


# Microbial Modulation of Coagulation Disorders in Venous Thromboembolism

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Anna Lichota <sup>1</sup>  
Krzysztof Gwozdziński <sup>2</sup>  
Eligia M Szewczyk <sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Microbiology and Microbiological Diagnostics, Faculty of Pharmacy, Medical University of Lodz, Lodz, Poland;

<sup>2</sup>Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland

**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.<sup>1</sup> 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.<sup>2,3</sup>

Other risk factor is sepsis, which is a serious epidemiological and therapeutic problem, mainly at intensive care units.<sup>4</sup> 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*).<sup>5</sup>

Correspondence: Anna Lichota  
Department of Pharmaceutical Microbiology and Microbiological Diagnostics, Faculty of Pharmacy, Medical University of Lodz, Pomorska 137, Lodz 90-235, Poland  
Tel +48 426779300  
Email [anna.lichota@umed.lodz.pl](mailto:anna.lichota@umed.lodz.pl)

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.<sup>6</sup> 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.<sup>7</sup>

As the studies of Patrakka et al<sup>8</sup> 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.<sup>9</sup> 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.<sup>10</sup> Sometimes, it is misdiagnosed and unrecognized even after the patient's death due to the lack of routine post-mortem examinations.<sup>11</sup> VTE is the third cardiovascular cause of death after myocardial infarction and stroke, and increases steeply with age.<sup>12</sup> It is estimated that almost

two-thirds of VTE cases are related to deep vein thrombosis (DVT), and 80% are proximal.<sup>13</sup> In the European population, there are about 70–140 DVT cases per 100 000 people a year.<sup>14</sup>

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.<sup>15</sup> 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.<sup>13</sup>

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.<sup>16</sup> Release of IL-6, IL-8 and tumor necrosis factor alpha (TNF- $\alpha$ ) cytokines is a response to systemic inflammation which leads to an increased risk of thrombosis.<sup>17</sup> It is believed that systemic infections increase the risk of deep vein thrombosis/pulmonary embolism about 2–20 times.<sup>5</sup> An additional risk factor for VTE is the age of the patients, the presence of malignant tumors, and hereditary features.<sup>3</sup> 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.<sup>18</sup> According to a recent study, there are over  $3.8 \times 10^{13}$  microorganisms living in different body sites.<sup>19</sup> The nature of the host-microbial interaction is complex and has a profound impact on human health.<sup>20</sup> 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.<sup>21,22</sup> 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.<sup>23</sup>

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.<sup>24–26</sup> 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.<sup>26</sup> In their studies, Qiu and colleagues<sup>27</sup> 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.<sup>28</sup> *Bacteroidetes* members originating from the oral microbiome like *Porphyromonas gingivalis* have been implicated in the development of atherosclerosis.<sup>29</sup> 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- $\kappa$ B) activity, and LPS – part of the outer membrane of the present periodontal bacteria: *Porphyromonas gingivalis* and *Tannerella forsythia*.<sup>30</sup> A functional role of the bacteria in thrombus growth in carotid artery was shown also in lipoprotein receptor-deficient mice.<sup>31</sup>

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.<sup>32–34</sup>

There are two pathways to the cascade of proteins in plasma that have an important role in host defense and hemostasis.<sup>35</sup> 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.<sup>36</sup> 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

**Table 1** Diversity of Bacteria and Fungi Inhabiting Human Body

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

**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<sup>20</sup> and Krüger W, Vielreicher S, Kapitan M, Jacobsen ID, Niemiec MJ. Fungal-Bacterial Interactions in Health and Disease. *Pathogens*. 2019;8(2):70.<sup>23</sup>

inflammatory response, generates and releases antimicrobial peptides, and immobilizes bacteria in clots.<sup>37,38</sup>

Additionally, microbes cause an inflammatory response in which the fibrinolytic system is involved.<sup>39</sup> 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).<sup>40,41</sup>

The bacterial infection leads to large changes in the balance between coagulation and fibrinolysis.<sup>42</sup> 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).<sup>43</sup> 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.

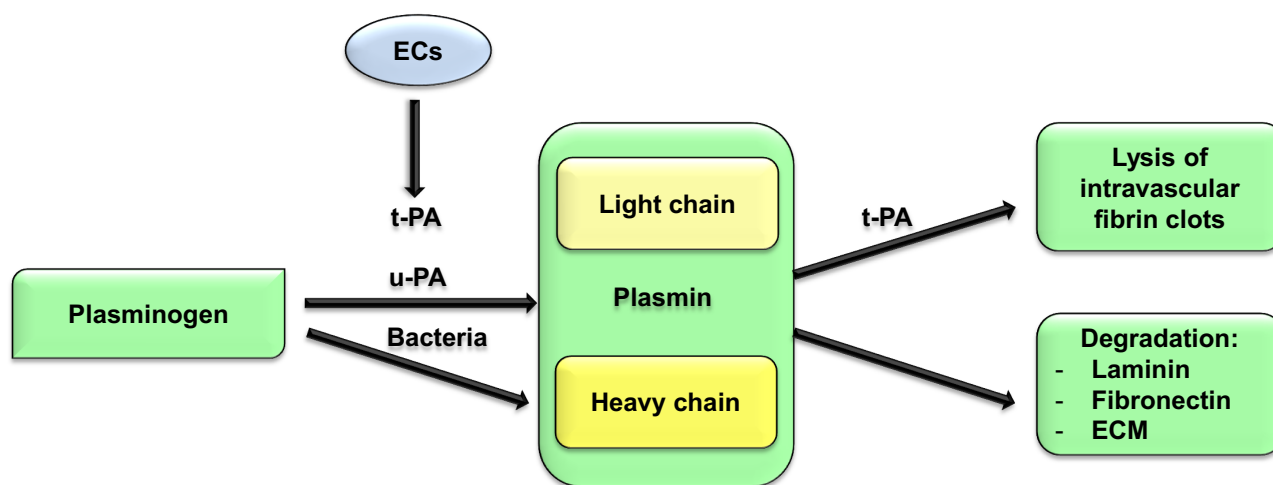
## Correlation of Oxidative Stress and Infections

Many studies have shown that inflammatory, immunological, hormonal, and metabolic reactions are involved in the pathogenesis of sepsis.<sup>44</sup> 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.<sup>45</sup> The relationship

between infection and the production of ROS has been clearly shown in numerous works.<sup>46</sup> 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.<sup>47</sup> 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).<sup>48</sup>

Chemical stimulation of cells by cytokines, xenobiotics, and bacterial invasion leads to ROS generation.<sup>49</sup> 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.<sup>50</sup> Another source is arachidonic acid metabolism and associated lipoxygenase expression, the other is NADPH oxidase (NOX).<sup>51</sup> 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.<sup>50</sup>

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.<sup>52</sup>



**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).<sup>53,54</sup>

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.<sup>55</sup> 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.<sup>56,57</sup> 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.<sup>58</sup> 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.<sup>59</sup> *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.<sup>60</sup> 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.<sup>61</sup>

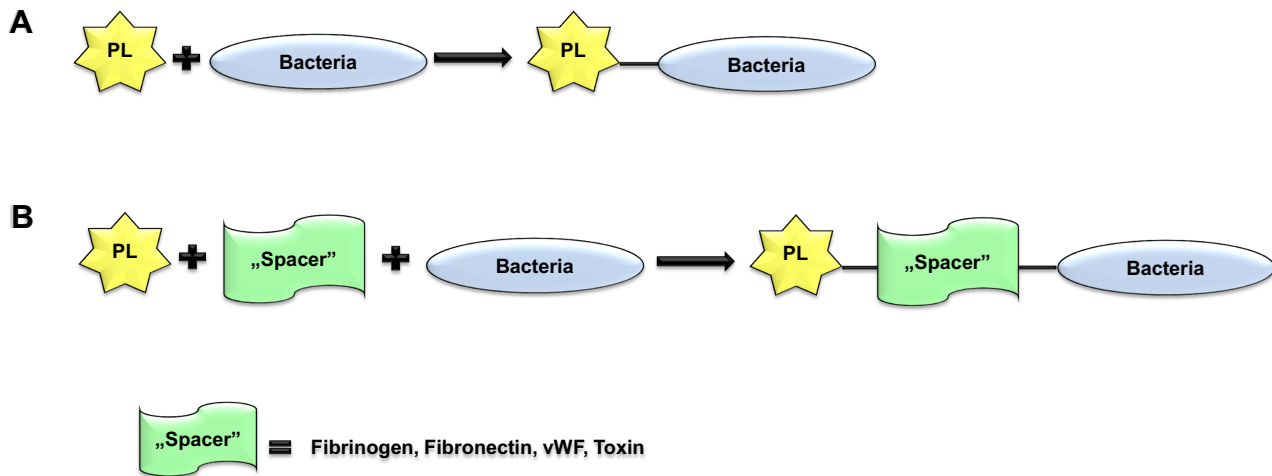
The increase in platelet activation may also be associated with a decrease in the release or/inactivation of nitric oxide and the release of platelet agonists. Substances released from platelets, such as ROS, including nitric oxide, cytokines, growth factors, chemokines, metalloproteinases, histamine, and selectin, are involved in the immune response and inflammatory reactions.<sup>62</sup> Platelet may release antibacterial molecules and can induce

apoptosis (phosphatidylserine exposure, caspase 3 activation, and depolarization of mitochondrial potential).<sup>9,63</sup>

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).<sup>9</sup> 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 glycylic aspartic acid.<sup>64</sup> Protein A exposed on the surface of bacteria *Staphylococcus aureus* can attach to the Fc $\gamma$ RII receptor exposed on the surface of platelet membrane and can lead to the release of serotonin and platelet aggregation. Both Fc $\gamma$ RII, required for *Staphylococcus aureus* adhesion, and aggregation induced by these bacteria depend on Fc $\gamma$ RII, which activates GPIIb-IIIa functions.<sup>65</sup> In turn, *Streptococcus sanguinis* can directly affect GPIIb and vWF receptors on the platelet surface.<sup>66</sup> *Helicobacter pylori* can also bind to a platelet using the vWF and GPIIb receptors.<sup>67</sup> However, in binding to platelet, bacteria most often use an IgG molecule that interacts with the Fc $\gamma$ RIIa platelet receptor.<sup>64</sup>

Binding of bacteria to platelets leads to their aggregation, as shown in the example of *Streptococcus sanguinis*, *Staphylococcus epidermidis*, or *Chlamydia pneumoniae*.<sup>9</sup> 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.<sup>68</sup> For example, *Staphylococcus aureus* forms aggregates with extracellular matrix (ECM) proteins, which include fibrinogen, fibronectin, and collagen.<sup>69</sup> The formation of platelet aggregates promotes the colonization of host tissues.<sup>70</sup> Many microorganisms can use blood clotting to their advantage, *Staphylococcus aureus* is one of these microorganisms.<sup>68</sup> On the other hand, platelets together with neutrophils form traps for bacteria, but this, in turn, leads to an increased risk of thrombosis.<sup>9</sup>

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- $\alpha$ ) during the response to inflammation. In turn, cytokines



**Figure 2** Direct or indirect adhesion of bacteria to platelets. **(A)** Direct binding of bacteria via GPIIb-IIIa or GPIb ( $\alpha$ - $\beta$ ) exposed on platelet surface. **(B)** Indirect binding of bacteria via spacer like fibrinogen or fibronectin and GPIIb-IIIa but also GPIb ( $\alpha$ - $\beta$ ) 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 Fc $\gamma$ RIIa 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<sup>17</sup> and platelets aggregation.<sup>71</sup>

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.<sup>9</sup> Excessive platelet activation leads to vascular thrombosis and is associated with acute coronary syndrome and ischemic stroke.<sup>62</sup> *Escherichia coli* releases Shiga-like toxin (verotoxin), which increases platelet aggregation. This toxin binds to the surface of platelets via glycosphingolipid receptors.<sup>72,73</sup> Using the intravital imaging method, it has been shown that *Staphylococcus aureus* toxin directly attacks platelets, leading to their aggregation in the circulation.<sup>74</sup>

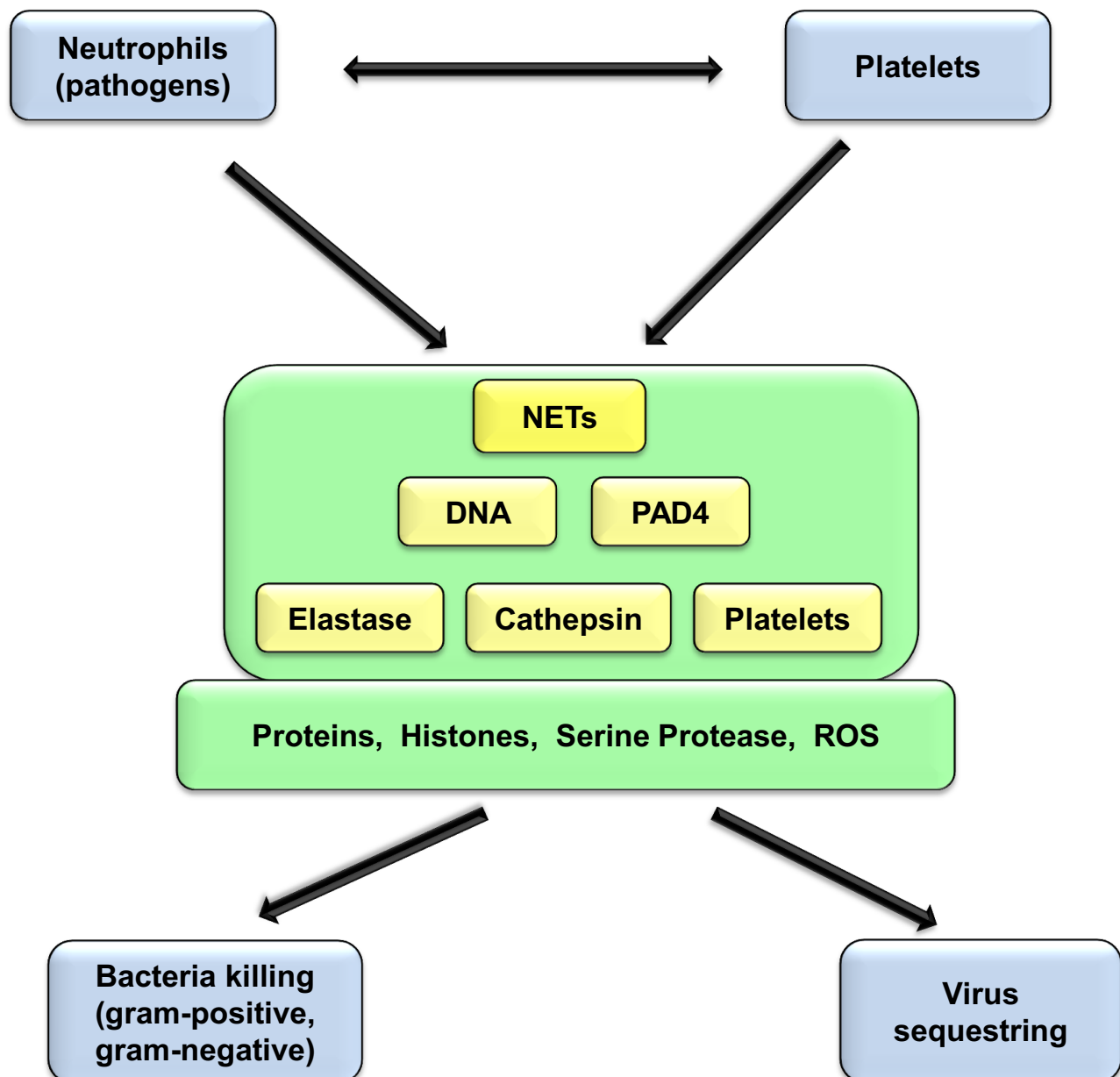
Platelets and neutrophils are necessary for the initiation and growth of thrombus. Platelets and neutrophils form NETs in thrombosis (Figure 3). The traps also contain other components such as extracellular DNA, proteins, histones, serine proteases, and others, which in total lead to platelet coagulation 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.<sup>75,76</sup> 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.<sup>77</sup> 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.<sup>78</sup> 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.<sup>79</sup> Microbiome disorders lead to many pathological conditions ranging from infectious diseases to cardiovascular and metabolic disorders, gastrointestinal diseases, and neurological diseases.<sup>80</sup> 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.<sup>81–84</sup>

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<sup>85</sup> 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.<sup>86</sup> 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.<sup>87</sup>

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.<sup>88</sup> 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.<sup>89</sup> 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- $\alpha$  production. Anthocyanins which modulate gut microbiota and are antiplatelet agents also have an importance in cardiovascular diseases prevention.<sup>88</sup> 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.<sup>90</sup>

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.<sup>91,92</sup> 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.<sup>93–95</sup> It was reported that TMAO enhances platelet hyperactivity and thrombosis risk.<sup>96</sup> 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.<sup>97,98</sup>

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.<sup>99</sup> The concentration of TMAO is also increased in the blood of people who eat food containing carnitine or lecithin.<sup>99,100</sup> 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.<sup>100</sup> Patients with high TMAO plasma levels are at an increased risk of myocardial infarction or stroke.<sup>99</sup>

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.<sup>101</sup> 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.<sup>89,102</sup> 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.<sup>5</sup> There are many well-known bacteria that cause infections and increase the risk of thrombotic complications, including ischemic stroke and myocardial infarction.<sup>103,104</sup> This group includes



*Helicobacter pylori*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, Epstein-Bar virus, herpesvirus and cytomegalovirus.<sup>103,105,106</sup>

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.<sup>105</sup> The risk of myocardial infarction was greatly increased within 30 days.<sup>103</sup> 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.<sup>107</sup>

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.<sup>5</sup> It has also been reported that various cardiovascular diseases may be associated with bacterial involvement as shown on the example of infective endocarditis.<sup>108</sup>

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.<sup>104</sup>

Infections caused by *Neisseria meningitidis* and *Staphylococcus aureus* lead to exacerbations of cardiovascular disease, including acute myocardial infarction.<sup>109</sup> *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.<sup>110</sup>

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.<sup>111,112</sup> 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.<sup>113</sup>

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.<sup>114</sup>

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.<sup>115</sup>

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.<sup>116</sup> 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.<sup>117</sup>

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.<sup>118</sup>

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.<sup>119,120</sup> Its incidence is high in patients with burns, taking steroids, and in injecting drug addicts.<sup>121,122</sup> Among drug addicts, who have been using intravenous drugs for many years, DVT may appear.<sup>123</sup> A rare case of DVT in the inferior vena cava and left iliac vein in an intravenous drug user has also been described.<sup>124</sup> Drug injections alone are not a risk factor for deep vein thrombosis; however, intravenous injection of these substances is an independent risk factor.<sup>125</sup> For example, heroin triggers an increase in  $\alpha$ -2 adrenergic receptor density platelets that can stimulate platelet aggregation dependent on adrenaline.<sup>126</sup> 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.<sup>127,128</sup> At the same time, an increase in fibrinogen concentration and leukocyte aggregation was observed.<sup>129</sup>

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- $\kappa$ B, 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- $\alpha$ , 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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## References

1. Wolberg AS, Rosendaal FR, Weitz JI, et al. Venous thrombosis. *Nat Rev Dis Primers*. 2015;1(1):15006. doi:10.1038/nrdp.2015.6
2. Suzuki N, Yoshioka N, Ohara T, et al. Risk factors for perioperative venous thromboembolism: a retrospective study in Japanese women with gynecologic diseases. *Thromb J*. 2010;8(1):17. doi:10.1186/1477-9560-8-17
3. Liem TK, Huynh TM, Moseley SE, et al. Symptomatic perioperative venous thromboembolism is a frequent complication in patients with a history of deep vein thrombosis. *J Vasc Surg*. 2010;52(3):651–657. doi:10.1016/j.jvs.2010.04.029
4. Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. *Virulence*. 2014;5(1):4–11. doi:10.4161/viru.27372
5. Beristain-Covarrubias N, Perez-Toledo M, Thomas MR, Henderson IR, Watson SP, Cunningham AF. Understanding infection-induced thrombosis: lessons learned from animal models. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.02569
6. Munford RS. Endotoxemia-menace, marker, or mistake? *J Leukoc Biol*. 2016;100(4):687–698. doi:10.1189/jlb.3RU0316-151R
7. Pretorius E, Mbotwe S, Bester J, Robinson CJ, Kell DB. Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. *J R Soc Interface*. 2016;13(122):20160539. doi:10.1098/rsif.2016.0539

8. Patrakka O, Pienimäki J, Tuomisto S, et al. Oral bacterial signatures in cerebral thrombi of patients with acute ischemic stroke treated with thrombectomy. *J Am Heart Assoc.* 2019;8(11). doi:10.1161/JAHA.119.012330
9. Hamzeh-Cognasse H, Damien P, Chabert A, Pozzetto B, Cognasse F, Garraud O. Platelets and infections - Complex interactions with bacteria. *Front Immunol.* 2015;6:1–18. doi:10.3389/fimmu.2015.00082
10. Tamowicz B, Mikstacki A, Urbanek T, Zawilska K. Mechanical methods of venous thromboembolism prevention – from the guidelines to the clinical practice. *Pol Arch Intern Med.* 2019. doi:10.20452/pamw.4482
11. Cohen A, Agnelli G, Anderson F, et al. Venous thromboembolism (VTE) in Europe. *Thromb Haemost.* 2007;98(10):756–764. doi:10.1160/TH07-03-0212
12. Adelborg K, Sundbøll J, Sørensen HT. Arterial cardiovascular events and mortality following venous thromboembolism. *Ann Transl Med.* 2015;3(9):117. doi:10.3978/j.issn.2305-5839.2015.04.11
13. Mazzolai L, Aboyans V, Ageno W, et al. Diagnosis and management of acute deep vein thrombosis: a joint consensus document from the European Society of Cardiology working groups of aorta and peripheral vascular diseases and pulmonary circulation and right ventricular function. *Eur Heart J.* 2018;39(47):4208–4218. doi:10.1093/eurheartj/ehx003
14. Raskob GE, Anghaisuksiri P, Blanco AN, et al. Thrombosis: a major contributor to global disease burden: ISTH steering committee for world thrombosis day the members of the ISTH steering committee for World Thrombosis Day. *Thromb Res.* 2014;134(5):931–938. doi:10.1016/j.thromres.2014.08.014
15. Youn YJ, Lee J. Chronic venous insufficiency and varicose veins of the lower extremities. *Korean J Intern Med.* 2019;34(2):269–283. doi:10.3904/kjim.2018.230
16. Lejay A, Koncar I, Diener H, Vega de Ceniga M, Chakfé N. Post-operative infection of prosthetic materials or stents involving the supra-aortic trunks: a comprehensive review. *Eur J Vasc Endovasc Surg.* 2018;56(6):885–900. doi:10.1016/j.ejvs.2018.07.016
17. Branchford BR, Carpenter SL. The role of inflammation in venous thromboembolism. *Front Pediatr.* 2018;6. doi:10.3389/fped.2018.00142
18. Integrative HM. The integrative human microbiome project: dynamic analysis of microbiome-host omics profiles during periods of human health and disease. *Cell Host Microbe.* 2014;16(3):276–289. doi:10.1016/j.chom.2014.08.014
19. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* 2016;14(8):e1002533. doi:10.1371/journal.pbio.1002533
20. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. *Genome Med.* 2016;8(1):51. doi:10.1186/s13073-016-0307-y
21. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol.* 2014;16(7):1024–1033. doi:10.1111/cmi.12308
22. Ahmad AF, Dwivedi G, O’Gara F, Caparros-Martin J, Ward NC. The gut microbiome and cardiovascular disease: current knowledge and clinical potential. *Am J Physiol Heart Circ Physiol.* 2019;317(5):H923–H938. doi:10.1152/ajpheart.00376.2019
23. Krüger W, Vielreicher S, Kapitan M, Jacobsen ID, Niemiec MJ. Fungal-bacterial interactions in health and disease. *Pathogens.* 2019;8(2):70. doi:10.3390/pathogens8020070
24. Paissé S, Valle C, Servant F, et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. *Transfusion.* 2016;56(5):1138–1147. doi:10.1111/trf.13477
25. Li Q, Wang C, Tang C, Zhao X, He Q, Li J. Identification and characterization of blood and neutrophil-associated microbiomes in patients with severe acute pancreatitis using next-generation sequencing. *Front Cell Infect Microbiol.* 2018;8. doi:10.3389/fcimb.2018.00005
26. Castillo DJ, Rifkin RF, Cowan DA, Potgieter M. The healthy human blood microbiome: fact or fiction? *Front Cell Infect Microbiol.* 2019;9. doi:10.3389/fcimb.2019.00148
27. Qiu J, Zhou H, Jing Y, Dong C. Association between blood microbiome and type 2 diabetes mellitus: a nested case-control study. *J Clin Lab Anal.* 2019;33(4):e22842. doi:10.1002/jcla.22842
28. Potgieter M, Bester J, Kell DB, Pretorius E. The dormant blood microbiome in chronic, inflammatory diseases. *FEMS Microbiol Rev.* 2015;39(4):567–591. doi:10.1093/femsre/fuv013
29. Nemati R, Dietz C, Anstadt EJ, et al. Deposition and hydrolysis of serine dipeptide lipids of Bacteroidetes bacteria in human arteries: relationship to atherosclerosis. *J Lipid Res.* 2017;58(10):1999–2007. doi:10.1194/jlr.m077792
30. Rangé H, Labreuche J, Louedec L, et al. Periodontal bacteria in human carotid atherothrombosis as a potential trigger for neutrophil activation. *Atherosclerosis.* 2014;236(2):448–455. doi:10.1016/j.atherosclerosis.2014.07.034
31. Kiouptsi K, Jäckel S, Pontarollo G, et al. The microbiota promotes arterial thrombosis in low-density lipoprotein receptor-deficient mice. *mBio.* 2019;10(5):e02298–19. doi:10.1128/mBio.02298-19
32. Samarasekara K, Munasinghe J. Dengue shock syndrome complicated with acute liver failure and kidney injury, infective endocarditis, and deep vein thrombosis: a case report. *J Med Case Rep.* 2018;12(1):321. doi:10.1186/s13256-018-1862-1
33. Dolapsakis C, Kranidioti E, Katsila S, Samarkos M. Cavernous sinus thrombosis due to ipsilateral sphenoid sinusitis. *BMJ Case Rep.* 2019;12(1):e227302. doi:10.1136/bcr-2018-227302
34. Periyah MH, Halim AS, Mat Saad AZ. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. *Int J Hematol Oncol Stem Cell Res.* 2017;11(4):319–327.
35. Smith SA, Travers RJ, Morrissey JH. How it all starts: initiation of the clotting cascade. *Crit Rev Biochem Mol Biol.* 2015;50(4):326–336. doi:10.3109/10409238.2015.1050550
36. Berends ETM, Kuipers A, Ravesloot MM, Urbanus RT, Rooijackers SHM. Bacteria under stress by complement and coagulation. *FEMS Microbiol Rev.* 2014;38(6):1146–1171. doi:10.1111/1574-6976.12080
37. Frick I-M, Björck L, Herwald H. The dual role of the contact system in bacterial infectious disease. *Thromb Haemost.* 2007;98(09):497–502. doi:10.1160/TH07-01-0051
38. Nickel KF, Renné T. Crosstalk of the plasma contact system with bacteria. *Thromb Res.* 2012;130:S78–S83. doi:10.1016/j.thromres.2012.08.284
39. Antoniak S. The coagulation system in host defense. *Res Pract Thromb Haemost.* 2018;2(3):549–557. doi:10.1002/rth2.12109
40. Bhattacharya S, Ploplis VA, Castellino FJ. Bacterial plasminogen receptors utilize host plasminogen system for effective invasion and dissemination. *J Biomed Biotechnol.* 2012;2012:1–19. doi:10.1155/2012/482096
41. Adivitiya A, Khasa YP. The evolution of recombinant thrombolytics: current status and future directions. *Bioengineered.* 2017;8(4):331–358. doi:10.1080/21655979.2016.1229718
42. Delano MJ, Ward PA. The immune system’s role in sepsis progression, resolution, and long-term outcome. *Immunol Rev.* 2016;274(1):330–353. doi:10.1111/imr.12499
43. Peetermans M, Vanassche T, Liesenborghs L, Lijnen RH, Verhamme P. Bacterial pathogens activate plasminogen to breach tissue barriers and escape from innate immunity. *Crit Rev Microbiol.* 2016;42(6):866–882. doi:10.3109/1040841X.2015.1080214

44. Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol*. 2008;8(10):776–787. doi:10.1038/nri2402
45. Duran-Bedolla J, de Oca-sandoval MA, Saldaña-Navor V, Villalobos-Silva JA, Rodriguez MC, Rivas-Arancibia S. Sepsis, mitochondrial failure and multiple organ dysfunction. *Clin Invest Med*. 2014;37(2):58. doi:10.25011/cim.v37i2.21087
46. Paiva CN, Bozza MT. Are reactive oxygen species always detrimental to pathogens? *Antioxid Redox Signal*. 2014;20(6):1000–1037. doi:10.1089/ars.2013.5447
47. Alberdi P, Cabezas-Cruz A, Prados PE, Rayo MV, Artigas-Jerónimo S, de la Fuente J. The redox metabolic pathways function to limit Anaplasma phagocytophilum infection and multiplication while preserving fitness in tick vector cells. *Sci Rep*. 2019;9(1):13236. doi:10.1038/s41598-019-49766-x
48. Ivanov AV, Bartosch B, Isagulians MG. Oxidative stress in infection and consequent disease. *Oxid Med Cell Longev*. 2017;2017:1–3. doi:10.1155/2017/3496043
49. Ray PD, Huang B-W, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal*. 2012;24(5):981–990. doi:10.1016/j.cellsig.2012.01.008
50. Qiao J, Arthur JF, Gardiner EE, Andrews RK, Zeng L, Xu K. Regulation of platelet activation and thrombus formation by reactive oxygen species. *Redox Biol*. 2018;14:126–130. doi:10.1016/j.redox.2017.08.021
51. Violi F, Pignatelli P. Platelet oxidative stress and thrombosis. *Thromb Res*. 2012;129(3):378–381. doi:10.1016/j.thromres.2011.12.002
52. Liu H, Wu J, Yao J, Wang H, Li S. The role of oxidative stress in decreased acetylcholinesterase activity at the neuromuscular junction of the diaphragm during sepsis. *Oxid Med Cell Longev*. 2017;2017:1–6. doi:10.1155/2017/9718615
53. Wang Q-S, Zheng Y-M, Dong L, Ho Y-S, Guo Z, Wang Y-X. Role of mitochondrial reactive oxygen species in hypoxia-dependent increase in intracellular calcium in pulmonary artery myocytes. *Free Radic Biol Med*. 2007;42(5):642–653. doi:10.1016/j.freeradbiomed.2006.12.008
54. Tafani M, Sansone L, Limana F, et al. The interplay of reactive oxygen species, hypoxia, inflammation, and sirtuins in cancer initiation and progression. *Oxid Med Cell Longev*. 2016;2016:1–18. doi:10.1155/2016/3907147
55. Yang S-L, Wu C, Xiong Z-F, Fang X. Progress on hypoxia-inducible factor-3: its structure, gene regulation and biological function (Review). *Mol Med Rep*. 2015;12(2):2411–2416. doi:10.3892/mmr.2015.3689
56. Ziello JE, Jovin IS, Huang Y. Hypoxia-inducible factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale J Biol Med*. 2007;80(2):51–60.
57. Weidemann A, Johnson RS. Biology of HIF-1 $\alpha$ . *Cell Death Differ*. 2008;15(4):621–627. doi:10.1038/cdd.2008.12
58. Malone PC. The aetiology of deep venous thrombosis. *QJM*. 2006;99(9):581–593. doi:10.1093/qjmed/hcl070
59. Prchal JT. Hypoxia and thrombosis. *Blood*. 2018;132(4):348–349. doi:10.1182/blood-2018-06-854976
60. Kempf VAJ, Lebedziejewski M, Alitalo K, et al. Activation of hypoxia-inducible factor-1 in bacillary angiomatosis. *Circulation*. 2005;111(8):1054–1062. doi:10.1161/01.CIR.0000155608.07691.B7
61. Yeung J, Li W, Holinstat M. Platelet signaling and disease: targeted therapy for thrombosis and other related diseases. *Pharmacol Rev*. 2018;70(3):526–548. doi:10.1124/pr.117.014530
62. Korzonek-Szlacheta I, Zubelewicz-Szkodzińska B, Gąsior M. Płytki krwi — ogniwo łączące zakrzepicę ze stanem zapalnym. *Folia Cardiol*. 2018;13(4):303–308. doi:10.5603/FC.2018.0068
63. Xu J, An Q, Yin W, Hu X. Platelet and immunity in transfusion medicine. In: *Transfusion Medicine and Scientific Developments*. InTech; 2017:55. doi:10.5772/intechopen.69135
64. Kerrigan SW. Platelet interactions with bacteria. in: *the non-thrombotic role of platelets in health and Disease*. InTech; 2015:65. doi:10.5772/60531
65. Kerrigan SW, Cox D. Platelet-bacterial interactions. *Cell Mol Life Sci*. 2010;67(4):513–523. doi:10.1007/s00018-009-0207-z
66. Kerrigan SW, Douglas I, Wray A, et al. A role for glycoprotein Ib in Streptococcus sanguis-induced platelet aggregation. *Blood*. 2002;100(2):509–516. doi:10.1182/blood.V100.2.509
67. Byrne MF, Kerrigan SW, Corcoran PA, et al. Helicobacter pylori binds von Willebrand factor and interacts with GPIb to induce platelet aggregation. *Gastroenterology*. 2003;124(7):1846–1854. doi:10.1016/S0016-5085(03)00397-4
68. McAdow M, Missiakas DM, Schneewind O. Staphylococcus aureus secretes coagulase and von willebrand factor binding protein to modify the coagulation cascade and establish host infections. *J Innate Immun*. 2012;4(2):141–148. doi:10.1159/000333447
69. Crosby HA, Kwiecinski J, Horswill AR. Staphylococcus aureus aggregation and coagulation mechanisms, and their function in host-pathogen interactions. In: *Advances in Applied Microbiology*; 2016:1–41. doi:10.1016/bs.aambs.2016.07.018
70. Thomer L, Schneewind O, Missiakas D. Pathogenesis of Staphylococcus aureus bloodstream infections. *Annu Rev Pathol*. 2016;11(1):343–364. doi:10.1146/annurev-pathol-012615-044351
71. Gros A, Ollivier V, Ho-Tin-Noé B. Platelets in inflammation: regulation of leukocyte activities and vascular repair. *Front Immunol*. 2015;5. doi:10.3389/fimmu.2014.00678
72. Rose PE, Armour JA, Williams CE, Hill FG. Verotoxin and neuraminidase induced platelet aggregating activity in plasma: their possible role in the pathogenesis of the haemolytic uraemic syndrome. *J Clin Pathol*. 1985;38(4):438–441. doi:10.1136/jcp.38.4.438
73. Guessou F, Marcinkiewicz M, Polanowska-Grabowska R, et al. Shiga toxin 2 and lipopolysaccharide induce human microvascular endothelial cells to release chemokines and factors that stimulate platelet function. *Infect Immun*. 2005;73(12):8306–8316. doi:10.1128/IAI.73.12.8306-8316.2005
74. Surewaard BGJ, Thanabalasuriar A, Zeng Z, et al.  $\alpha$ -Toxin induces platelet aggregation and liver injury during Staphylococcus aureus sepsis. *Cell Host Microbe*. 2018;24(2):271–284.e3. doi:10.1016/j.chom.2018.06.017
75. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. *Blood*. 2014;123(18):2768–2776. doi:10.1182/blood-2013-10-463646
76. König MF, Andrade F. A critical reappraisal of neutrophil extracellular traps and NETosis mimics based on differential requirements for protein citrullination. *Front Immunol*. 2016;7. doi:10.3389/fimmu.2016.00461
77. Lazzaretto B, Fadeel B. Intra- and extracellular degradation of neutrophil extracellular traps by macrophages and dendritic cells. *J Immunol*. 2019;203(8):2276–2290. doi:10.4049/jimmunol.1800159
78. Scheithauer TPM, Dallinga-Thie GM, de Vos WM, Nieuwdorp M, van Raalte DH. Causality of small and large intestinal microbiota in weight regulation and insulin resistance. *Mol Metab*. 2016;5(9):759–770. doi:10.1016/j.molmet.2016.06.002
79. Mondot S, Lepage P. The human gut microbiome and its dysfunctions through the meta-omics prism. *Ann N Y Acad Sci*. 2016;1372(1):9–19. doi:10.1111/nyas.13033
80. Vinchi F. Thrombosis prevention. *Hemasphere*. 2019;3(1):e165. doi:10.1097/HS9.0000000000000165
81. Shivaji S. We are not alone: a case for the human microbiome in extra intestinal diseases. *Gut Pathog*. 2017;9(1):13. doi:10.1186/s13099-017-0163-3
82. Khan AA, Shrivastava A, Khurshid M. Normal to cancer microbiome transformation and its implication in cancer diagnosis. *Biochim Biophys Acta Rev Cancer*. 2012;1826(2):331–337. doi:10.1016/j.bbcan.2012.05.005



83. Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autism spectrum disorder. *Biol Psychiatry*. 2017;81(5):411–423. doi:10.1016/j.biopsych.2016.08.024
84. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315(8):801. doi:10.1001/jama.2016.0287
85. Cheng X, Zhang L, Xie NC, Xu HL, Lian YJ. Association between small-intestinal bacterial overgrowth and deep vein thrombosis in patients with spinal cord injuries. *J Thromb Haemost*. 2017;15(2):304–311. doi:10.1111/jth.13583
86. Wigg AJ. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut*. 2001;48(2):206–211. doi:10.1136/gut.48.2.206
87. Shanab AA, Scully P, Crosbie O, et al. Small intestinal bacterial overgrowth in nonalcoholic steatohepatitis: association with toll-like receptor 4 expression and plasma levels of interleukin 8. *Dig Dis Sci*. 2011;56(5):1524–1534. doi:10.1007/s10620-010-1447-3
88. Wang Z, Zhao Y. Gut microbiota derived metabolites in cardiovascular health and disease. *Protein Cell*. 2018;9(5):416–431. doi:10.1007/s13238-018-0549-0
89. Yang K, Du C, Wang X, et al. Indoxyl sulfate induces platelet hyperactivity and contributes to chronic kidney disease-associated thrombosis in mice. *Blood*. 2017;129(19):2667–2679. doi:10.1182/blood-2016-10-744060
90. Brown JM, Hazen SL. Microbial modulation of cardiovascular disease. *Nat Rev Microbiol*. 2018;16(3):171–181. doi:10.1038/nrmicro.2017.149
91. Yancey PH. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J Exp Biol*. 2005;208(15):2819–2830. doi:10.1242/jeb.01730
92. Velasquez M, Ramezani A, Manal A, Raj D. Trimethylamine N-oxide: the good, the bad and the unknown. *Toxins*. 2016;8(11):326. doi:10.3390/toxins8110326
93. Haghikia A, Li XS, Liman TG, et al. Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arterioscler Thromb Vasc Biol*. 2018;38(9):2225–2235. doi:10.1161/ATVBAHA.118.311023
94. Qi J, You T, Li J, et al. Circulating trimethylamine N-oxide and the risk of cardiovascular diseases: a systematic review and meta-analysis of 11 prospective cohort studies. *J Cell Mol Med*. 2018;22(1):185–194. doi:10.1111/jcmm.13307
95. Roncal C, Martinez-Aguilar E, Orbe J, et al. Trimethylamine N-oxide (TMAO) predicts cardiovascular mortality in peripheral artery disease. *Sci Rep*. 2019;9(1):15580. doi:10.1038/s41598-019-52082-z
96. Zhu W, Gregory JC, Org E, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell*. 2016;165(1):111–124. doi:10.1016/j.cell.2016.02.011
97. Wang Z, Roberts AB, Buffa JA, et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell*. 2015;163(7):1585–1595. doi:10.1016/j.cell.2015.11.055
98. Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev*. 2014. doi:10.1111/1574-6976.12075
99. Tang WHW, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013;368(17):1575–1584. doi:10.1056/NEJMoal109400
100. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013;19(5):576–585. doi:10.1038/nm.3145
101. Hung S, Kuo K, Wu C, Tarn D-C. Indoxyl sulfate: a novel cardiovascular risk factor in chronic kidney disease. *J Am Heart Assoc*. 2017;6(2):102–110. doi:10.1161/JAHA.116.005022
102. Karbowska M, Kaminski TW, Znorko B, et al. Indoxyl sulfate promotes arterial thrombosis in rat model via increased levels of complex TF/VII, PAI-1, platelet activation as well as decreased contents of SIRT1 and SIRT3. *Front Physiol*. 2018;9. doi:10.3389/fphys.2018.01623
103. Dalager-Pedersen M, Søgaard M, Schönheyder HC, Nielsen H, Thomsen RW. Risk for myocardial infarction and stroke after community-acquired bacteremia. *Circulation*. 2014;129(13):1387–1396. doi:10.1161/CIRCULATIONAHA.113.006699
104. Putot A, Chague F, Manckoundia P, Cottin Y, Zeller M. Post-infectious myocardial infarction: new insights for improved screening. *J Clin Med*. 2019;8(6):827. doi:10.3390/jcm8060827
105. Fugate JE, Lyons JL, Thakur KT, Smith BR, Hedley-Whyte ET, Mateen FJ. Infectious causes of stroke. *Lancet Infect Dis*. 2014;14(9):869–880. doi:10.1016/S1473-3099(14)70755-8
106. Roquer J, Cuadrado-Godia E, Giralte-Steinthauer E, et al. Previous infection and stroke: a prospective study. *Cerebrovasc Dis*. 2012;33(4):310–315. doi:10.1159/000335306
107. Tralhão A, Póvoa P. Cardiovascular events after community-acquired pneumonia: a global perspective with systematic review and meta-analysis of observational studies. *J Clin Med*. 2020;9(2):414. doi:10.3390/jcm9020414
108. Beynon RP, Bahl VK, Prendergast BD. Infective endocarditis. *BMJ*. 2006;333(7563):334–339. doi:10.1136/bmj.333.7563.334
109. Musher DM, Abers MS, Corrales-Medina VF. Acute infection and myocardial infarction. *N Engl J Med*. 2019;380(2):171–176. doi:10.1056/NEJMra1808137
110. Kastrop CJ, Boedicker JQ, Pomerantsev AP, et al. Spatial localization of bacteria controls coagulation of human blood by ‘quorum acting’. *Nat Chem Biol*. 2008;4(12):742–750. doi:10.1038/nchembio.124
111. Cray SE, Buchanan GR, Drake CE, Journeycake JM. Venous thrombosis and thromboembolism in children with osteomyelitis. *J Pediatr*. 2006;149(4):537–541. doi:10.1016/j.jpeds.2006.06.067
112. Gonzalez BE, Teruya J, Mahoney DH, et al. Venous thrombosis associated with staphylococcal osteomyelitis in children. *Pediatrics*. 2006;117(5):1673–1679. doi:10.1542/peds.2005-2009
113. Lee C-Y, Lee Y-S, Tsao P-C, Jeng M-J, Soong W-J. Musculoskeletal sepsis associated with deep vein thrombosis in a child. *Pediatr Neonatol*. 2016;57(3):244–247. doi:10.1016/j.pedneo.2013.09.004
114. Mantadakis E, Plessa E, Vouloumanou EK, Michailidis L, Chatzimichael A, Falagas ME. Deep venous thrombosis in children with musculoskeletal infections: the clinical evidence. *Int J Infect Dis*. 2012;16(4):e236–e243. doi:10.1016/j.ijid.2011.12.012
115. Arduini A, Zammit VA, Bonomini M. Identification of trimethylamine N-oxide (TMAO)-producer phenotype is interesting, but is it helpful? *Gut*. 2018;2019. doi:10.1136/gutjnl-2018-318000
116. Vail GM, Xie YJ, Haney DJ, Barnes CJ. Biomarkers of thrombosis, fibrinolysis, and inflammation in patients with severe sepsis due to community-acquired pneumonia with and without *Streptococcus pneumoniae*. *Infection*. 2009;37(4):358–364. doi:10.1007/s15010-008-8128-6
117. Violi F, Cangemi R, Calvieri C. Pneumonia, thrombosis and vascular disease. *J Thromb Haemost*. 2014;12(9):1391–1400. doi:10.1111/jth.12646
118. Johansson D, Shannon O, Rasmussen M. Platelet and neutrophil responses to Gram positive pathogens in patients with bacteremic infection. *PLoS One*. 2011;6(11):e26928. doi:10.1371/journal.pone.0026928



119. Andes DR, Urban AW, Acher CW, Maki DG. Septic thrombosis of the basilic, axillary, and subclavian veins caused by a peripherally inserted central venous catheter. *Am J Med.* 1998;105(5):446–450. doi:10.1016/S0002-9343(98)00287-3
120. Sato A, Nakamura I, Fujita H, et al. Peripheral venous catheter-related bloodstream infection is associated with severe complications and potential death: a retrospective observational study. *BMC Infect Dis.* 2017;17(1):434. doi:10.1186/s12879-017-2536-0
121. Denis Spelman, MBBS, FRACP, FRCPA M. Suppurative (septic) thrombophlebitis. Literature review current through. 2018. Available from: <https://www.uptodate.com/contents/suppurative-septic-thrombophlebitis>.
122. Cox ER, Amoroso A, Gilliam BL. Pannus attack: septic thrombophlebitis. *Am J Med.* 2012;125(12):1175–1177. doi:10.1016/j.amjmed.2012.08.002
123. Weinberg G. Upper-extremity suppurative thrombophlebitis and septic pulmonary emboli. *JAMA.* 1978;240(14):1519. doi:10.1001/jama.1978.03290140061029
124. Xin Koh Y, Kian Chng J, Guan Tan S. A rare case of septic deep vein thrombosis in the inferior vena cava and the left iliac vein in an intravenous drug abuser. *Ann Vasc Dis.* 2012;5(3):389–392. doi:10.3400/avd.cr.12.00036
125. Cornford CS, Mason JM, Inns F. Deep vein thromboses in users of opioid drugs: incidence, prevalence, and risk factors. *Br J Gen Pract.* 2011;61(593):e781–e786. doi:10.3399/bjgp11X613115
126. Kwiatkowska W, Knysz B, Gąsiorowski J, Witkiewicz W. Deep vein thrombosis of the lower limbs in intravenous drug users. *Postepy Higieny I Medycyny Doswiadczalnej.* 2015;69:510–520. doi:10.5604/17322693.1150215
127. Antonova N, Zvetkova E, Ivanov I, Savov Y. Hemorheological changes and characteristic parameters derived from whole blood viscometry in chronic heroin addicts. *Clin Hemorheol Microcirc.* 2008;39(1–4):53–61. doi:10.3233/CH-2008-1068
128. Huisjes R, Bogdanova A, van Solinge WW, Schiffelers RM, Kaestner L, van Wijk R. Squeezing for life – properties of red blood cell deformability. *Front Physiol.* 2018;9. doi:10.3389/fphys.2018.00656
129. Galante A, DeLuca A, Pietroiusti A, et al. Effects of opiates on blood rheology. *J Toxicol Clin Toxicol.* 1994;32(4):411–417. doi:10.3109/15563659409011042

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