Coagulation in Patients with Severe Sepsis

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Abstract

In the majority of patients with severe sepsis, systemic activation of coagulation is present. Increasing evidence points to an extensive cross-talk between coagulation and inflammation that may play an important role in the pathogenesis of sepsis. Inflammation not only leads to activation of coagulation, but coagulation also considerably affects inflammatory activity. Molecular pathways that contribute to inflammation-induced activation of coagulation have been precisely identified. Proinflammatory cytokines and other mediators are capable of activating the coagulation system and downregulating important physiological anticoagulant pathways. Activation of the coagulation system and ensuing thrombin generation is dependent on expression of tissue factor on activated mononuclear cells and endothelial cells, and is insufficiently counteracted by TFPI. Simultaneously, endothelial-bound anticoagulant mechanism, in particular the protein C system, is shutoff by proinflammatory cytokines. In addition, fibrin removal is severely inhibited, because of inactivation of the fibrinolytic system, caused by an upregulation of its main inhibitor, plasminogen activator inhibitor type 1 (PAI-1). Increased fibrin formation and impaired removal lead to (micro)vascular thrombosis, which may result in tissue ischemia and subsequent organ damage. The cornerstone of the management of coagulation in sepsis is the specific and vigorous treatment of the underlying disorder. Strategies aimed at the inhibition of coagulation activation may theoretically be justified and have been found beneficial in experimental and initial clinical studies. Heparin may be an effective anticoagulant approach and alternative strategies comprise restoration of physiological anticoagulant pathways.

Keywords
- coagulation
- inflammation
- sepsis
- tissue factor
- antithrombin
- protein C
- fibrinolysis

Sepsis is a clinical syndrome that is caused by an infection, often associated with bacteremia and characterized by the presence of systemic signs and symptoms of inflammation.[1] When sepsis leads to organ failure, the term *severe sepsis* is used. The incidence of sepsis is estimated to be approximately 2.5 per 1,000 in the Western world and shows a rapid 8.7% annual increase over the past 20 years.[2] Total in-hospital mortality of sepsis is around 20%, whereas severe sepsis is associated with mortality rates of 40 to 50%.[3] Treatment of sepsis is focused on adequate antibiotic therapy, source control, and appropriate supportive care and organ function replacement, if required.

Virtually all patients with sepsis have coagulation abnormalities. These abnormalities range from subtle activation of coagulation that can only be detected by sensitive markers for coagulation factor activation to somewhat stronger coagulation activation that may be detectable by a small decrease in platelet count and subclinical prolongation of global clotting times to fulminant disseminated intravascular coagulation (DIC), characterized by simultaneous widespread microvascular thrombosis and profuse bleeding from various sites.[4,5] Septic patients with severe forms of DIC may present with manifest thromboembolic disease or clinically less apparent microvascular fibrin deposition that predominantly presents

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as multiple organ dysfunction.[5,6] Alternatively, severe bleeding may be the leading symptom,[7] but quite often a patient with DIC has simultaneous thrombosis and bleeding. Bleeding is caused by consumption and subsequent exhaustion of coagulation proteins and platelets, because of the ongoing activation of the coagulation system.[8] In its most severe form this combination may present as the Waterhouse-Friderichsen syndrome, commonly seen during fulminant meningococcal septicemia, although many other microorganisms may cause this clinical state.[9]

Incidence of Coagulation Abnormalities in Sepsis

Clinically relevant coagulation abnormalities may occur in 50 to 70% of patients with sepsis, whereas approximately 35% of patients will meet the criteria for DIC (see further).[1,10] In general, the incidence of thrombocytopenia (platelet count $<150 \times 10^9$/L) in critically ill medical patients is 35 to 50%.[11,12] Typically, the platelet count decreases during the first 4 days on the intensive care unit.[13] Sepsis is a clear risk factor for thrombocytopenia in critically ill patients and the severity of sepsis correlates with the decrease in platelet count.[14] Main factors that contribute to thrombocytopenia in patients with sepsis are impaired platelet production, increased consumption or destruction, or sequestration in the spleen or at the endothelial level. Impaired production of platelets in the bone marrow may seem contradictory to the high levels of platelet production-stimulating proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, and high concentration of circulating thrombopoietin in patients with sepsis, which theoretically should stimulate megakaryopoiesis in the bone marrow.[15] However, in a substantial number of patients with sepsis marked hemophagocytosis may occur, consisting of active phagocytosis of megakaryocytes and other hematopoietic cells by monocytes and macrophages, hypothetically because of stimulation with high levels of macrophage colony stimulating factor (M-CSF) in sepsis.[16] Platelet consumption probably also plays an important role in patients with sepsis, because of ongoing generation of thrombin. Platelet activation, consumption, and destruction may also occur at the endothelial site as a result of the extensive endothelial cell-platelet interaction in sepsis, which may vary between different vascular beds in various organs.[17] A prolonged global coagulation time (such as the prothrombin time [PT] or the activated partial thromboplastin time [aPTT]) occurs in 14 to 28% of patients.[18] Other coagulation test abnormalities include high fibrin split products (in 99% of patients with sepsis)[19,20] and low levels of coagulation inhibitors, such as antithrombin and protein C (90% of sepsis patients).[20,21]

Pathogenetic Pathways in the Coagulopathy of Sepsis

In recent years the mechanisms involved in the pathological derangement of coagulation in patients with sepsis have become increasingly clear. Apparently, various mechanisms at different sites in the hemostatic balance act simultaneously toward a procoagulant state. It has become clear that the most important mediators that orchestrate this imbalance of the coagulation system during sepsis are cytokines.[22] Increasing evidence points to extensive cross-talk between these two systems, whereby inflammation leads not only to activation of coagulation, but coagulation also considerably affects inflammatory activity.[23] Interestingly, systemic activation of coagulation and inflammation in sepsis can have some organ-specific manifestations that are relevant for the specific organ dysfunction as a consequence of severe sepsis.[24]

The principal initiator of thrombin generation in sepsis is tissue factor. The evidence that points to a pivotal role of the tissue factor/factor VIIa system in the initiation of thrombin generation comes from studies of human endotoxemia or cytokinemia, which did not show any change in markers for activation of the contact system.[25] Furthermore, abrogation of the tissue factor/factor VII(a) pathway by monoclonal antibodies specifically directed against tissue factor or factor VIIa activity resulted in a complete inhibition of thrombin generation in endotoxin-challenged chimpanzees and prevented the occurrence of DIC and mortality in baboons, that were infused with *Escherichia coli*.[26,27] However, other than in severe meningococcemia,[28] it has proved difficult to demonstrate ex vivo tissue factor expression on monocytes of septic patients or experimental animals systemically exposed to microorganisms. It has been shown, however, that low-dose endotoxemia in healthy subjects results in an 125-fold increase in tissue factor mRNA levels in blood monocytes.[29] Another source of tissue factor may be its localization on polymorphonuclear cells,[30] although it is unlikely that these cells actually synthesize tissue factor in substantial quantities.[31] Based on the observation of transfer of tissue factor from leukocytes to activated platelets on a collagen surface in an ex vivo perfusion system, it is hypothesized that this "blood borne" tissue factor is transferred between cells through microparticles derived from activated mononuclear cells.[32]

Platelets play a pivotal role in the pathogenesis of coagulation abnormalities in sepsis. Platelets can be activated directly, for example by proinflammatory mediators, such as platelet activating factor.[33] Once thrombin is formed, this will activate additional platelets. Activation of platelets may also accelerate fibrin formation by another mechanism. The expression of P-selectin on the platelet membrane not only mediates the adherence of platelets to leukocytes and endothelial cells but also enhances the expression of tissue factor on monocytes.[34] The molecular mechanism of this effect relies on nuclear factor kappa-B (NFκB) activation, induced by binding of activated platelets to neutrophils and mononuclear cells. P-selectin can be relatively easily shed from the surface of the platelet membrane and soluble P-selectin levels have been shown to be increased during systemic inflammation.[34]

In general, activation of coagulation is regulated by three major anticoagulant pathways: antithrombin, the protein C system, and tissue factor pathway inhibitor (TFPI). During sepsis-induced activation of coagulation, the function of all three pathways can be impaired. Experimental models

indicate that at the time of maximal activation of coagulation in sepsis, the fibrinolytic system is largely shutoff. The acute fibrinolytic response to inflammation is the release of plasminogen activators, in particular tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), from storage sites in vascular endothelial cells. However, this increase in plasminogen activation and subsequent plasmin generation is counteracted by a delayed but sustained increase in plasminogen activator inhibitor type 1 (PAI-1).[35] Of interest, studies have shown that a functional mutation in the *PAI-1* gene, the 4G/5G polymorphism, not only influenced the plasma levels of PAI-1, but it was also linked to clinical outcome of meningococcal septicemia. Patients with the 4G/4G genotype had significantly higher PAI-1 concentrations in plasma and an increased risk of death.[36] Further investigations demonstrated that the PAI-1 polymorphism did not influence the risk of contracting meningitis as such, but probably increased the likelihood of developing septic shock from meningococcal infection.[37]

Inflammation and the Coagulopathy of Sepsis

Similar to almost all systemic inflammatory responses to infection, the derangement of coagulation and fibrinolysis in sepsis is mediated by several cytokines. Most proinflammatory cytokines have been shown to activate coagulation in vitro. In patients with sepsis, high levels of cytokines are detectable in the circulation and experimental bacteremia or endotoxemia results in the transient enhancement of serum levels of these cytokines.[25] Consecutively, tumor necrosis factor becomes first detectable, followed by an increase in circulating levels of interleukin-6 (IL-6) and interleukin 1 (IL-1). Several experimental and clinical studies have focused on the roles of these cytokines in the pathogenesis of DIC.

Because TNF is the first cytokine to appear in the circulation after infusion of bacteria or endotoxin and exerts potent procoagulant effects in vitro, it was initially thought that activation of coagulation was mediated by TNF. However, in studies using various strategies to block TNF activity, it became clear that the endotoxin-induced increase in TNF could be completely abolished whereas activation of coagulation was unchanged, although the effects on anticoagulant pathways and fibrinolysis seemed to be driven by TNF.[25] Also, in baboons infused with a lethal dose of *E. coli*, treatment with an anti-TNF antibody had little or no effect on fibrinogen consumption.[38] Moreover, clinical studies in septic patients with an anti-TNF monoclonal antibody did not show a beneficial effect of this treatment.[39] In subsequent studies the role of IL-6 was investigated. It could be shown that infusion of a monoclonal anti–IL-6 antibody resulted in the complete abrogation of endotoxin-induced activation of coagulation in chimpanzees.[40] In addition, studies in cancer patients receiving recombinant IL-6 indicated that indeed thrombin is generated following the injection of this cytokine.[41] Thus, these data suggest that IL-6 rather than TNF is relevant as a mediator for the induction of the procoagulant response in DIC. Though IL-1 is a potent agonist of tissue factor expression in vitro, its role has not been clarified in vivo. Administration of a IL-1 receptor antagonist partly blocked the procoagulant response in a sepsis model in baboons and treatment of patients with an IL-1 receptor inhibitor-reduced thrombin generation.[42] However, most procoagulant changes after an endotoxin challenge occur well before IL-1 becomes detectable in the circulation, leaving a potential direct role of IL-1 in coagulation activation in sepsis an unresolved issue.

Coagulation proteases and protease inhibitors not only interact with coagulation protein zymogens, but also with specific cell receptors to induce signaling pathways. In particular, protease interactions that affect inflammatory processes may be important in sepsis. The most important mechanisms by which coagulation proteases influence inflammation is by binding to so-called protease-activated receptors (PARs), of which four types (PAR 1–4) have been identified—all belonging to the family of transmembrane domain, G-protein coupled receptors.[43] A peculiar feature of PARs (in contrast to most other receptors of the superfamily) is that they serve as their own ligand. Proteolytic cleavage by an activated coagulation factor leads to exposure of a neo-amino terminus that activates the same receptor (and possibly adjacent receptors), initiating transmembrane signaling. PARs 1, 3, and 4 are thrombin receptors whereas PAR 2 cannot bind thrombin but can be activated by the tissue factor-factor VIIa complex, factor Xa, and trypsin. PAR 1 can also serve as receptor of the tissue factor-factor VIIa complex and factor Xa.

There is also considerable cross-talk between physiological anticoagulant pathways and inflammatory mediators. Antithrombin can act as a mediator of inflammation, for example, by direct binding to neutrophils and other leucocytes and thereby attenuating cytokine and chemokine receptor expression.[44] In addition, there is mounting evidence that the protein C system also has an important function in modulating inflammation.[45] Indeed, activated protein C has been found to inhibit endotoxin-induced production of TNF-α, IL-1β, IL-6, and IL-8 by cultured monocytes/macrophages.[46] Further, activated protein C abrogates endotoxin-induced cytokine release and leukocyte activation in rats in vivo.[47] Blocking the protein C pathway by a monoclonal antibody in septic baboons exacerbates the inflammatory response, as evidenced by increased levels of proinflammatory cytokines and more leukocyte infiltration and tissue destruction at histological analysis.[48] Mice with a one-allele targeted disruption of the protein C gene (resulting in heterozygous protein C deficiency) not only have a more severe coagulation response to endotoxin but also demonstrate significant differences in inflammatory responses, as shown by higher levels of circulating proinflammatory cytokines.[49]

Diagnostic Approach to the Coagulopathy in Sepsis

It is important to realize that apart from DIC, there are several other reasons for coagulation abnormalities in patients with sepsis (►**Table 1**). Although thrombocytopenia is common in patients with severe sepsis, this may also be caused by

Table 1 Causes of coagulation abnormalities in critically ill patients

Thrombocytopenia
Sepsis
DIC
Massive blood loss
Thrombotic microangiopathy
Heparin-induced thrombocytopenia
Immune thrombocytopenia
Drug-induced thrombocytopenia
Abnormal global coagulation times[a]
Coagulation factor deficiency
• Synthesis: liver failure
• Loss: massive bleeding
• Consumption: DIC
Vitamin K deficiency
Use of vitamin K antagonists
Use of unfractionated heparin
inhibiting antibody and/or antiphospholipid antibody

Abbreviation: DIC, disseminated intravascular coagulation.
[a]Prothrombin time and/or activated partial thromboplastin time.

other (sometimes simultaneously occurring) diseases, such as immune thrombocytopenia, medication-induced bone marrow depression, heparin-induced thrombocytopenia, or thrombotic microangiopathies.[50] It is very important to properly diagnose these causes of thrombocytopenia, as they may require distinctive treatment strategies.[17] Laboratory tests can be helpful in differentiating the coagulopathy in sepsis from various other hemostatic disorders, such as vitamin K deficiency or liver failure. Because such conditions, however, may also occur simultaneously with for example DIC, this differentiation is not always simple.[51,52]

According to the current understanding of sepsis-associated coagulation abnormalities, the determination of soluble fibrin in plasma appears to be crucial.[53,54] In general, the sensitivity of these assays for severe coagulation activation or DIC is relatively higher than the specificity. Indeed, initial clinical studies indicate that if the concentration of soluble fibrin has increased above a defined threshold, a diagnosis of DIC can be made.[19,55] Most of the clinical studies show a sensitivity of 90 to 100% for the diagnosis of DIC but a rather low specificity.[56] Fibrin degradation products (FDPs) may be detected by specific ELISA tests or by latex agglutination assays, allowing rapid and bedside determination in emergency cases.[57] None of the available assays for FDPs discriminates between degradation products of cross-linked fibrin and fibrinogen degradation, which may cause spuriously high results.[58] The specificity of high levels of FDPs is therefore limited, and many other conditions, such as trauma, recent surgery, inflammation, or venous thromboembolism, are associated with elevated FDPs. Recently developed tests are specifically aimed at the detection of neoantigens on degraded cross-linked fibrin. One of such tests detects an epitope related to plasmin-degraded cross-linked γ-chain, resulting in fragment D-dimer. These tests better differentiate degradation of cross-linked fibrin from fibrinogen or fibrinogen degradation products.[59]

Consumption of coagulation factors leads to low levels of coagulation factors in patients with sepsis. In addition, impaired synthesis, for example, due to impaired liver function or a vitamin K deficiency, and loss of coagulation proteins due to massive bleeding, may play a role as well.[60] Although the accuracy of the measurement of one-stage clotting assays in DIC has been contested (due to the presence of activated coagulation factors in plasma), the level of coagulation factors appears to correlate well with the severity of DIC.[60] Measurement of fibrinogen has been widely advocated as a useful tool for the diagnosis of DIC but in fact is not very helpful to diagnose DIC in most cases.[8,61] Fibrinogen acts as an acute-phase reactant, and despite ongoing consumption, plasma levels can remain well within the normal range for a long time. In a consecutive series of patients the sensitivity of a low fibrinogen level for the diagnosis of DIC was only 28% and hypofibrinogenemia was detected in very severe cases of DIC only. Sequential measurements of fibrinogen might be more useful and provide diagnostic clues.

Thromboelastography (TEG) is a method that has been developed decades ago and provides an overall picture of ex vivo coagulation. Modern techniques, such as rotational thromboelastography (ROTEG), enable bedside performance of this test and have again become popular recently in acute care settings.[62] The theoretical advantage of TEG over conventional coagulation assays is that it provides an idea of platelet function as well as fibrinolytic activity. Hyper- and hypocoagulability as demonstrated with TEG was shown to correlate with clinically relevant morbidity and mortality in several studies,[63,64] although its superiority over conventional tests has not unequivocally been established.[65] Also, TEG seems to be overly sensitive to some interventions in the coagulation system, such as administration of fibrinogen, of which the therapeutic benefit remains to be established. There are no systematic studies on the diagnostic accuracy of TEG for the diagnosis of DIC; however, the test may be useful for assessing the global status of the coagulation system in critically ill patients.

For the diagnosis of the most extreme form of coagulation activation in critically ill patients, DIC, a simple scoring system has been developed.[66] The score can be calculated based on routinely available laboratory tests, that is, platelet count, prothrombin time, a fibrin-related marker (usually D-dimer), and fibrinogen. Tentatively, a score of 5 or more is compatible with DIC, whereas a score of less than 5 may be indicative but is *not* affirmative for nonovert DIC. By using receiver-operating characteristics curves, an optimal cutoff for a quantitative D-dimer assay was determined, thereby optimizing sensitivity and the negative predictive value of the system.[56] Prospective studies show that the sensitivity of the DIC score is 93%, and the specificity is 98%.[67,68] The severity of DIC according to this scoring system is related to the mortality

in patients with sepsis.[69] Linking prognostic determinants from critical care measurement scores such as Acute Physiology and Chronic Health Evaluation (APACHE-II) to DIC scores is an important means to assess prognosis in critically ill patients. Similar scoring systems have been developed in Japan.[70]

Supportive Treatment of Coagulation Abnormalities in Sepsis

The keystone of the treatment of hemostatic abnormalities in patients with sepsis is the specific treatment of the sepsis by appropriate antibiotics and control of the infectious source. However, in many cases additional supportive treatment, aimed at circulatory and respiratory support and replacement of organ function, is required. Coagulation abnormalities may proceed, even after proper treatment has been initiated. In those cases, supportive measures to manage the coagulation disorder may be considered, and they may positively affect morbidity and mortality. The increase in the insight into the various mechanisms that play a role in the coagulation abnormalities associated with sepsis has indeed been accommodating in the development of such supportive management strategies.

Low levels of platelets and coagulation factors may increase the risk of bleeding. However, plasma or platelet substitution therapy should not be instituted on the basis of laboratory results alone; it is indicated only in patients with active bleeding and in those requiring an invasive procedure or otherwise at risk for bleeding complications.[71] The presumed efficacy of treatment with plasma, fibrinogen, cryoprecipitate, or platelets is not based on randomized controlled trials but appears to be rational therapy in bleeding patients or in patients at risk for bleeding with a significant depletion of these hemostatic factors.[72] It may be necessary to use large volumes of plasma to correct the coagulation defect. Coagulation factor concentrates, such as prothrombin complex concentrate, may overcome this obstacle, but these compounds may lack essential factors, such as factor V. Moreover, in older literature caution is advocated with the use of prothrombin complex concentrates in DIC, as it may worsen the coagulopathy due to small traces of activated factors in the concentrate. It is, however, not clear whether this is still relevant for the concentrates that are currently in use. Specific deficiencies in coagulation factors, such as fibrinogen, may be corrected by administration of purified coagulation factor concentrates.

Experimental studies have shown that heparin can at least partly inhibit the activation of coagulation in sepsis.[73] Uncontrolled case series in patients with sepsis and DIC have claimed to be successful. However, a beneficial effect of heparin on clinically important outcome events in patients with DIC has never been demonstrated in controlled clinical trials.[74] Also, the safety of heparin treatment is debatable in DIC patients who are prone to bleeding. Therapeutic doses of heparin are indicated in patients with clinically overt thromboembolism or extensive fibrin deposition, like purpura fulminans or acral ischemia. Patients with sepsis may benefit from prophylaxis to prevent venous thromboembolism, which may not be achieved with standard low-dose subcutaneous heparin.[75]

In view of the deficient state of physiological anticoagulant pathways in patients with sepsis, restoration of these inhibitors may be a rational approach.[76] Because activated protein C and antithrombin are the most important physiologic inhibitors of coagulation and based on successful preclinical results, the use of concentrates of these factors in patients with sepsis has been studied relatively intensively. Most of the randomized controlled trials concern patients with sepsis, septic shock, or both. All trials showing some beneficial effect in terms of improvement of laboratory parameters, shortening of the duration of DIC, or even improvement in organ function, however, failed to reduce mortality.

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