

Microvascular endothelial dysfunction: a renewed appreciation of sepsis pathophysiology

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Abstract

Severe sepsis, defined as sepsis associated with acute organ dysfunction, results from a generalized inflammatory and procoagulant host response to infection. Coagulopathy in severe sepsis is commonly associated with multiple organ dysfunction, and often results in death. The molecule that is central to these effects is thrombin, although it may also have anticoagulant and antithrombotic effects through the activation of Protein C and induction of prostacyclin. In recent years, it has been recognized that chemicals produced by endothelial cells play a key role in the pathogenesis of sepsis. Thrombomodulin on endothelial cells converts Protein C to Activated Protein C, which has important antithrombotic, profibrinolytic and anti-inflammatory properties. A number of studies have shown that Protein C levels are reduced in patients with severe infection, or even in inflammatory states without infection. Because coagulopathy is associated with high mortality rates, and animal studies have indicated that therapeutic intervention may result in improved outcomes, it was rational to initiate clinical studies.

Keywords: Activated Protein C, antithrombotic, coagulation, endothelial cells, fibrinolysis, inflammation, organ dysfunction, sepsis

Introduction

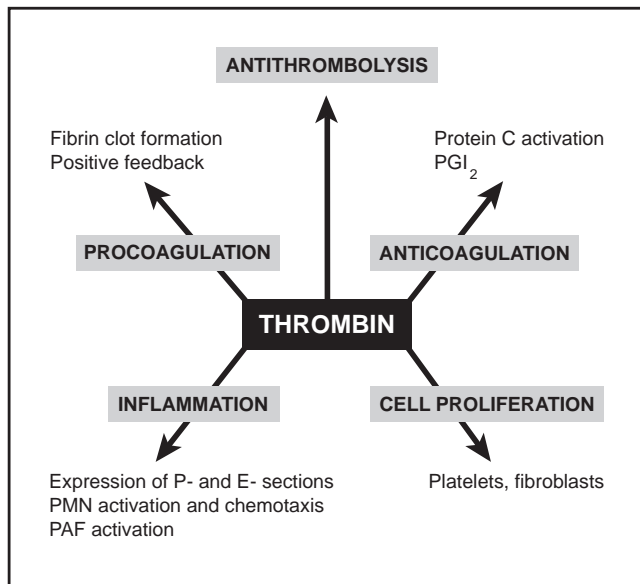
Sepsis, the host response to infection, involves a series of clinical, hematological, inflammatory and metabolic responses that can ultimately lead to organ failure. Severe sepsis is typically associated with activation of the coagulation system, leading to deposition of thrombin in the microvasculature. This process is associated first with activation, and then with inhibition of fibrinolysis. It is also associated with an inflammatory response and changes in microvascular permeability. These alterations, which are recognized by the clinician as disseminated intravascular coagulation (DIC), are commonly associated with multiple organ dysfunction, and have important prognostic implications. DIC, therefore, can be seen as both a thrombotic

and a bleeding disorder, as an index of disease severity, or as an inflammatory disorder.

Interaction of coagulation and inflammation

It is now recognized that acute sepsis involves a complex interaction between the coagulation system and the inflammatory system that may result in organ dysfunction. The molecule that may be considered central to these effects is thrombin. In addition to its well-known procoagulation effects, thrombin may have anticoagulation effects through Protein C activation and induction of prostacyclin (Fig. 1). Thrombin may also cause cell proliferation and inflammation through induction of adhesion molecules and platelet-activating factor activation.

Figure 1

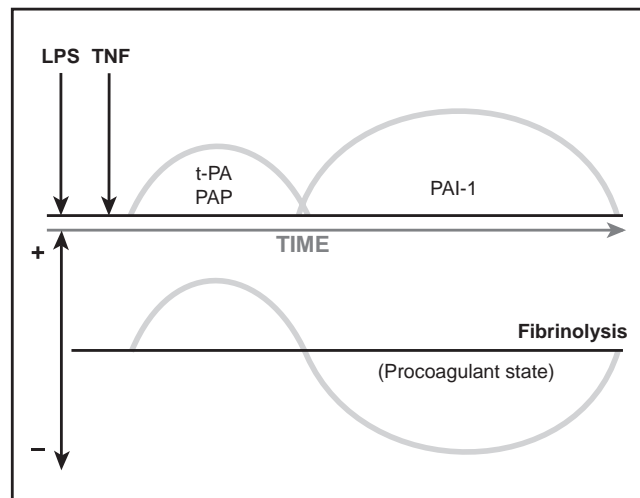


Central role of thrombin in acute sepsis. PAF, platelet-activating factor; PGI₂, prostacyclin; PMN, polymorphonuclear leukocyte.

The interaction between coagulation and inflammation has been demonstrated in a number of animal studies in which anticoagulant therapies, including heparin [1,2], antithrombin [3,4], tissue factor (TF) pathway inhibitor [5–7] and Activated Protein C [8], were shown to influence the inflammatory response. It is also now clear that both the coagulation and the fibrinolytic systems may be altered in acute sepsis, with the balance tipped in favour of procoagulation (through increased TF and thrombin expression, with decreased activation of thrombomodulin and Protein C) and antifibrinolysis (through plasminogen activator inhibitor [PAI]-1 expression). In experimental models in which very small doses of endotoxin or tumour necrosis factor (TNF) are given to volunteers, there is initially a profibrinolytic response, followed by an antifibrinolytic response, which corresponds to a procoagulant state (Fig. 2).

Considering the coagulation cascade as a whole, it is the extrinsic pathway (via TF and thrombin activation) rather than the intrinsic pathway that is of primary importance in sepsis. Once coagulation has been triggered by TF activation, leading to thrombin formation, this can have further procoagulant effects, because thrombin itself can activate factors VIII, IX and X. This is normally balanced by the production of anticoagulant factors, such as TF pathway inhibitor, antithrombin and Activated Protein C. However, there are other contributors to the processes of pro-/anticoagulation and inflammation, including monocytes, macrophages, platelets and, importantly, endothelial cells.

Figure 2



Experimental model of sepsis in volunteers: Effect of small doses of endotoxin (lipopolysaccharide [LPS]) or TNF on the fibrinolysis system. PAI, plasmin-antiplasmin complex; t-PA, tissue plasminogen activator.

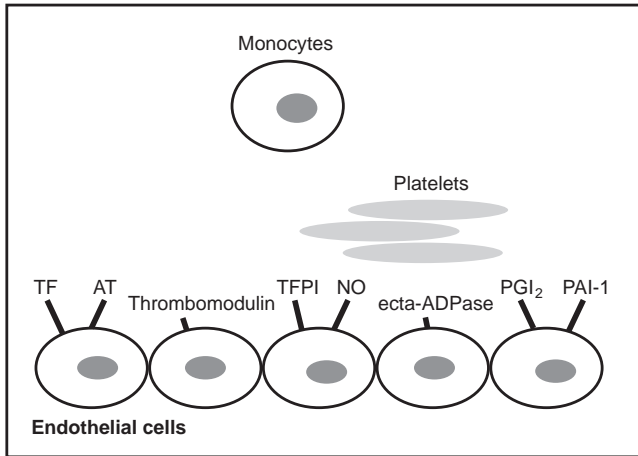
Role of endothelial cells

In recent years, it has been recognized that endothelial cells play a key role in the pathogenesis of sepsis, and that they produce important regulators of both coagulation and inflammation. They can express or release a number of substances, such as TF, endothelin-1 and PAI-1, which promote the coagulation process, as well as other substances, such as antithrombin, thrombomodulin, nitric oxide and prostacyclin, which inhibit it (Fig. 3). Thrombomodulin is to anticoagulation what TF is to coagulation. That is, the binding of thrombin to thrombomodulin on endothelial cells results in Protein C activation. Once activated, Protein C binds to its cofactor, Protein S (either freely circulating or bound to C4b-binding protein), and Activated Protein C inhibits the coagulation factors Va and VIIIa. Activated Protein C may also neutralize PAI-1, resulting in increased fibrinolysis, which represents an important difference between Activated Protein C and other anticoagulant substances, such as antithrombin. Activated Protein C also has important anti-inflammatory properties. It may inhibit E-selectin-mediated cell adhesion, or generation of interleukin-1 and TNF by monocytes, and may also uncouple the lipopolysaccharide (endotoxin) interaction with CD14 receptors.

Decreased Protein C in disease

Protein C is the source of Activated Protein C. Although Protein C is a biomarker or indicator of sepsis, it has no known specific biological activity. Protein C is converted to Activated Protein C in the presence of normal endothelium. In patients with severe sepsis, the vascular endothelium becomes damaged, levels of endothelial

Figure 3



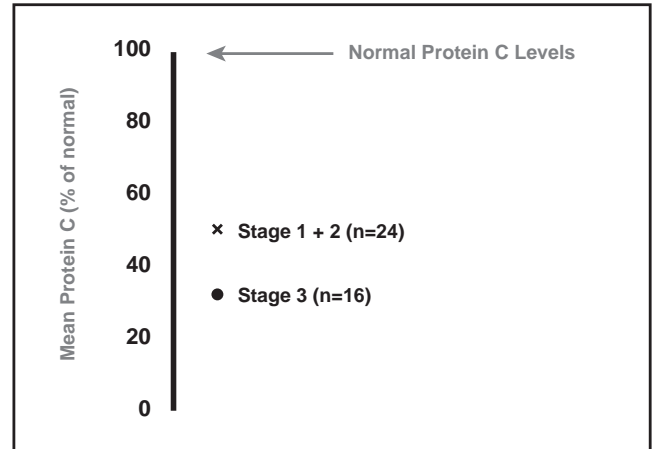
Procoagulant and anticoagulant substances that are expressed/released by endothelial cells. AT, antithrombin; NO, nitric oxide; PGI₂, prostacyclin; TFPI, TF pathway inhibitor.

thrombomodulin significantly decrease, and the body's ability to convert Protein C to Activated Protein C diminishes. Only when activated does Protein C have antithrombotic, profibrinolytic and anti-inflammatory properties.

A number of studies have now shown that Protein C levels are reduced in patients with severe infection. In the study by Powars *et al* [9], Protein C levels were reduced to approximately 50% of normal levels in patients with moderate meningococcaemia, and to approximately 30% of normal levels in critically ill patients (Fig. 4). Similarly, a study by Hemmer *et al* [10] in patients with malaria showed that those with complications had much higher levels of TNF and greatly reduced Protein C activity (Fig. 5). In patients who have undergone bone marrow transplantation, low antithrombin and Protein C levels are also the best predictors of multiple organ dysfunction [11]. In a subgroup of 70 patients with sepsis included in an ibuprofen trial [12], 90% were shown to have low Protein C levels and most also had other alterations in coagulation factors (elevated D-dimers, low fibrinogen level and low platelet count). In that study, there was a significant correlation between the increase in Protein C levels from 0 to 44 h, and 30-day mortality ($P < 0.01$). Furthermore, mortality was higher in patients with persistently low Protein C levels than in those whose Protein C levels recovered spontaneously without any treatment (45% versus 26%).

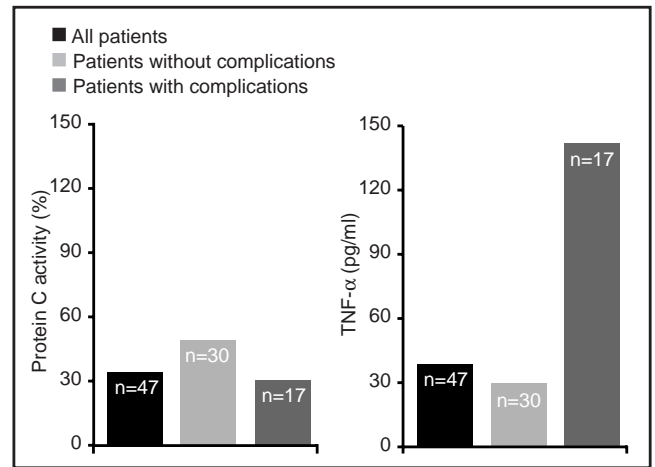
Coagulation abnormalities can occur in all types of infection, including both Gram-positive and Gram-negative bacterial infections [13–15], or even in the absence of infection, such as in inflammatory states secondary to trauma or neurosurgery [16]. Interestingly, they can also occur in patients with localized disease, such as those with

Figure 4



Protein C levels (% of normal) in patients with meningococcaemia. Adapted from Powars *et al* [9].

Figure 5



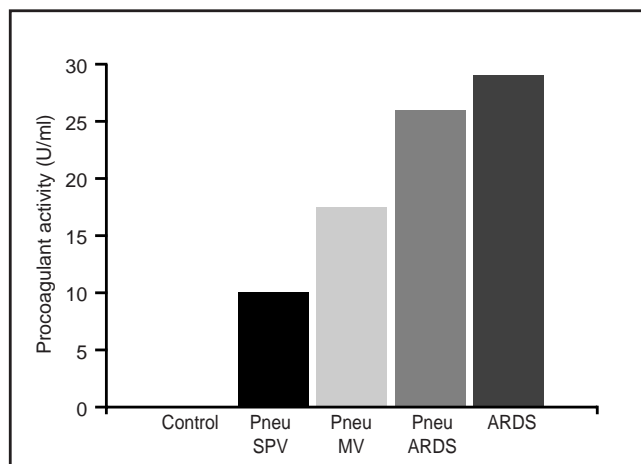
TNF-α levels (normal range <15 pg/ml) and Protein C activity (normal range 60–140%) in malaria. Adapted from Hemmer *et al* [10].

respiratory infection [17]. In a study by Günther *et al* [17], procoagulant activity in bronchial lavage fluid from patients with pneumonia or acute respiratory distress syndrome was found to be increased compared with that from control individuals, with a correlation between the severity of respiratory failure and level of coagulant activity (Fig. 6).

Rationale for clinical studies

It is well documented that DIC in septic shock is associated with higher mortality rates than is septic shock without DIC (77% versus 32%) [18]. Higher Sequential Organ Failure Assessment scores [19] and PAI-1 levels

Figure 6



Coagulation abnormalities in pneumonia (Pneu) and acute respiratory distress syndrome (ARDS; $n = 222$). SPV, spontaneous ventilation; MV, mechanical ventilation. Adapted from Günther *et al* [17].

[20] have also been correlated with increased mortality. Furthermore, coagulation is inter-related with inflammation. Because animal studies indicated that therapeutic intervention may result in improved outcome, a number of clinical studies were initiated.

Recent clinical studies with antithrombin and Activated Protein C

Early clinical studies with antithrombin III (ATIII) achieved promising results. In a study by Fourrier *et al* [21], patients who were treated with ATIII had a shorter duration of DIC with no side effects, and no change in Protein C or Protein S levels. However, a meta-analysis of three placebo-controlled, randomized clinical trials with ATIII in sepsis [22] demonstrated a nonsignificant reduction in 30-day all-cause mortality. A phase II clinical trial has identified the optimum dosage regimen for recombinant human Activated Protein C, and this has subsequently been used in a large, multicenter, placebo-controlled, phase III trial.

Conclusion

Severe sepsis, defined as sepsis associated with acute organ dysfunction, results from a generalized inflammatory and procoagulant host response to infection. The ultimate cause of acute organ dysfunction in sepsis is injury to the vascular endothelium, which can result in microvascular coagulopathy.

The extrinsic pathway (via TF and thrombin activation) is of primary importance in sepsis. Thrombomodulin on endothelial cells converts Protein C to Activated Protein C, which has multiple mechanisms of action that support its anti-inflammatory, antithrombotic and profibrinolytic properties. In patients with severe sepsis, the vascular endothelium

becomes damaged, levels of endothelial thrombomodulin significantly decrease, and the body's ability to convert Protein C to Activated Protein C diminishes.

The complex interactions between coagulation and inflammation support the development and use of strategies that are aimed at improving endothelial cell function, and thus providing therapeutic intervention to modulate inflammation and coagulopathy in patients with sepsis and organ dysfunction. Early clinical studies using recombinant human Activated Protein C appear promising.

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