

# Sepsis-Associated Coagulopathy

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## ABSTRACT

Systemic inflammatory activation in sepsis often leads to coagulation activation, but the relationship is bilateral, as coagulation also modulates the inflammatory response. This close association has significant consequences for the pathogenesis of microvascular thrombosis and organ dysfunction in sepsis. While coagulation activation can be beneficial for immune defense, it can also be detrimental once it becomes widespread and uncontrolled. The knowledge of the pathophysiologic mechanisms involved in the interaction between infection and coagulation may lead to the better timing for the administration of targeted antithrombotic therapies in septic patients. This brief review highlights the pathophysiologic pathways leading to the prothrombotic state in sepsis and the mechanisms that play a role in the interaction between infection and coagulation.

**Keywords:** sepsis, coagulopathy, thrombosis

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## INTRODUCTION

Sepsis is a major public health concern, with an increasing incidence reported in the US and increasing mortality worldwide [1,2]. Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection [3,4]. Haematologic disturbances are well described in critical care patients [5]. In the vast majority of cases, sepsis is associated with disturbances of haemostasis, ranging from mild laboratory changes to widespread microvascular thrombosis, contributing to organ dysfunction [6]. Eventually, bleeding occurs due to disseminated intravascular coagulation (DIC), induced platelet destruction, and increased release of coagulation factors [6].

Increasing evidence points to an extensive interrelationship between inflammation and coagulation. Inflammation leads to coagulation activation, but the relationship between inflammation and coagulation is bidirectional, and activation of the coagulation cascade modulates inflammatory activity [7]. When control mechanisms are overwhelmed, the inflammation expression and coagulation become systemic, leading to endothelial dysfunction, microvascular thrombosis, ischemia, organ dysfunction, and death [7,8]. In this review article, the primary pathophysiologic mechanisms of sepsis-associated coagulopathy and the rela-

tionship between coagulation and inflammation are summarized.

## INFLAMMATION INDUCED ACTIVATION OF COAGULATION

The key event in the pathophysiology of sepsis-associated coagulopathy is the systemic inflammatory response to the infection [6]. The main mechanisms of coagulation derangement during sepsis are tissue factor mediated thrombin generation and a dysfunction of the normal physiologic anticoagulant and fibrinolytic mechanisms such that enhanced fibrin formation is followed by impaired fibrin removal [7]. The main components involved in inflammatory-induced coagulation activation are tissue factor, micro-particles, and platelets [9]. In the light of recent research, neutrophil extracellular traps (NETs) seem to have important roles in inflammation-induced coagulation [10].

### Tissue factor (TF)

In health, the cells constitutively expressing TF are not found in direct contact with blood. TF comes in contact with blood normally when the vessel wall is damaged, or pathologically when endothelial cells or circulating blood cells start expressing TF under the effect of pro-inflammatory cytokines [8,11]. After contact with

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blood, TF forms a complex with FVIIa. The TF-FVIIa complex activates FX and FIX, leading to thrombin generation with subsequent fibrin formation and platelet activation [12].

The central role of TF in the initiation of inflammation-induced coagulation has been demonstrated in several experimental models; blocking of TF-FVIIa activity in animals completely inhibited coagulation activation and, in lethal models, prevented DIC and reduced mortality [11,13,14].

During endotoxemia and sepsis, TF expression responsible for coagulation activation occurs both in haematopoietic and non-haematopoietic cells [11]. Monocytes are the main cells expressing TF under the effect of proinflammatory cytokines, mostly under the effect of IL6. TF-dependent thrombin generation was abrogated after the inhibition of IL6, underlining the important role of this cytokine in sepsis-associated coagulopathy [15].

Many research studies demonstrated that neutrophils or eosinophils cannot express TF but can acquire it from monocyte-derived microparticles [11,16,17]. TF expression by human platelets is still a matter of debate, but recent work showed increased expression of TF mRNA on platelets from septic patients compared to healthy controls [18]. Non-haematopoietic cells expressing TF also contribute to the coagulation activation, as increased vasculature permeability will lead to higher exposure of TF from extravascular sources to blood [11,19]. TF expression by endothelial cells has been demonstrated mostly by *in vitro* studies [11,20].

Alternatively, spliced TF is a soluble variant of TF with inflammatory activity and relatively low coagulant function [21]. Pro-inflammatory cytokines induce the expression and release of alternatively spliced TF from endothelial cells, making it a useful marker of inflammation-induced coagulation activation [22].

### **Microparticles (MPs)**

MPs are cell membrane-derived particles with diameters between 0.1 and 2  $\mu\text{m}$  released from activated or apoptotic cells. MPs are often released during inflammatory states from different cell types: endothelial cells, platelets, erythrocytes, monocytes. The actions of MPs depend on their cellular origin, but they seem to play important roles in coagulation activation and in intercellular communication [23,24]. MPs are present at low levels in healthy individuals and have a large

spectrum of effects, as their activity reflects their composition and cellular origin [24].

Animal studies have shown that most of the circulating MPs in sepsis are derived from platelets (85%), with a minority originating from endothelial cells and monocytes [25]. Higher procoagulant TF activity on MPs in patients with community-acquired febrile *E. coli* urinary tract infections and bacteraemia compared to healthy individuals was demonstrated in human studies [26]. The membranes of MPs contain two pro-coagulants, phosphatidylserine (a pro-coagulant phospholipid) and tissue factor. Phosphatidylserine enhances the pro-thrombinase activity (FVaXa), offering a surface for amplification and propagation of thrombin generation [24]. In sepsis, MPs exert both pro-thrombotic and pro-inflammatory actions, contributing to the pro-thrombotic state and to generalized microvascular injury. Plasma levels of MPs may have prognostic value in different patient populations, as MPs can be regarded as markers of thrombosis, inflammation and endothelial dysfunction [24,27].

The relative contributions of the intrinsic and extrinsic pathways to MP-initiated thrombin generation were assessed using FVII-deficient plasma or antibodies to block TF effects, revealing the important role for FXI and the intrinsic pathway in the MP-induced pro-coagulant activity [28].

### **Platelets**

An important role in the inflammation-induced activation of coagulation is played by platelets, activated by pro-inflammatory mediators, such as platelet activating factor, or by already formed thrombin. Activated platelets express P-selectin on the membrane, which mediates the adherence of platelets to leukocytes and endothelial cells and enhances the expression of tissue factor on monocytes [29].

The amplification phase of clot formation usually takes place on phospholipid surfaces presented by activated platelets. Microparticles bearing tissue factor and the P-selectin glycoprotein ligand-1 (PSGL-1, a protein expressed by leukocytes) readily bind to activated platelets through an interaction between PSGL-1 within the particle and its natural counter-receptor, P-selectin, expressed by platelets. As a result, activated platelets offering a phospholipid surface and tissue factor abundant microparticles form a complex, leading to a thrombin burst which favours a pro-thrombotic state [9].

Sepsis induces complex alterations in platelet function. Decreased aggregation was noted even in septic patients with a normal platelet count. This reduced aggregation was independent of the amount of thrombin generated, was related to the sepsis severity and was associated with preserved platelet functions such as adhesion or secretion [30]. Animal studies showed that the coagulation cascade is not hindered by decreased platelet counts. Animals with severe thrombocytopenia displayed enhanced coagulation activation, endothelial cell activation and vascular inflammation [31]. Severe thrombocytopenia in sepsis is associated with increased mortality [31,32]. The main role in coagulation and endothelial cell activation is played by the systemic inflammatory response, not by the platelets, but platelets are important in modulating this inflammatory response [32].

### Network microbial trapping

Neutrophils are effective against pathogens by phagocytosis, degranulation or the release of intracellular components such as DNA, histones, and antimicrobial proteins, forming neutrophil extracellular traps (NETs) [33]. Activated neutrophils release NETs, important as an innate defence mechanism as they catch and prevent spreading of microorganisms [34]. On the other hand, the excessive expression of NETs seems to have a significant contribution to endothelial dysfunction and organ injuries in sepsis [33]. NETs are present in septic patients and, in laboratory animals with induced experimental sepsis, are correlated with organ dysfunction as assessed by the SOFA score [34]. During infections, platelets stimulate neutrophils to release NETs, but during sepsis, platelets activate the neutrophils sequestered in the microvasculature, releasing NETs, which trap bacteria, but can also lead to endothelial and tissue damage [10,35]. It has been demonstrated that neutrophils from septic patients release TF-bearing NETs at the sites of inflammation resulting in the localized activation of the coagulation cascade and thrombin generation, contributing to organ dysfunction [10,36].

## ■ DYSFUNCTION OF ANTICOAGULANT MECHANISMS

The main anticoagulant pathways that regulate the coagulation process are antithrombin (AT), the protein C system (APC) and tissue factor pathway inhibitor

(TFPI) [37]. Dysfunction of anticoagulant mechanisms is important in the inflammation-induced pro-thrombotic state [37,38].

### Antithrombin (AT)

AT mainly inhibits FXa and thrombin and to a lesser degree TF-FVIIa and FIXa. In the presence of heparin, AT activity is increased 1000 fold, but without heparin, AT activity is slow. Like heparin, endogenous glycosaminoglycans on the vessel wall promote AT-mediated inhibition of coagulation enzymes as well [38]. Septic patients usually have low levels of circulating antithrombin from the beginning of the septic process [39]. Several factors contribute to this phenomenon, such as decreased AT synthesis, degradation by elastase from activated neutrophils and consumption by the formation of the thrombin-antithrombin complexes as a consequence of increased thrombin generation [38]. Proinflammatory cytokines cause reduced synthesis and expression of glycosaminoglycans on the endothelium, also contributing to decreased AT function [38]. Experimental studies suggest that antithrombin may have anti-inflammatory properties independent of the anticoagulant activity [40].

### Protein C system

Circulating Protein C is activated by endothelial thrombomodulin bound to thrombin. The *endothelial protein C receptor* (EPCR), and cofactor protein S amplify its activation several folds [38,41]. Once activated, protein C proteolytically cleaves Factor Va and VIIIa, promotes fibrinolysis by blocking plasminogen activator inhibitor 1 (PAI-1) and thrombin activatable fibrinolysis inhibitor (TAFI) [41]. In patients with severe inflammation, protein C levels are low due to decreased synthesis, increased degradation by neutrophil elastases and consumption. Furthermore, a significant downregulation of TM and EPCR on endothelial cell surface due to inflammatory cytokines diminishes protein C activation [38].

Typically, in plasma protein S is complexed with a complement protein, C4b binding protein, which is increased in septic patients resulting in a relative protein S deficiency. This low free protein S level found in sepsis amplifies the malfunction of the protein C system. [8,32,40]. Besides the anticoagulant and profibrinolytic activity, Activated Protein C (APC) has anti-inflammatory actions, demonstrated in a significant number of studies [7,42].

### Tissue Factor Pathway Inhibitor (TFPI)

TFPI typically controls thrombin generation via the TF pathway. TFPI is predominantly found bound to the endothelium via proteoglycans (PGs) which play a major role in the anticoagulant function of TFPI [22]. Coagulation inhibition by TFPI occurs by forming a stable complex with factor Xa, followed by FXa-dependent binding of TF-VIIa [22]. In sepsis, TFPI is both consumed and degraded, quickly leading to TFPI deficiency due to its relatively small concentration in plasma [22]. Pro-inflammatory cytokines reduce the synthesis of PGs on the endothelial surface resulting in a decrease in TFPI function during severe inflammation [22]. In conclusion, both plasma levels and function of TFPI are low in sepsis, leading to a pro-coagulant tendency demonstrated in experimental studies [43].

### ■ DYSFUNCTION OF FIBRINOLYTIC MECHANISMS

Fibrinolysis is essential in order to maintain vasculature patency. The key enzyme for clot degradation is plasmin, generated from plasminogen by different proteases, most notably, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Plasmin formation is increased in the presence of fibrin, as plasminogen and tPA binding to lysine residues formed in fibrin enhances the process of plasmin generation [44]. The maintenance of a precise balance between coagulation and fibrinolysis is vital for normal haemostasis. The main inhibitors of the fibrinolytic process are  $\alpha$ -2-antiplasmin (which binds free plasmin), PAI-1 and PAI-2 (produced in the liver and responsible for binding free tPA and uPA and limiting fibrinolysis) and thrombin activatable fibrinolysis inhibitor (TAFI), which cleaves exposed lysine residues from fibrin and other substrates [44].

Experimental sepsis models have shown an initial transient increase in the pro-fibrinolytic response due to the release of plasminogen activators, followed by a fibrinolytic shutdown generated by increased levels of PAI-1 in plasma synthesized by endothelial cells and monocytes as a response to cytokine stimulation [41,45,46].

In vitro studies have demonstrated that TAFI is activated in sepsis mainly by thrombin, but also by plasmin, trypsin, and elastases. Normally, TAFI is activated by proteases at a very low rate, but the speed of acti-

vation increases in the presence of thrombomodulin, heparin or glycosaminoglycans [47]. Animal studies on endotoxin-induced sepsis showed that TAFI was activated mainly by thrombin-thrombomodulin complex [47]. Due to reduced thrombomodulin expression on endothelial cells in sepsis, TAFI activation is reduced, in contrast to the increased release of PAI-1 from activated endothelial cells [48]. It was suggested that in early sepsis PAI-1 is responsible for fibrinolysis inhibition and TAFI probably contributes to the procoagulant stage in later stages [48].

The fibrinolytic shutdown caused by increased PAI-1 levels, TAFI activity and insufficient activation of fibrinolysis by plasmin and/or neutrophil elastases is associated with organ dysfunction and poor outcomes in septic patients [49,50]. Human studies revealed increased specific activity of factor XIII in septic patients compared to healthy controls, contributing to the increased resistance of fibrin clots to fibrinolysis [51]. In this way, both decreased fibrinolysis and increased clot resistance work together to promote microvascular thrombosis and organ dysfunction in septic patients.

### The reciprocal relationship between coagulation and inflammation

Inflammation leads to activation of coagulation; in response, coagulation, once activated, modulates inflammatory pathways. This was underlined in a study demonstrating that the activation of coagulation by the administration of recombinant factor VII triggers IL6 and 8 responses in healthy humans [52]. The protease-activated receptors (PARs) on immune cells activated by tissue factor and other coagulation factors modulate pro- and anti-inflammatory pathways, playing a pivotal role in the cross-talk between coagulation and inflammation [53]. Not only the coagulation factors but also other components of the coagulation process, like platelets or natural anticoagulants, have important effects on inflammation.

Besides their role in haemostasis, platelets play an important role in the modulation of the inflammatory/immune response. Platelets are able to bind infectious agents, to sense pathogen-associated molecular patterns, to express and secrete pro- and anti-inflammatory molecules, to initiate and modulate immune functions [54]. Histones, a major component of neutrophil extracellular traps, are able to stimulate platelet activation, leading to pro-coagulant and pro-inflammatory responses [10,55].

Independent of its anticoagulant function, AT possesses anti-inflammatory properties. AT increases the production of prostacyclin from endothelial cells and decreases the adhesion of leukocytes to endothelial cells by binding to receptors on neutrophils, monocytes, and lymphocytes. The overall effect is a decrease in endothelial cell production of chemokines, cytokines and in the severity of capillary leakage [37,38,40].

APC is another natural anticoagulant with anti-inflammatory properties. During sepsis, APC is able to reduce circulating damage-associated molecular patterns (DAMPs) by decreasing apoptosis of endothelial cells and lymphocytes. APC also inhibits the inflammatory response of monocytes, leukocytes, and neutrophils inhibit leukocyte chemotaxis and decreases neutrophils adherence to endothelial cells [7,8,56]. APC helps to maintain the integrity of the endothelium, prevents capillary leak and decreases in blood pressure during severe infections [56].

Considering this close relationship between coagulation and inflammation, it is not surprising that inhibiting the activity of the TF/VIIa pathway reduced the release of interleukin 6 (IL-6) and IL-8 during severe bacteraemia [13]. However, human studies showed that infusion of TFPI completely blocked endotoxin-induced coagulation activation, without affecting endotoxin-induced inflammatory pathways or the fibrinolytic pathways [57,58].

Because it leads to decreased blood supply to organs, thrombosis was considered harmful, but it was recently shown that thrombosis plays a significant role in the early immune defence system [59]. Activation of coagulation and fibrin clot formation is a host-defence strategy useful to stop bacterial spreading during sepsis explained by four mechanisms:

1. Immunothrombosis limits microbial dissemination by containing microbes within thrombi.
2. Thrombi form protective barricades inside and/or around blood vessels that limit microbial movement in and out of the vessels.
3. Fibrin, fibrinogen and their degradation products promote recruitment and activation of leukocytes locally.
4. Intravascular thrombi yield a distinct compartment where antimicrobial peptides are concentrated and have increased actions against pathogens [53,59].

## ■ THERAPEUTIC IMPLICATIONS

Apart from classical biomarkers, coagulation or fibrinolysis-related biomarkers are studied as prognostic markers of sepsis [60,61]. Worsening coagulopathy is associated with increased severity of organ failure and mortality [62]. In order to avoid organ failure, and following promising results of Phase II studies, anticoagulant therapies were tested in septic patients [9]. Large randomized trials investigating the efficacy of recombinant activated protein C (rAPC), plasma-derived antithrombin concentrates and recombinant tissue factor pathway inhibitor (rTFPI) failed to show a reduction in mortality [63-65]. However, post-hoc analysis of databases revealed beneficial effects of anticoagulants in subgroups of patients with sepsis-induced disseminated intravascular coagulation [66,67]. Thus, in order to improve outcomes using anticoagulant therapy, proper patient selection by rapid diagnosis of early-stage sepsis-induced DIC is mandatory. Timing of treatment is also important because coagulation activation has beneficial effects as long as it doesn't become uncontrolled.

## ■ CONCLUSIONS

Whenever the systemic inflammatory response is activated there is a concomitant associated degree of coagulation activation. TF-triggered coagulation activation and impairment of anticoagulant and fibrinolytic pathways are the main mechanisms of sepsis-associated coagulopathy. Like inflammation, coagulation activation is also important in the immune defence against pathogens. In the same manner as the inflammatory response, the intensity and regulation of coagulation activation in sepsis determines patients' outcome.

The precise identification of the moment when coagulation activation becomes detrimental in septic patients would allow a more focused approach of antithrombotic therapy in sepsis, with better chances for outcome improvement.

## ■ CONFLICT OF INTEREST

Nothing to declare

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