

# Inflammation, Sepsis, and the Coagulation System

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## Abstract

Sepsis has been a major health problem for centuries and it is still the leading cause of hospital deaths. Several studies in the past decades have identified numerous biochemical abnormalities in severe cases, and many of these studies provide evidence of the perturbation of the hemostatic system. This can result in complications, such as disseminated intravascular coagulation that can lead to multiorgan failure. Nevertheless, large clinical studies have demonstrated that the simple approach of inhibiting the coagulation processes by any means fails to provide significant improvement in the survival of septic patients. A cause of this failure could be the fact that in sepsis the major clinical problems result not primarily from the presence of the infective agent or enhanced coagulation but from the complex dysregulated systemic host response to pathogens. If this overt reaction is not fully deciphered, appropriate interference is highly unlikely and any improvement by conventional therapeutic interventions would be limited. Cellular activation in sepsis can be targeted by novel approaches like inhibition of the heterotypic cellular interactions of blood cells by targeting surface receptors or posttranscriptional control of the hemostatic system by noncoding ribonucleic acid (RNA) molecules. Stable RNA molecules can affect the expression of several proteins. Thus, it can be anticipated that modulation of microRNA production would result in a multitude of effects that may be beneficial in septic cases. Here, we highlight some of the recent diagnostic possibilities and potential novel routes of the dysregulated host response.

## Keywords

- ▶ sepsis
- ▶ inflammation
- ▶ biomarker
- ▶ p-selectin
- ▶ procoagulant platelets
- ▶ microRNAs

## Introduction

Patients may react to inflammatory stimuli with an overexpression of a variety of proinflammatory mediators. This results in a clinical syndrome characterized by fever, tachycardia, tachypnea, and massive leukocytosis, namely, the systemic inflammatory response syndrome (SIRS). SIRS is the final common pathway of inflammation. It is difficult to distinguish SIRS caused by sterile inflammation from sepsis that is defined as SIRS induced by a verifiable infection.<sup>1</sup> It is a life-threatening condition that annually claims at least 11 million lives worldwide out of the nearly 48 to 50 million

intensive care unit (ICU) septic patients. Although the initiating insult in most cases is clear being a bacterial, viral, or fungal infection, the major clinical problem results from the dysregulated systemic host response to these microbial pathogens.<sup>2</sup> This leads to a disproportionate inflammatory response and may lead to critical organ dysfunction affecting particularly the guts, lungs, kidneys, liver, heart, and the brain. This is the primary cause for sepsis becoming the most common cause of death in hospitals.<sup>3</sup> When the septic response leads to complex circulatory, metabolic, and cellular abnormalities, the term septic shock is applied where the mortality ratio still reaches around 40%. The severity of

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sepsis is categorized by two commonly used clinical scoring systems: the acute physiology and chronic health evaluation II (APACHE II) and the sequential/sepsis-related organ failure assessment (SOFA) scores that assess several organ systems based on clinical and laboratory data.

## Fundamental Immunological Mechanisms in Sepsis

The basic pathomechanism of sepsis has recently been extensively reviewed by Arora et al.<sup>2</sup> In sepsis, the innate immune system is activated in response to pathogens via binding discrete epitopes designated as pathogen-associated molecular patterns (PAMPs) to pattern recognition receptors called toll-like receptors (TLRs). Activation of these receptors triggers intracellular signaling that stimulates the activation of transcription factors, such as the nuclear factor-kappa B (NF- $\kappa$ B) and interferon regulatory factor pathways to release inflammatory cytokines.<sup>4</sup> Several studies demonstrated that the compensatory anti-inflammatory response occurs after hyperinflammation. The initial cytokine storm is responsible for the observed symptoms and early death is due to multiple organ dysfunction.<sup>5</sup> Initially, there is an abrupt innate immune response by phagocytic cells followed by a much slower adaptive immune response. Sepsis-induced immunosuppression, also called “immune exhaustion,” involves the apoptotic depletion of immune cells.<sup>6</sup> The innate and adaptive immune cells undergo apoptosis, contributing to reduced clearance of invading pathogens.<sup>7</sup> Apoptotic depletion of CD4<sup>+</sup> T cells results in decreased cytokine production and these cells can develop a state of functional unresponsiveness referred to as “exhaustion” due to prolonged antigen exposure and altered differentiation of memory T cells.<sup>8</sup> There is an increase in T regulatory suppressor cells with a concomitant loss of effector T cells and because of the suppression of cell-based immunity, the mortality associated with the late phase of sepsis is due to acquired secondary and opportunistic infections, such as *Candida*.<sup>9</sup> The immune system also stimulates endothelial cells and contributes to microcirculatory failure that intertwines in sepsis pathophysiology.

## Laboratory Background for Sepsis Investigation

Although sepsis is defined as a dysregulated host response to infection, our ability to discriminate adaptive and maladaptive immune response is still limited<sup>10</sup> as we lack clinical tools to quantify the balance between hyper- and hypo-inflammation. Various biomarkers are available for the diagnosis, prognosis estimation, and follow-up of sepsis; however, these laboratory parameters may show inherent limitations. The most frequently used tests are leukocyte count, C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6), and IL-6 has become even more widely used in recent years during the coronavirus disease 2019 (COVID-19) pandemic. Septic patients are commonly characterized by the SOFA score that incorporates the following

three laboratory parameters: platelet count, creatinine level, and total bilirubin concentration. Furthermore, for the evaluation of inflammatory and infectious conditions, basic data provided by hematology analyzers are commonly utilized in addition to platelet count, like the mean platelet volume (MPV), platelet distribution width (PDW), platelet-to-large cell ratio, and plateletcrit. Changes in these parameters are not only associated with occurrence of inflammatory diseases but also occur during severe infections.<sup>11–13</sup> These laboratory assays are always complemented by a panel of hemostasis tests to screen for the potential presence of disseminated intravascular coagulation (DIC). This is a real threat as clinically relevant hemostatic alterations may occur in 50 to 70% of septic patients, and approximately one-third of these patients actually meet the criteria for DIC.<sup>14</sup> Based on the circumstances and local possibilities, the DIC test panel implies a large fraction of the following laboratory assays: prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen assay, D-dimer assay, fibrin monomer (FM) test, and antithrombin activity with the investigation of blood smear for the quantitative determination of schistocytes. It was also found that sepsis-associated DIC has different biomarkers than DIC associated with other diseases like hematologic malignancies. Namely, IL-6 and thrombopoietin (TPO) were found to be elevated in the former but not in the latter, and IL-6 and TPO also promoted platelet activation *in vitro*.<sup>15</sup> This may lead to the consideration that, while a basic DIC panel is the same for all cases regardless of the eliciting cause, it may be worth extending the DIC panel to be more specific to the underlying disease. Numerous current and potential future clinical laboratory assays are summarized in [Table 1](#).

## Hemostatic System Activation in Sepsis

### Platelet-Associated Proinflammatory Events

Forty years ago, an antibody clone was developed that was found to be reactive only with activated but not resting platelets<sup>16</sup> and later it became evident that this clone identified the platelet surface P-selectin receptor that now represents one of the classical platelet activation markers. During subsequent years, its expression has been found to be elevated by flow cytometry in numerous diseases including sepsis with enhanced platelet activation.<sup>17–19</sup> This surface protein can detach from the platelet surface and is detectable in the plasma by appropriate immunoassays in soluble form. Although soluble P-selectin level is thought to be primarily associated with platelet activation, the same P-selectin molecule is released upon endothelial cell activation as that is synthesized and stored in the Weibel–Palade bodies of the endothelial lining.<sup>20</sup> Measurement of soluble P-selectin was found to be useful in patients with SIRS not admitted for an underlying infective problem as their soluble P-selectin levels were significantly related to the subsequent development of infection.<sup>21</sup> The expression of P-selectin on the platelet membrane mediates the aforementioned adherence of platelets to leukocytes, and P-selectin on endothelial cells enhances the rolling of platelets on stimulated

**Table 1** Laboratory tests used in sepsis diagnosis as well as for prognosis of multiorgan failure

	Laboratory biomarkers for sepsis		
	Chemistry	Hematology	Hemostasis
Emergency tests	Na <sup>+</sup> , K <sup>+</sup> , Cl <sup>-</sup> , Ca <sup>2+</sup> , Mg <sup>2+</sup> , osmolality, CRP, PCT, IL-6, lactate, creatinine, urea, uric acid, AST, ALT, bilirubin, total protein, albumin	<i>Hematology analyzer:</i> WBC, RBC, HGB, HCT, PLT, MPV, PDW, P-LCR <i>Microscopy:</i> schistocyte	DIC panel (PT, APTT, TT, fibrin-monomer, D-dimer), fibrinogen, fibrin degradation product
Special tests	IL-1 $\beta$ , IL-8, IL-10, IL-18, TNF- $\alpha$ , LBP, haptoglobin, amyloid-A, fibronectin, TPO, ferritin, $\alpha$ 1-antitrypsin, $\alpha$ 2-macroglobulin, mannose binding lectin, resistin, NGAL, NT-proBNP, S100B, NSE, hepcidin	<i>Hematology analyzer:</i> immature granulocyte, immature platelet fraction <i>Flow cytometry:</i> platelet P-selectin, monocyte CD64	Coagulation factors, vWF, TFPI, AT-III, protein C, PAI-1, thrombomodulin, ADAMTS13, rotational and viscoelastic thromboelastometry
Potential future tests	<i>Blood tests:</i> ICAM-1, VCAM-1, ELAM-1, E-selectin, VEGF, HMGB-1, MIF, MIP, presepsin, neopterin, pentraxin-3, KIM-1, PD-L1, ROS <i>Urine tests:</i> IGFBP-7, TIMP-2	<i>Hematology analyzer:</i> MDW <i>Flow cytometry:</i> CD48, CD68, TNFR, C5aR, TREM-1 mHLA-DR, PEVs	suPAR, NETs, TGT, micro-RNAs, long noncoding RNAs, MP-TF

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with thrombospondin motifs-13; APTT, activated partial thromboplastin time; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AT-III, antithrombin III; C5aR, complement component 5a receptor; CRP, C-reactive protein; DIC, disseminated intravascular coagulation; ELAM-1, endothelial leukocyte adhesion molecule; HCT, hematocrit; HGB, hemoglobin; HMGB-1, high-mobility-group protein B1; ICAM-1, intercellular adhesion molecule 1; IGFBP-7, insulinlike growth factor binding protein 7; IL, interleukin; KIM-1, kidney injury molecule-1; LBP, lipopolysaccharide binding protein; MP-TF, microparticle tissue factor complex; MDW, monocyte distribution width; mHLA-DR, monocytic human leukocyte antigen DR; MIF, migration inhibitory factor; MIP, macrophage inflammatory protein; MPV, mean platelet volume; NETs, neutrophil extracellular traps; NGAL, neutrophil gelatinase-associated lipocalin; NSE, neuron-specific enolase; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAI-1, plasminogen activator inhibitor; PCT, procalcitonin; PD-L1, programmed cell death ligand-1; PDW, platelet distribution width; PEVs, platelet-derived extracellular vesicles; PLT, platelet count; PT, prothrombin time; P-LCR, platelet-to-large cell ratio; RBC, red blood cell; ROS, reactive oxygen species; S100B, calcium-binding protein B; suPAR, soluble urokinase plasminogen activator receptor; TFPI, tissue factor pathway inhibitor; TGT, thrombin generation test; TIMP-2, tissue inhibitor of metalloproteinase-2; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; TNFR, tumor necrosis factor receptor; TPO, thrombopoietin; TREM-1, triggering receptor expressed on myeloid cells 1; TT, thrombin time; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor; WBC, white blood cell.

endothelium<sup>22</sup> and the expression of tissue factor (TF) on monocytes<sup>23</sup> in association with an unfavorable prognosis of sepsis already reported in early studies.<sup>24</sup> Furthermore, activated platelets express CD40 ligand (CD40L) and—similarly to P-selectin—shed this molecule into the bloodstream. Platelet-derived CD40L can bind to CD40 exposed on their surface and neutrophils leading to more platelet activation.<sup>25,26</sup> It can also interact with endothelial CD40 leading to stimulation of endothelial cells to upregulate expression of various other adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), and to release the chemokine CCL2, also referred to monocyte chemoattractant protein 1, thereby promoting recruitment of myeloid cells. An instrument, suitable for detection of platelet activation, for example, flow cytometry, can provide a comprehensive panel of tests that identify various aspects of platelet reactivity. In addition to P-selectin and CD40L expression, apoptosis with loss of plasma membrane asymmetry and phosphatidylserine (PS) exposure as well as abnormal index values of mitochondrial membrane potential (Mmp) in patients diagnosed with sepsis were extensively analyzed and the platelet PS exposure was notable. This parameter was found to be associated

with the pathological mechanism of sepsis, which was evident by its correlation with clinical scores.<sup>27</sup> When stratifying patients with sepsis and septic shock by clinical condition, the APACHE II score significantly correlated with platelet Mmp and platelet PS positivity. Platelets participate in the thrombotic processes by cellular interactions, but these anucleate cells also contain a functional set of ribonucleic acids (RNAs), such as messenger RNAs (mRNAs). As the main initiator of blood coagulation processes is TF, it is of particular importance that TF mRNA was found in platelets, which means these cells can de novo synthesize the protein present in circulating platelets<sup>28</sup> and thus express TF activity upon agonist stimulation.<sup>29</sup> It has also been described that live bacteria and bacterial toxins can directly induce TF mRNA production in platelets, thereby directly contributing to its procoagulant activity.<sup>30</sup> This results in the formation of the P-selectin TF coagulation triad.<sup>31</sup>

#### Leukocyte-Related Hyperinflammatory Events

The longest known function of leukocytes, that is, their participation in septic processes, is the elimination of pathogens via innate and adaptive immunocompetent cells. However, there are clear connections with the involvement of

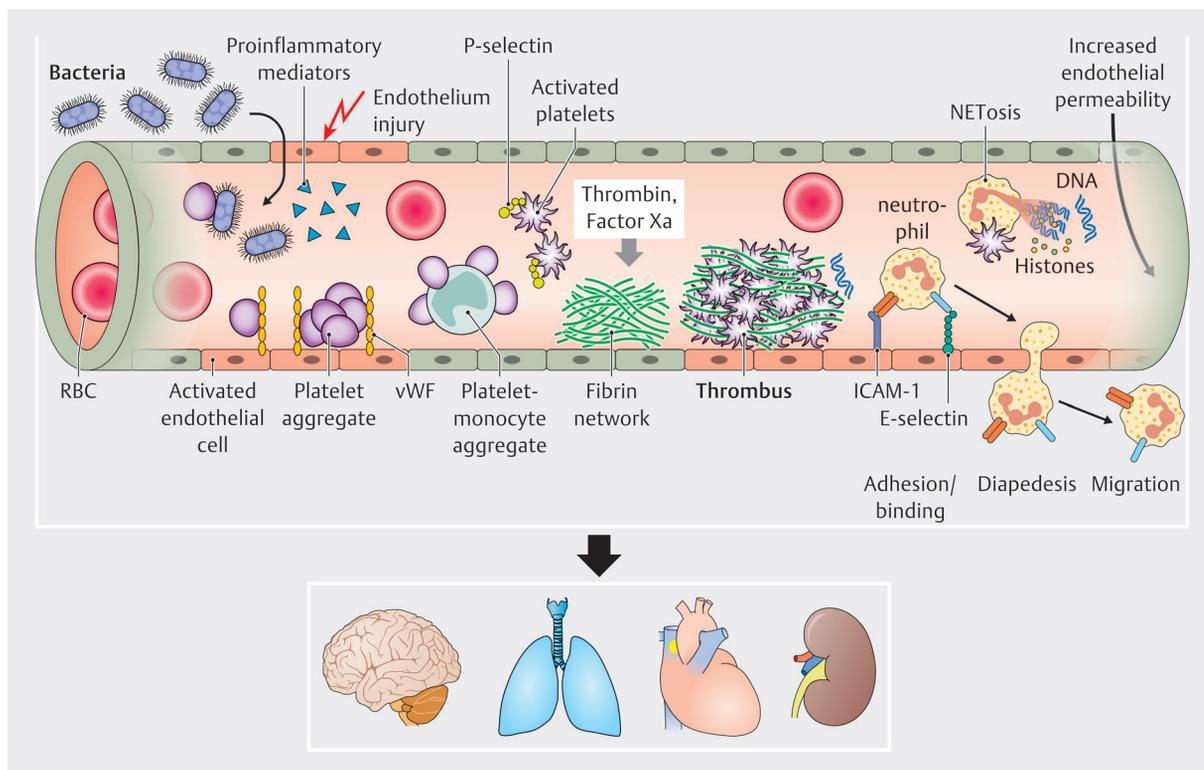
hemostatic mechanisms primarily involving the myeloid cells. Activated platelets form heterotypic cell aggregates mostly with monocytes and neutrophils. The percentage of monocyte–platelet aggregates, revealed by flow cytometry, was found to be a very sensitive indirect marker of platelet activation both in cardiovascular and septic conditions.<sup>32</sup> Monocytes and neutrophils attach to activated platelets mainly through P-selectin via its counterreceptor PSGL-1 (P-selectin glycoprotein ligand-1), a dimeric mucin that is constitutively expressed on leukocytes. Hence, experiments aimed at blocking either of these molecules with a monoclonal antibody resulted in a complete inhibition of the initial interaction between platelets and neutrophils.<sup>33</sup> Neutrophils also participate in host defense mechanisms against sepsis via the generation of neutrophil extracellular traps (NETs). At their first description, two decades ago, it was observed that the presence of NETs correlated with the severity of organ dysfunction in sepsis.<sup>34</sup> NETs are released extracellularly from activated neutrophils in response to both infection and the sterile inflammatory processes. These fibrous structures then trap and kill pathogens within their matrices as part of the host defense mechanism. The principal component of NETs is the DNA and treating NETs with DNases indeed destroys these components. There are several proteins that build up NETs, primarily histones, and components

of the primary neutrophil granules like cathepsin G, elastase, myeloperoxidase (MPO), and a nuclear protein, the high mobility group box 1 (HMGB1). NETs subsequently can activate endothelial cells and thus further affect hemostatic processes during sepsis.<sup>35</sup> More recently, interesting mechanistic discoveries have been made by using a novel assay for NET detection, where the authors proved that IL-8-induced pathway is a major event in NET formation, thereby opening the theoretical possibility of reducing NET formation with the aid of IL-8 inhibitors.<sup>36</sup> There is a large body of literature dealing with TF upregulation in inflammatory states and sepsis in monocytes and endothelial cells; however, that falls beyond the frames of the recent review.

The versatile mechanisms of different cellular interplays that participate in these processes are illustrated in **Fig. 1**.

### Coagulation-Associated Events

The fact that sepsis is one of the main causes of DIC has been textbook data for several decades. This implies that the fluid phase of coagulation is activated and the coagulation factors are consumed, causing prolongation of the clotting times, PT and APTT. Fibrinogen, a well-known coagulation factor, is also known to be an acute-phase protein that is rapidly produced following the onset of infection and inflammation. It serves as a protective barrier by acting as bacteria-



**Fig. 1** Schematic figure on various subsequent cellular events that are initiated upon sepsis. Due to the accumulation of bacteria via the bloodstream, not only leukocytes but also platelets and endothelial cells easily get activated, leading to cell dysfunctions: platelets become hyperreactive to produce platelet–platelet and platelet–leukocyte aggregates, injured endothelial cells may be swollen, and permeability of the vasculature can increase resulting in diapedesis of neutrophils and fluid leakage. Stimulated neutrophils bound to activated platelets may produce NETs with the release of DNA, histones, and other mediators. Apart from cellular phenomena, humoral alterations also occur in sepsis via enhanced thrombin generation and cytokine storm. All these mechanisms may cause thrombus formation and related ischemic damage of variable organs, such as the brain, lungs, heart, and kidneys. DNA, deoxyribonucleic acid; ICAM-1, intercellular adhesion molecule 1; NETs, neutrophil extracellular traps; RBC, red blood cell; vWF, von Willebrand factor.

entrapping fibrin matrices, which activate the host immune system either directly or indirectly; thus, fibrinogen plays a major role in hemostasis and antimicrobial host defense by limiting bacterial growth and mediation of host bacterial killing.<sup>37</sup> Hyperfibrinogenemia in septic patients was a consequence of increased fibrinogen production.<sup>38</sup> However, a large part of septic patients in several studies displayed a decreasing trend of fibrinogen concentration as time progressed due to severe coagulopathy. In such patients, the 28-day mortality rate was higher than that in patients with nondecreasing fibrinogen trends.<sup>39</sup> Thus, it is reasonable to assume that an elevated level of fibrinogen reflects a favorable compensation of the human body and avoidance of the slide into DIC, and even predicts better survival than patients with normal fibrinogen.<sup>40</sup> In addition to fibrinogen, the D-dimer level was found to be a useful predictor in sepsis in patients with very different ages. A subgroup analysis indicated that its level was strongly and stably associated with in-hospital mortality independent of age and sex in septic children.<sup>41</sup> In elderly patients suffering from sepsis, the combination of PaO<sub>2</sub>/FiO<sub>2</sub>, SOFA score, and D-dimer represented a promising tool and biomarker for predicting 28-day mortality.<sup>42</sup>

### Lessons on Coagulation Learned from Experimental Sepsis Models

One key disadvantage of human studies is the large variability in the underlying clinical conditions as well as the effect of applied therapy on laboratory parameters. Severity of the disease has been linked to several laboratory parameters in clinical studies, but the kinetics of these changes can be better followed when sepsis is elicited under controlled conditions in an animal experiment. Experimental animal models have increasingly been used in the past decades.<sup>43</sup> A relatively well-standardized way to study sepsis is to use an animal model that mostly replicates severe human sepsis. Unlike rodents, murine sepsis models with cecal ligation and puncture (CLP)<sup>44</sup> or porcine sepsis models by administering LPS/live bacteria are indeed relatively close to human sepsis and thus have been used in the past for this disease model. We also performed experiments in a lethal 4-hour pig model after live *E. Coli* injection. We observed that both the core temperature and the modified shock index were increased significantly by 4 hours compared with baseline data. In addition, the absolute lymphocyte count was significantly decreased, and a considerable difference was noted between the treated and control group already at 2 hours after sepsis induction. The lobularity index of the white blood cells (WBCs) decreased significantly at both 2 and 4 hours referring to the appearance of immature WBCs in the bloodstream.<sup>45</sup> Furthermore, we studied mitochondrial membrane changes in platelets as mitochondria play an important role in cellular survival and apoptotic death upon sepsis.<sup>46</sup> In sepsis, mitochondria play a crucial role in the maintenance of endothelial cell homeostasis,<sup>47</sup> where dysfunction can lead to both micro- and macrovascular complications. Dysfunctional mitochondria can contribute

to the development of a hyperinflammatory state<sup>48</sup> by increasing reactive oxygen species (ROS) production during infection. Studying platelet mitochondrial function can also serve as a model of mitochondrial changes in other not-easily-accessible cells like endothelial cells. Mitochondrial membrane depolarization not only may reflect the abnormality of aerobic metabolism but can also be associated with the mitochondrial pathway of apoptosis. We found a significant drop in mitochondrial function in the septic group by 2 hours, indicating mitochondrial dysfunction and potentially platelet apoptosis in sepsis. We also observed that plasma samples drawn at 2 hours from septic animals induced a significant PS expression in washed human red blood cells. This demonstrates that soluble substances that are capable of inducing a procoagulant surface on cells are released during the *E. Coli* challenge.<sup>45</sup> As hypo- and hypercoagulable features are simultaneously present in sepsis, a well-suited technique to study sepsis-related hemostatic events is the thrombin generation assay (TGA). By using various modifications of this technique, we observed that at 4 hours after sepsis induction, thrombin generation initiated and finished significantly earlier, while the thrombin peak was higher compared with the baseline values in the group who received *E. Coli*. The thrombin peak increased significantly by 2 hours according to the increased amount of generated thrombin.<sup>49</sup> These results also support the presence of an initial and short-lived hypercoagulable phase in sepsis (→Fig. 2). Others described increased thrombin generation within 3 hours after CLP in a murine sepsis model and described a marked reduction in thrombin generation by 6 and 24 hours.<sup>50</sup> We suppose that human studies usually fail to demonstrate the initial hypercoagulability in sepsis, which might be caused by the delay between early changes in the coagulation system and clinical presentation of septic symptoms. In a relatively recent pilot report,<sup>51</sup> it was found that a high frequency of mitochondrial DNA mutations was detectable in pediatric cases of sepsis, raising the possibility that mitochondrial dysfunction in sepsis may have a genetic basis.

### Clinical Trials to Alleviate the Enhanced Coagulation in Sepsis

Anticoagulant therapies were once expected to be a beneficial adjunctive therapy in sepsis. However, despite the numerous studies focusing on anticoagulant treatment against sepsis, these therapies continue to remain a matter of dispute. Most large randomized controlled clinical trials concluded that these therapeutic attempts failed to provide a meaningful improvement.<sup>52</sup> The largest trials on the efficacy of unfractionated heparin in sepsis showed no difference in 28-day mortality between the treatment and control groups,<sup>53</sup> just as another large trial using low-dose heparin in patients with sepsis-induced coagulopathy.<sup>54</sup> Multiple authors have verified that antithrombin is decreased in sepsis and that this decrease correlates with lethal outcome. Thus, large expectations preceded the trial when recombinant antithrombin was used in septic patients. However, the

Sample type	Reagent	Parameters of thrombin generation				Coagulation status
		lagtime	ttPeak	peak	ETP	
PPP	TF+PL+	↓	↓	↓	↓	hypocoagulation
PPP	TF-PL+	↓	↓	↓	↓	hypocoagulation
PPP	TF-PL-	↓	↓	↑	ns	hypercoagulation
PRP	TF+	ns	↓	ns	ns	platelet activation

**Fig. 2** In the septic pig model, the detectability of changes on coagulation depends largely on the composition of the reagent used for TGA. In the presence of TF and PL or only PL (TF+PL+ or TF-PL+), the hypocoagulable state can be easily detected in a plasma (PPP) by TGA. Here, the time parameters of TGA, such as lagtime and time to peak (ttPeak) are shortened, and the amount of formed thrombin is less, which is characterized by the decreasing thrombin peak and endogenous thrombin potential (ETP). The presence of a hypercoagulable state however, can only be detected by the sensitization of TGA with no added exogenous TF and PL (TF-PL-). As a result the following changes could be observed, the time parameters (lagtime and time to peak (ttPeak)) were shortened and thrombin peak was increased. Upon platelet activation, the thrombin formation reaches its peak value earlier in a platelet rich plasma (PRP) due to PL appearing on the cell surface and exogenously added TF (TF+).

KyberSept trial, the largest of these trials, that enrolled over 2,000 patients with severe sepsis showed no difference in 28-day mortality between patients receiving antithrombin and placebo.<sup>55</sup> Another agent that was introduced in sepsis with high expectations was the recombinant human activated protein C (PC), since reduced activation of PC facilitates a prothrombotic phenotype in sepsis.<sup>56</sup> This was the only anticoagulant agent so far that was recommended for use against severe sepsis in the Surviving Sepsis Campaign guidelines, following the results of the PROWESS (Recombinant human protein C Worldwide Evaluation in Severe Sepsis) trial, which showed a significant reduction in mortality with recombinant activated PC (drotrecogin alfa, Xigris) versus placebo.<sup>57</sup> Nevertheless, subsequent large-scale trials showed no significant reduction in mortality with this drug and finally it was withdrawn from the market in 2011. Another possibility was the recombinant soluble thrombomodulin (rTM) that is a relatively novel anticoagulant agent released to the market in 2008. In patients with sepsis, rTM binds to thrombin, promotes the activation of PC, and exhibits anticoagulant effects by inhibiting further thrombin generation.<sup>58</sup> In a phase 3, multinational, multicenter trial called the Sepsis Coagulopathy Asahi Recombinant LE Thrombomodulin (SCARLET) trial, 800 patients with sepsis-induced coagulopathy who fulfilled the following criteria were studied: (1) at least one sepsis-associated organ dysfunction, (2) prolongation of the international normalized ratio (INR) of greater than 1.4, and (3) reduction of platelet count. This study reported no statistically significant difference in 28-day mortality between the rTM and placebo groups.<sup>59</sup> Taken all these unfavorable news together, it seems highly unlikely that an effective treatment can be introduced until proper mechanisms in sepsis-induced coagulopathy are clarified. Simple laboratory approaches, like the ones listed earlier, may not be sufficient to delineate the

underlying processes in sepsis. Today there is persistently a high mortality associated with sepsis and specific therapies may be based on a more complex evaluation of the disturbed host responses, for example, by using the “omics” technology. By using this technique, it was discovered that there are two different sepsis response signatures, one of which is associated with an immunosuppressed phenotype and a higher mortality rate.<sup>60</sup> The authors have also shown that this categorization in response is feasible based on the predictive value of only a handful of genes. Whether such approaches may become more widely used and can aid in a more personalized treatment in sepsis may be answered in the coming years.

### A Potential Novel Pathway in Interfering with Sepsis Induced Coagulopathy

Epigenetic changes have long been known to affect and determine pathological conditions via posttranscriptional modifications of DNA and histones as well as the interference with transcription via noncoding RNA. A portion of these noncoding RNA molecules are small and stable RNA molecules designated as microRNAs (miRNAs) typically consist of around 22 nucleotides in length and are conserved across multiple animal species, indicating the evolutionary importance of these molecules as modulators of critical biological pathways and processes.<sup>61</sup> These miRNAs inhibit translation as they can cause direct degradation of their target mRNAs, and they do not require perfect complementarity for target recognition, so a single miRNA is responsible for the regulation of multiple mRNAs. Computational analysis indicates that the total number of miRNAs could be more than 1% of the total translated genes, and more than 30% of protein-coding genes may be targeted by miRNAs.<sup>62</sup> Upon maturation, miRNAs are translocated to the RNA-induced silencing

complex (RISC), bind the complementary sequences of downstream targets, and prevent the translation of target genes through translational repression or mRNA degradation.<sup>63</sup> Circulating miRNAs are present in the bloodstream in microvesicle-associated or protein-bound forms with distinct cell origin,<sup>64</sup> and consequently they may be suitable laboratory biomarkers in sepsis. Since the largest pool of microvesicles is derived from platelets, a substantial ratio of cell-free miRNAs is transferred from activated platelets to other cell types and interfere with gene expression.<sup>65</sup> As these regulatory processes can be targeted by silencing RNA molecules as well as mimics, it is important to characterize the miRNA changes during sepsis so that these processes can be appropriately interfered. We have described in a series of septic patients by a TaqMan OpenArray technique that out of 390 platelet miRNAs, 121 were significantly decreased and 61 were upregulated in sepsis versus controls.<sup>66</sup> Septic platelets showed attenuated miR-26b, which were associated with disease severity and mortality. The P-selectin (SELP) mRNA level was elevated in sepsis, especially in platelets with increased MPV values causing a higher P-selectin expression. MiRNA formation can be regulated as decreased Dicer1 enzyme level—a key factor in miRNA formation<sup>67</sup>—generated lower platelet miR-26b expression with higher SELP mRNA level, while calpeptin could restore miR-26b using MEG-01 cells among in vitro septic conditions. We could conclude that decreased miR-26b in megakaryocytes and platelets contributes to an increased level of platelet activation status in sepsis.<sup>66</sup> MiRNA can be present not only as a “soluble plasma oligonucleotide” but also as a cargo in microvesicles released by activated cells, primarily platelets, and these microvesicles can be taken up by various cell types.<sup>68,69</sup> Nevertheless, further studies focusing on the pathological role and therapeutic potentials of miRNAs in sepsis are required.<sup>70</sup>

## Conclusion

We are not yet able to entirely understand and modulate the functions of the immune system in severe clinical situations that may lead to SIRS or sepsis. In the search for diagnostic and treatment approaches, it has become very important to study the pathogenesis of distinct protein and nucleic acid proinflammatory mediators. This mini-review attempts to summarize some major issues of hyperinflammation, abnormal coagulation, and cellular activation that can be detected by different laboratory techniques to estimate the severity and outcome of sepsis.

### Author Contributions

J.K. supervised a part of the study and wrote the manuscript. I.B.D. set up laboratory assays regarding thrombin generation and performed experiments. Z.F. performed microRNA measurements and other laboratory tests. B.N. Jr. provided ideas for experiments, supervised a part of the sepsis studies, and critically reviewed this manuscript.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

## References

- 1 Kaukonen K-M, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med* 2015;372(17):1629–1638
- 2 Arora J, Mendelson AA, Fox-Robichaud A. Sepsis: network pathophysiology and implications for early diagnosis. *Am J Physiol Regul Integr Comp Physiol* 2023;324(05):R613–R624
- 3 Liu XL, Wang XZ, Liu XX, et al. Low-dose heparin as treatment for early disseminated intravascular coagulation during sepsis: a prospective clinical study. *Exp Ther Med* 2014;7(03):604–608
- 4 Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124(04):783–801
- 5 Osuchowski MF, Welch K, Siddiqui J, Remick DG. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. *J Immunol* 2006;177(03):1967–1974
- 6 Hotchkiss RS, Swanson PE, Freeman BD, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit Care Med* 1999;27(07):1230–1251
- 7 Sharma A, Yang WL, Matsuo S, Wang P. Differential alterations of tissue T-cell subsets after sepsis. *Immunol Lett* 2015;168(01):41–50
- 8 Martin MD, Badovinac VP, Griffith TS. CD4 T cell responses and the sepsis-induced immunoparalysis state. *Front Immunol* 2020;11:1364
- 9 Otto GP, Sossdorf M, Claus RA, et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. *Crit Care* 2011;15(04):R183–R183
- 10 Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016;315(08):801–810
- 11 Shen G, Li S, Shao Z, et al. Platelet indices in patients with acute appendicitis: a systematic review with meta-analysis. *Updates Surg* 2021;73(04):1327–1341
- 12 Jiang SQ, Huang QF, Xie WM, Lv C, Quan XQ. The association between severe COVID-19 and low platelet count: evidence from 31 observational studies involving 7613 participants. *Br J Haematol* 2020;190(01):e29–e33
- 13 Leli C, Di Matteo L, Gotta F, et al. Clinical utility of platelet count for screening of malaria. *New Microbiol* 2020;43(02):89–92
- 14 Levi M, de Jonge E, van der Poll T. Sepsis and disseminated intravascular coagulation. *J Thromb Thrombolysis* 2003;16(1-2):43–47
- 15 Shimizu M, Konishi A, Nomura S. Examination of biomarker expressions in sepsis-related DIC patients. *Int J Gen Med* 2018;11:353–361
- 16 McEver RP, Martin MN. A monoclonal antibody to a membrane glycoprotein binds only to activated platelets. *J Biol Chem* 1984;259(15):9799–9804
- 17 Kappelmayer J, Nagy B Jr, Miszti-Blasius K, Hevessy Z, Setiadi H. The emerging value of P-selectin as a disease marker. *Clin Chem Lab Med* 2004;42(05):475–486
- 18 Zonneveld R, Martinelli R, Shapiro NI, Kuijpers TW, Plötz FB, Carman CV. Soluble adhesion molecules as markers for sepsis and the potential pathophysiological discrepancy in neonates, children and adults. *Crit Care* 2014;18(02):204

- 19 McEver RP. Selectins: initiators of leucocyte adhesion and signaling at the vascular wall. *Cardiovasc Res* 2015;107(03):331–339
- 20 McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DFJ. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J Clin Invest* 1989;84(01):92–99
- 21 Schrijver IT, Kemperman H, Roest M, Kesecioglu J, de Lange DW. Soluble P-selectin as a biomarker for infection and survival in patients with a systemic inflammatory response syndrome on the intensive care unit. *Biomark Insights* 2017;12:1177271916684823
- 22 Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci U S A* 1995;92(16):7450–7454
- 23 Shebuski RJ, Kilgore KS. Role of inflammatory mediators in thrombogenesis. *J Pharmacol Exp Ther* 2002;300(03):729–735
- 24 Osterud B, Flaegstad T. Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. *Thromb Haemost* 1983;49(01):5–7
- 25 Rahman M, Roller J, Zhang S, et al. Metalloproteinases regulate CD40L shedding from platelets and pulmonary recruitment of neutrophils in abdominal sepsis. *Inflamm Res* 2012;61(06):571–579
- 26 Stokes KY, Granger DN. Platelets: a critical link between inflammation and microvascular dysfunction. *J Physiol* 2012;590(05):1023–1034
- 27 Zhang C, Shang X, Yuan Y, Li Y. Platelet-related parameters as potential biomarkers for the prognosis of sepsis. *Exp Ther Med* 2023;25(03):133
- 28 Schwertz H, Tolley ND, Foulks JM, et al. Signal-dependent splicing of tissue factor pre-mRNA modulates the thrombogenicity of human platelets. *J Exp Med* 2006;203(11):2433–2440
- 29 Panes O, Matus V, Sáez CG, Quiroga T, Pereira J, Mezzano D. Human platelets synthesize and express functional tissue factor. *Blood* 2007;109(12):5242–5250
- 30 Rondina MT, Schwertz H, Harris ES, et al. The septic milieu triggers expression of spliced tissue factor mRNA in human platelets. *J Thromb Haemost* 2011;9(04):748–758
- 31 Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. *J Thromb Haemost* 2005;3(08):1590–1596
- 32 Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* 2001;104(13):1533–1537
- 33 Evangelista V, Smyth SS, Michelson A. Interactions between platelets, leukocytes, and endothelium. In: *Platelets*. 3rd ed. London: Elsevier; 2013:295–312
- 34 Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004;303(5663):1532–1535
- 35 Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: is immunity the second function of chromatin? *J Cell Biol* 2012;198(05):773–783
- 36 Abrams ST, Morton B, Alhamdi Y, et al. A novel assay for neutrophil extracellular trap formation independently predicts disseminated intravascular coagulation and mortality in critically ill patients. *Am J Respir Crit Care Med* 2019;200(07):869–880
- 37 Ko YP, Flick MJ. Fibrinogen is at the interface of host defense and pathogen virulence in *Staphylococcus aureus* infection. *Semin Thromb Hemost* 2016;42(04):408–421
- 38 Omiya K, Sato H, Sato T, et al. Albumin and fibrinogen kinetics in sepsis: a prospective observational study. *Crit Care* 2021;25(01):436
- 39 Mori K, Tsujita Y, Yamane T, Eguchi Y. Decreasing plasma fibrinogen levels in the intensive care unit are associated with high mortality rates in patients with sepsis-induced coagulopathy. *Clin Appl Thromb Hemost* 2022;28:10760296221101386
- 40 Yao C, Zhang G, Zhang N, et al. Fibrinogen is associated with prognosis of critically ill patients with sepsis: a study based on Cox regression and propensity score matching. *Mediators Inflamm* 2023;2023:7312822
- 41 Wang G, Liu J, Xu R, Liu X. Elevated plasma D-dimer levels are associated with the poor prognosis of critically ill children. *Front Pediatr* 2022;10:1001893
- 42 Li T, Hu WQ, Li X, et al. Prognostic value of PaO<sub>2</sub>/FiO<sub>2</sub>, SOFA and D-dimer in elderly patients with sepsis. *Integr Med Res* 2022;50(06):1–10
- 43 Poli-de-Figueiredo LF, Garrido AG, Nakagawa N, Sannomiya P. Experimental models of sepsis and their clinical relevance. *Shock* 2008;30(Suppl 1):53–59
- 44 Roller J, Wang Y, Rahman M, et al. Direct in vivo observations of P-selectin glycoprotein ligand-1-mediated leukocyte-endothelial cell interactions in the pulmonary microvasculature in abdominal sepsis in mice. *Inflamm Res* 2013;62(03):275–282
- 45 Tóth J, Debreceni IB, Berhés M, et al. Red blood cell and platelet parameters are sepsis predictors in an *Escherichia coli* induced lethal porcine model. *Clin Hemorheol Microcirc* 2017;66(03):249–259
- 46 Yamakawa K, Ogura H, Koh T, et al. Platelet mitochondrial membrane potential correlates with severity in patients with systemic inflammatory response syndrome. *J Trauma Acute Care Surg* 2013;74(02):411–417, discussion 418
- 47 Dolmatova EV, Wang K, Mandavilli R, Griendling KK. The effects of sepsis on endothelium and clinical implications. *Cardiovasc Res* 2021;117(01):60–73
- 48 van Horssen J, van Schaik P, Witte M. Inflammation and mitochondrial dysfunction: a vicious circle in neurodegenerative disorders? *Neurosci Lett* 2019;710:132931
- 49 Tóth J, Debreceni IB, Deák Á, et al. Characteristics of thrombin generation in a fulminant porcine sepsis model. *Thromb Res* 2017;158:25–34(b)
- 50 Wang Y, Braun OO, Zhang S, Luo L, Norström E, Thorlacius H. Dynamic changes in thrombin generation in abdominal sepsis in mice. *Shock* 2014;42(04):343–349
- 51 Park J, Kang E, Kang S, et al. Mitochondrial gene mutations in pediatric septic shock. *Pediatr Res* 2021;90(05):1016–1022
- 52 Umemura Y, Nishida T, Yamakawa K, Ogura H, Oda J, Fujimi S. Anticoagulant therapies against sepsis-induced disseminated intravascular coagulation. *Acute Med Surg* 2023;10(01):e884
- 53 Jaimes F, De La Rosa G, Morales C, et al. Unfractionated heparin for treatment of sepsis: a randomized clinical trial (The HETRASE Study). *Crit Care Med* 2009;37(04):1185–1196
- 54 Liu V, Escobar GJ, Greene JD, et al. Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA* 2014;312(01):90–92
- 55 Warren BL, Eid A, Singer P, et al; KyberSept Trial Study Group. Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial. *JAMA* 2001;286(15):1869–1878
- 56 Faust SN, Levin M, Harrison OB, et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *N Engl J Med* 2001;345(06):408–416
- 57 Bernard GR, Vincent JL, Laterre PF, et al; Recombinant human protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;344(10):699–709
- 58 Van de Wouwer M, Collen D, Conway EM. Thrombomodulin-protein C-EPCR system: integrated to regulate coagulation and inflammation. *Arterioscler Thromb Vasc Biol* 2004;24(08):1374–1383
- 59 Vincent JL, Francois B, Zabolotskikh I, et al; SCARLET Trial Group. Effect of a recombinant human soluble thrombomodulin on

- mortality in patients with sepsis-associated coagulopathy: the SCARLET randomized clinical Trial. *JAMA* 2019;321(20):1993–2002
- 60 Davenport EE, Burnham KL, Radhakrishnan J, et al. Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Respir Med* 2016;4(04):259–271
- 61 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116(02):281–297
- 62 Christopher AF, Kaur RP, Kaur G, Kaur A, Gupta V, Bansal P. MicroRNA therapeutics: discovering novel targets and developing specific therapy. *Perspect Clin Res* 2016;7(02):68–74
- 63 Nilsen TW. Mechanisms of microRNA-mediated gene regulation in animal cells. *Trends Genet* 2007;23(05):243–249
- 64 Chen X, Liang H, Zhang J, Zen K, Zhang CY. Secreted microRNAs: a new form of intercellular communication. *Trends Cell Biol* 2012;22(03):125–132
- 65 Águila S, Cuenca-Zamora E, Martínez C, Teruel-Montoya R. MicroRNAs in platelets: should I stay or should I go? In: Kerrigan SW, ed. *Platelets*. London:: IntechOpen; 2020
- 66 Szilágyi B, Fejes Z, Póliska S, et al. Reduced miR-26b expression in megakaryocytes and platelets contributes to elevated level of platelet activation status in sepsis. *Int J Mol Sci* 2020;21(03):866
- 67 Elgheznawy A, Shi L, Hu J, et al. Dicer cleavage by calpain determines platelet microRNA levels and function in diabetes. *Circ Res* 2015;117(02):157–165
- 68 Laffont B, Corduan A, Plé H, et al. Activated platelets can deliver mRNA regulatory Ago2•microRNA complexes to endothelial cells via microparticles. *Blood* 2013;122(02):253–261
- 69 Laffont B, Corduan A, Rousseau M, et al. Platelet microparticles reprogram macrophage gene expression and function. *Thromb Haemost* 2016;115(02):311–323
- 70 Maiese A, Scatena A, Costantino A, et al. Expression of MicroRNAs in sepsis-related organ dysfunction: a systematic review. *Int J Mol Sci* 2022;23(16):9354