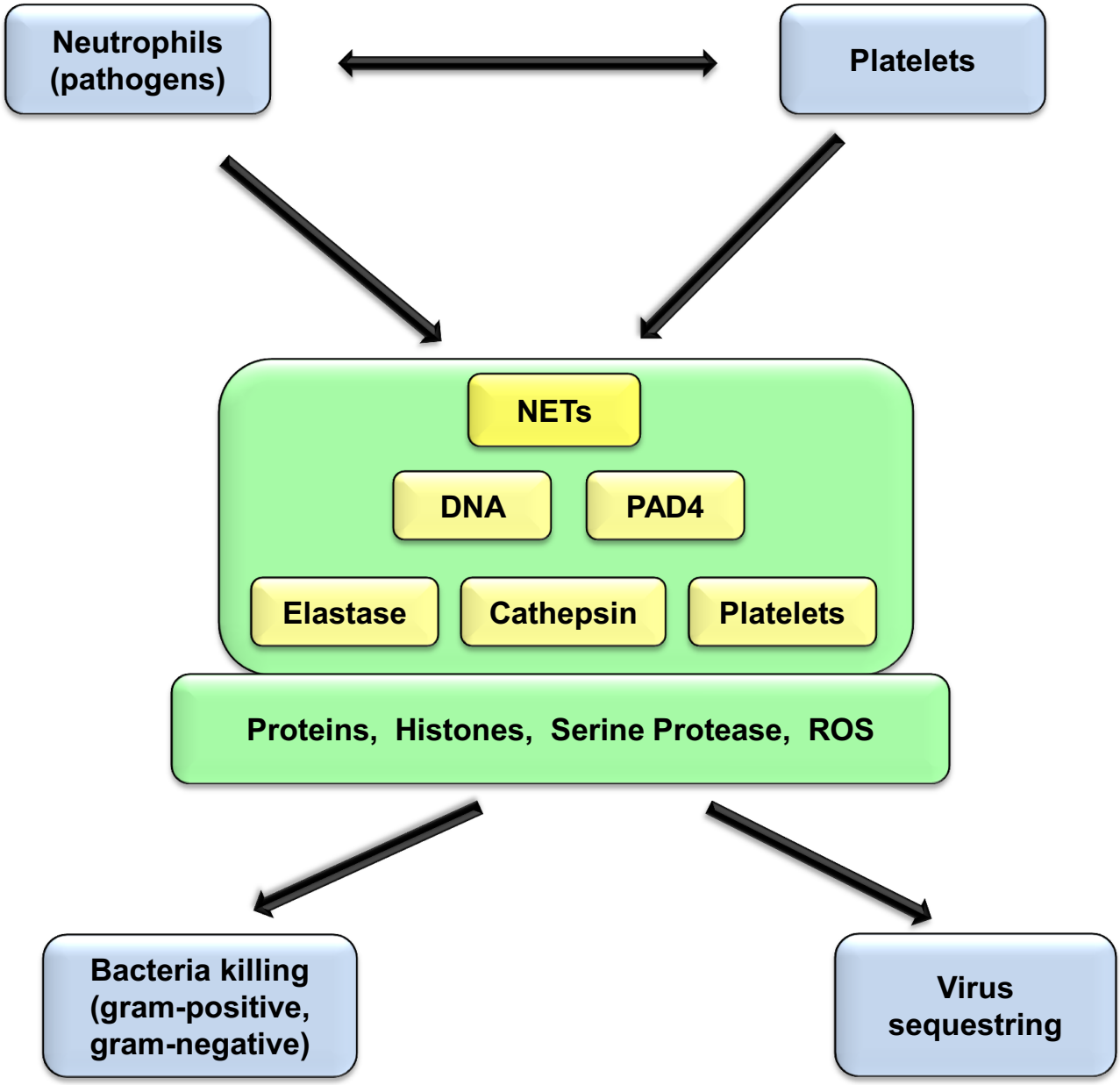

Figure 3 The formation of NETs in response to infection is associated with the interaction of platelets with neutrophils containing cathepsin, DNA and protein components such as histones and serine proteases, PAD4, elastase and others. ROS also participate in NETs formation.
Abbreviations: NETs, neutrophil extracellular traps; DNA, deoxyribonucleic acid; PAD4, peptidylarginine deiminase type 4; ROS, reactive oxygen species.

and thromboembolism. A known example is small intestinal bacterial overgrowth (SIBO), a malabsorption syndrome, which is referred to as an increase in the number of bacteria in the proximal part of small bowel or dysbiosis manifested as the presence of bacteria characteristic for colon. Cheng et al[85] showed the DVT as a dangerous complication associated with SIBO during posttraumatic hospitalization in patients after spinal cord injury. SIBO syndrome leads to increased bowel permeability with enhanced adsorption of LPS and other bacterial products.[86] It is suggested that testing and treatment for SIBO may be beneficial for suffering patients and may prevent deep venous thrombosis. The reason for that is not precisely known but similarly to other diseases associated with SIBO, it may be a gut inflammation state caused by bacterial products. Increased expression of pro-inflammatory cytokines as IL-1a and IL-1b and increased production of IL-8 have been shown as a result of toll-like receptor 4 (TLR-4) for LPS expression on cells.[87]

Intestinal bacteria are an inseparable anatomical and physiological element of the human body. They are involved in the biosynthesis of metabolically active compounds by regulating processes in health but also have a role in the pathogenesis of diseases. Apart of LPS which is a constituent part of the cell wall of all gram-negative bacteria, gut microbiome bacteria can derive metabolites that have clinical relevance in disease pathogenesis. These are TMAO, uremic toxins, short-chain fatty acids (SCFA), phytoestrogens, anthocyanins, or bile acids. The bioactive molecules may have an important role in thrombosis as well as other cardiovascular diseases.[88] They can be accumulated in blood, influencing many processes. Bacteria can also act directly producing enzymes activating blood clotting factors. Many families of colon bacteria, for example *Clostridiaceae, Enterobacteriaceae*, can produce urease, many can produce tryptophanase (ie *Clostridiaceae, Enterobacteriaceae*, and *Verrucomicrobiaceae*) or produce short-chain fatty acids (i.e *Lactobacillaceae, Bifidobacteriaceae, Prevotellaceae*). Uremic toxin-indoxyl sulfate, when bound to albumin, enhances platelet activities and increases response to collagen and thrombin which in consequence leads to thrombosis.[89] Produced by bacteria, short-chain acids contribute to blood pressure control and phytoestrogens may have prothrombotic and intermediately triggering pro-inflammatory and anti-inflammatory action connected with TNF-α production. Anthocyanins which modulate gut microbiota and are antiplatelet agents also have an importance in cardiovascular diseases prevention.[88] Changes in human microbiome have a significant impact on the development of cardiovascular diseases, including thrombosis.

TMAO and Thrombosis Risk

Microorganisms inhabiting the gut affect the physiology and metabolism throughout the body. It has been shown that the host-microbiome has produced various substances such as trimethylamine (TMA), short-chain fatty acids, and secondary bile acids that can affect cardiovascular disease. The modification of interaction between the host and its microflora may likely contribute to the prevention and/or treatment of cardiovascular diseases.[90]

TMAO is a known metabolite in animals resulting from the oxidation of trimethylamine. TMAO is found in marine animals, such as fish, sharks, rays, mollusks, and crustaceans, including deep-sea fishes and crustaceans as a protector against high pressure, which can damage the proteins.[91,92] TMA of bacterial origin is oxidized to TMAO by flavin-containing monooxygenase (FMO). This enzyme is expressed in the liver and oxidizes xenobiotics containing amines or sulfides. TMAO has been identified as an independent risk factor of cardiovascular disease, as well as other disorders.[93–95] It was reported that TMAO enhances platelet hyperactivity and thrombosis risk.[96] TMA is produced by the anaerobic gram-positive *Ruminococcus* bacteria belonging to the *Clostridia* class occurring in a significant number in the human intestinal microflora.[97,98]

The presented research on a group of 513 people after cardiovascular events, of which about 70% were smokers, showed high levels of TMAO in the blood.[99] The concentration of TMAO is also increased in the blood of people who eat food containing carnitine or lecithin.[99,100] Bacteria of the Acinetobacter species present in the human intestine can convert dietary carnitine into TMAO. High concentrations of carnitine are present in red meat, soy, dietary supplements, and even in energy drinks.[100] Patients with high TMAO plasma levels are at an increased risk of myocardial infarction or stroke.[99]

In addition to TMA, the intestinal microflora also produces another toxin, indoxyl sulfate (IS). Enterobacteria, by fermentation of tryptophan, produce indole, which is hydroxylated in the liver to indoxyl by enzyme CYP250, CYP2E1, and converted to IS by sulfotransferase. In healthy individuals, IS is excreted in the urine through tubular secretion. In patients with chronic kidney disease (CKD), its concentration in plasma can be increased almost 80 times.[101] Thrombosis is a common complication of CKD, but the mechanism of thrombosis is not fully understood. Higher platelet activity was demonstrated in animal models that increased with the increase of serum concentration of IS leading to thrombosis.[89,102] TMAO and IS produced in the gut with the help of a microbiome may be an independent risk factor of cardiovascular disease.

Clinical Aspects

Thrombosis may be a consequence of an improperly treated infection. However, the mechanisms associated with the induction of thrombosis by infections are poorly understood, as are the mechanisms of infection control by the host in the spread of the pathogen. Thrombosis initiated by pathogens is associated with the release of inflammatory agents activating platelets, which may be accompanied by damage to the endothelium, leading to fibrin deposition and the consequence of thrombus formation.[5] There are many well-known bacteria that cause infections and increase the risk of thrombotic complications, including ischemic stroke and myocardial infarction.[103,104] This group includes

Helicobacter pylori, Chlamydia pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Epstein-Bar virus, herpesvirus and cytomegalovirus.[103,105,106]

It was reported that ischemic stroke may occur within the first few days after respiratory or urinary tract infections, as well as in the case of chronic bronchitis.[105] The risk of myocardial infarction was greatly increased within 30 days.[103] In turn, bacteremic infections of *Neisseria meningitidis* and *Staphylococcus aureus* led to an exacerbation of cardiovascular disease such as acute myocardial infarction and unstable angina. This event is usually referred to as thrombophlebitis and can be caused by many different pathogens. This process is often referred to as thrombo-inflammation.[107]

Acute respiratory or urinary tract infections lead to ischemic stroke. Many bacteria such as *Helicobacter pylori, Chlamydia pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli* increase the risk associated with the stroke.[5] It has also been reported that various cardiovascular diseases may be associated with bacterial involvement as shown on the example of infective endocarditis.[108]

Relationships between pneumonia due to *Haemophilus influenzae* and infection with other bacteria and myocardial infarction have been confirmed. The risk of myocardial infarction was the highest at the beginning of the infection and correlated with the severity of the disease. An increased risk of myocardial infarction also occurred in urinary tract infections and bacteraemia.[104]

Infections caused by *Neisseria meningitidis* and *Staphylococcus* aureus lead to exacerbations of cardiovascular disease, including acute myocardial infarction.[109] *Bacillus cereus* and *Bacillus anthracis* lead to blood clotting within a few minutes in clustered bacterial cells. However, the process of blood clotting by *Bacillus anthracis* needed the release of the InhA1 zinc metalloprotease. This enzyme directly activated prothrombin and factor X (FX) without the factor XII (FXII) pathway or TF pathway.[110]

The adhesion of bacteria to the surface of the midblock has an important role in the formation of a clot. *Staphylococcus aureus* bacteria release various exotoxins that, when interacting with cell membranes, lead to platelet activation and aggregation and smooth muscle contraction. Both of these factors can trigger thrombosis. In turn, the coagulase enzyme interacting with fibrinogen leads to plasma coagulation. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common pathogen involved in venous thrombosis with osteomyelitis. It was shown that diffuse soft tissue infection, septic arthritis, osteomyelitis and myositis can lead to deep vein thrombosis in children.[111,112] Vascular complications induced by MRSA were more frequent and more severe compared to methicillin-sensitive *Staphylococcus aureus* (MSSA) infections, especially in patients with lung involvement. MRSA infections also showed significantly higher markers of inflammation and required longer hospitalizations.[113]

Because the clinical manifestations of deep musculoskeletal infections (MSI) usually overlap with DVT symptoms, the incidence of DVT as a complication of MSI may be difficult to assess. The most common infection with MSI was osteomyelitis, and the dominant pathogen was MRSA. Pulmonary involvement, probably due to septic embolism, was seen in 65% of enrolled children.[114]

The gram-negative bacterium *Burkholderia pseudomallei* causing melioidosis has a different range of infection symptoms, with pneumonia and bacteremia being the most common presentations. However, deep vein thrombosis and PE caused by *Burkholderia pseudomallei* are rarely reported in the literature, but their effects can be fatal. Diabetes is an additional risk factor for DVT and PE.[115]

Systemic coagulation disorders were observed not only in sepsis, but also in pneumonia. And the symptoms were associated with the activation of coagulation and inhibition of anticoagulants.[116] In the course of the research, it was found that bacteria or bacterial lipopolysaccharides participate in the activation of platelets and promote thrombus formation, thus complicating the clinical course of pneumonia. Platelets can interact with gram-negative and gram-positive bacteria through direct binding through the membrane surface receptors of platelets and bacterial surface protein.[117]

In addition, various bacteria, including gram-positive bacteria isolated from bacteremia patients, induce platelet-neutrophil complex formation in addition to platelet activation and aggregation. Epidemiological studies have shown that pneumonia and other respiratory infections are increasing the risk of cardiovascular diseases associated with thrombosis, such as myocardial infarction, ischemic stroke, and venous thrombosis. The occurrence of myocardial infarction and stroke was observed within 48 hours of admission to hospital, with a frequency of 1% to even 11%. Additional risk factors for heart attack would be patient's age, previous cardiovascular events, and a high rate of pneumonia. Pneumonia can also lead to DVT and PE. Lung infection is further complicated by platelet aggregation and activation of the coagulation system, which is associated with the expression of TF and down regulation of the activated C protein.[118]

Purulent peripheral thrombophlebitis is not a very common occurrence but accounts for approximately 7% of cases. It can occur after the introduction of an intravenous catheter or with persistent bacteremia.[119,120] Its incidence is high in patients with burns, taking steroids, and in injecting drug addicts.[121,122] Among drug addicts, who have been using intravenous drugs for many years, DVT may appear.[123] A rare case of DVT in the inferior vena cava and left iliac vein in an intravenous drug user has also been described.[124] Drug injections alone are not a risk factor for deep vein thrombosis; however, intravenous injection of these substances is an independent risk factor.[125] For example, heroin triggers an increase in α-2 adrenergic receptor density platelets that can stimulate platelet aggregation dependent on adrenaline.[126] In contrast, opioid substances are decreasing antithrombin activity and blood viscosity due to an increase in aggregation of RBCs and platelets and a change in their deformability.[127,128] At the same time, an increase in fibrinogen concentration and leukocyte aggregation was observed.[129]

Infections leading to pneumonia, urinary tract infections and bacteremia increase the risk of thrombotic complications such as ischemic stroke and myocardial infarction.

Conclusion

Recent research results indicate a significant participation of microbes inhabiting the human body in metabolism, and thus physiology, pathophysiology, and pathogenesis of diseases. In this article, we have demonstrated that they have a direct, as well as an indirect, contribution to the development of thrombotic diseases such as ischemic stroke, myocardial infarction and VTE and PE. These processes are intensified in the course of infectious diseases, but they can also be a result of a disturbance in the composition of the microbiome, ie dysbiosis, or of microbial populations that appear transiently in the blood vessels. It is necessary to look for the relationships between these processes, especially at the metabolomic level, the explanation of which can contribute to a better diagnosis and treatment of thrombotic diseases.

Abbreviations

AChE, acetylcholinesterase; ATP, adenosine triphosphate; CFU, colony forming unit; CKD, chronic kidney disease; COX, cyclooxygenase; CYP, cytochrome P; DNA, deoxyribonucleic acid; DT, digestive tract; DVT, deep vein thrombosis; ECM, extracellular matrix; ECs, endothelial cells; ETC, electron transport chain; FMO, flavin-containing monooxygenase; GP, glycoprotein; HIF-1, hypoxia-inducible factor-1; Ig, immunoglobulin; IL, interleukin; IS, indoxyl sulfate; LPS, lipopolysaccharide; MAC, membrane attack complex; MRSA, methicillin-resistant *Staphylococcus aureus*; MSI, musculoskeletal infections; MSSA, methicillin-sensitive *Staphylococcus aureus*; NADPH, nicotinamide-adenine dinucleotide phosphate; NETs, neutrophil extracellular traps; NF-κB, nuclear factor kappa B; NGS, next-generation sequencing; NMJ, neuromuscular junction; NOX, NADPH oxidase; PAD4, peptidylarginine deiminase type 4; PE, pulmonary embolism; PKC, protein kinase C; PL, platelets; RBCs, red blood cells; ROS, reactive oxygen species; SCFA, short-chain fatty acids; SIBO, small intestinal bacterial overgrowth; SK, streptokinase; TBARS, thiobarbituric acid reactive substances; TF, tissue factor; TLR-4, toll-like receptor 4; TMA, trimethylamine; TMAO, trimethylamine N-oxide; TNF-α, tumor necrosis factor alfa; t-PA, tissue plasminogen activator; u-PA, urokinase plasminogen activator; VASP, vasodilator-stimulated phosphoprotein; VTE, venous thromboembolism; vWF, von Willebrand factor; WMGS, whole metagenome shotgun.

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