The haematological manifestations of sepsis

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Introduction

Sepsis is associated with the activation of a number of host defence mechanisms that lead to profound metabolic changes. The haematological changes primarily involve leucocytes, platelets and the haemostasis system. The well-known leucocyte alterations entail neutrophilia or neutropenia which are directly related to the ongoing infection. These will not be discussed in this article. This review will focus on thrombocytopenia and alterations in the haemostasis system, especially disseminated intravascular coagulation (DIC), also known as consumptive coagulopathy or consumptive thrombohaemorrhagic disorder.

Thrombocytopenia

Bacterial sepsis is often accompanied by thrombocytopenia. This may or may not be associated with DIC, although DIC is in most instances the underlying cause, especially in patients with severe thrombocytopenia (<50 × 10^9/L). The mechanisms underlying thrombocytopenia in the absence of DIC are unknown. Platelet interaction with bacteria or endotoxin, inhibition of thrombopoiesis and immunological platelet damage also occur. DIC contributes to bleeding and microvascular thrombosis, leading to multiple organ failure. Tissue factor release, primarily mediated by tumour necrosis factor, activates the clotting system; fibrinolysis is initially activated, but later becomes inhibited by the release of plasminogen-activator inhibitor (PAI-1), further fostering multiple organ failure. Most septic patients have compensated, chronic DIC, detectable by assays of molecular markers; the earliest signs are already found during the systemic inflammatory response syndrome. Compensated DIC later becomes decompensated with rapid consumption of factors including inhibitors such as antithrombin III (AT III) and proteins C and S. AT III concentrations of <60–70% of the normal values predict outcome. Management of DIC must address the underlying disease, interrupt the activated haemostasis system and replace consumed coagulation constituents. Interruption of haemostasis with heparin may be attempted, but bleeding may worsen. Administration of a natural anticoagulant, such as AT III, may arrest clotting without concomitant risk of bleeding. In several animal models of DIC, AT III concentrations shortened the duration of DIC and reduced multiple organ failure and mortality. Similar benefits have been reported in early studies of patients with DIC, especially in the absence of sepsis. Studies are under way to determine whether outcome will improve if patients with sepsis are treated before the development of shock and plasma AT III concentrations are maintained at 100–150% of normal.

Disseminated intravascular coagulation

DIC, with disseminated fibrin deposits in the micro-circulation of various organ systems, is a frequent, if not invariable, finding in patients in septic shock. This
DIC in sepsis
Most if not all patients with sepsis have compensated DIC, and a smaller number have the decompensated form. This is triggered by bacteria, both Gram-negative and Gram-positive, by endotoxins and by viruses. The mechanisms are complex and involve the direct activation of factor XII, the first contact factor of the intrinsic pathway of clotting, activation of platelets with subsequent procoagulant release, direct endothelial damage by endotoxins with the exposure of clot-promoting surfaces and release of granulocyte procoagulant material, especially elastase. More recently it has been shown that the activation of the haemostasis system in septic patients is primarily due to the release of tissue factor and, therefore, due to an activation of the extrinsic pathway of clotting. Tumour necrosis factor (TNF) seems to be the most important mediator for the release of tissue factor from its locations. It is conceivable that in sepsis all mechanisms affecting the extrinsic and intrinsic pathways of clotting become operative leading to DIC, compensated or decompensated. These mechanisms include release of tissue factor, activation of factor XII, activation of platelets, endothelial damage and elastase release from granulocytes.

The fibrinolytic system is also activated in sepsis and plays an important role in the regulation of fibrin deposition in the microcirculation. Several studies have demonstrated in septic patients the release of tissue-type plasminogen activator which activates the fibrinolytic system, at least initially in sepsis. As sepsis progresses, there is an increased release of plasminogen activator inhibitor type 1 (PAI-1) which blocks plasmin generation and thus contributes to fibrin deposition in the microcirculation, and subsequent multiple organ failure.

Diagnosis of DIC
The clinical diagnosis of DIC is difficult to establish, but its presence may be suspected when a patient suffers from a disorder known to trigger DIC (see above), when a patient displays a sudden and unexpected diffuse bleeding tendency and when multiple organ failure develops from unexplained causes. The suspicion can be confirmed by appropriate laboratory tests, although routinely available tests will usually only diagnose decompensated DIC. However, the laboratory manifestations may be due to a variety of underlying disorders. Furthermore, the laboratory criteria are arbitrary, and for this reason there is no consensus on how to establish the definitive diagnosis.

Since DIC may occur at any time, most laboratories rely on readily available tests that are not specific for DIC. Table I lists the tests performed by most laboratories to diagnose DIC. Activated partial thromboplastin time...
(APTT) and prothrombin time (PT) are usually prolonged in patients with decompensated DIC. This reflects the consumption of several procoagulant clotting factors and gives non-specific evidence for DIC. Most laboratories perform platelet counts and determine fibrinogen concentrations which should be decreased in patients with decompensated, acute DIC. If time allows, serial determinations may be helpful to demonstrate the drop in these two constituents over time. Platelet counts are decreased in 98% of patients with DIC, and 50% of these have counts of <50 x 10^9/L. Fibrinogen concentrations are usually below normal levels in patients with decompensated DIC but may be still normal in the early stages of the syndrome. A gain, low platelet counts and fibrinogen concentrations are not specific for DIC and are found in many patients with disorders other than DIC. For this reason most laboratories perform determinations of fibrinogen (FSPs) and D-dimers. FSPs are produced when fibrinogen and fibrin are digested proteolytically by plasmin; they thus indicate an activation of the fibrinolytic system (not the clotting system!). D-dimer tests are a measure of fibrin breakdown products. Their presence in plasma indicates that fibrin was formed from fibrinogen, i.e. the presence of thrombin (or activation of the clotting system), and that fibrin was digested by plasmin, i.e. an activation of the fibrinolytic system. D-dimer tests therefore correlate best with DIC, but are not specific. Patients who have had recent invasive surgical procedures or those with extensive deep venous thrombosis and pulmonary embolism may have elevated D-dimer concentrations. An alternative or additional test would be the determination of fibrin monomers, which are formed from fibrinogen by cleavage of fibrinopeptides A and B by thrombin. A positive test would thus indicate an activated clotting system.

It is therefore, important to take the patient’s clinical situation, underlying disease and the status of hepatic and bone marrow function into account when interpreting laboratory findings that suggest DIC. Unfortunately, these common laboratory tests only suggest acute (or decompensated) DIC. In patients with compensated (or chronic) DIC these laboratory tests are less reliable. APTT and PT may be normal or reduced, fibrinogen concentrations may be normal or elevated and FSP and D-dimer concentrations may or may not be elevated. The latter two tests are better performed using ELISA-based assays; the routinely used latex agglutination tests may not be sufficiently sensitive. Platelet counts may be normal, but are mostly low, especially as time progresses.

It is obvious that with the use of routinely available test procedures, compensated DIC may not be identified. This accounts for many literature reports where ‘DIC’ was not seen or only seen in few septic patients. It is very likely that all patients with sepsis would have DIC, but in most of them it is the compensated form. This form of DIC can only be diagnosed with the aid of newer assay techniques that identify, for example, inhibitor–enzyme complexes (thrombin–antithrombin III complexes) or activation peptides (prothrombin fragments 1 and 2 (F1+2)) (Table II). These ELISA-based assays are very sensitive, highly accurate and reproducible. A gain, caution must be exercised when interpreting these markers because minor invasive procedures will lead to elevated plasma concentrations.

More recently it has been found that thrombin-antithrombin III complexes and F1+2 concentrations rise within hours of a febrile infectious event and that the concentrations rise further as sepsis develops and progresses. Highest concentrations are found in patients with septic shock. These data suggest that there is evidence early in an infectious event for an accelerated systemic activation of the haemostasis system that should be termed compensated DIC. As sepsis develops the process will accelerate.

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<tr>
<th>Test</th>
<th>Result</th>
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<tr>
<td>Activated partial thromboplastin time (APTT)</td>
<td>prolonged</td>
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<tr>
<td>Prothrombin time (PT)</td>
<td>prolonged</td>
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<tr>
<td>Platelet count</td>
<td>decreased</td>
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<td>Fibrinogen concentration</td>
<td>decreased</td>
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<td>Fibrinogen split products (FSPs)</td>
<td>elevated</td>
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<td>Fibrin monomer</td>
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<td>D-dimer</td>
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<th>Test</th>
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<tr>
<td>Activated partial thromboplastin time (APTT)</td>
<td>normal or short</td>
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<td>Prothrombin time (PT)</td>
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<td>Platelet count</td>
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<td>Fibrinogen levels</td>
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<td>D-dimer</td>
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<td>Thrombin–antithrombin III complex</td>
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<td>Fragments 1 and 2</td>
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Depend on laboratory procedures used.
and will eventually deteriorate into a decompensated form of DIC with its associated high mortality.

Coagulation parameters and outcome of sepsis

Since the haemostasis system is activated in septic patients, several studies have attempted to assess certain coagulation factors in relation to patient survival. A thrombomodulin concentrations have been most widely studied. A thrombin is one of the most important regulators of the clotting system and is consumed when the enzymes responsible for clotting are produced and when its rate of utilization is greater than its rate of synthesis by liver parenchymal cells. As indicated above, increased antithrombin consumption leads to more clotting which, in turn, leads to more antithrombin consumption. There have been numerous reports of decreased antithrombin concentrations in septic patients. The first report to suggest a relationship between antithrombin concentrations and outcome in a limited number of patients, was published in 1984. W e subsequently studied this relationship in a large number of surgical and trauma patients, with or without sepsis. Generally, patients with fatal sepsis had the lowest antithrombin concentrations. Septic patients had significantly lower (P < 0.001) antithrombin concentrations than patients with an infection but no sepsis. The latter group had, in turn, significantly lower concentrations (P < 0.001) than trauma patients without infections. A thrombin concentrations of <60% of normal values were associated with a >90% mortality. A thrombin plasma concentrations were found to predict mortality with 74% accuracy. Similar data on antithrombin have recently been published. In this report factor VIIa concentrations were also analysed. Factor VIIa is the key enzyme of the extrinsic pathway of clotting which is mediated through the release of tissue factor from damaged endothelium, monocytes and macrophages. Factor VIIa concentrations of >0.8 ng/mL and antithrombin concentrations of <70% at the onset of fever predicted lethal outcome with a specificity of 75% and 85%, respectively, and a sensitivity of 100% and 85%, respectively. These data support the concept that activation of the clotting system with the generation of thrombin is an integral part of infection and sepsis.

The relationship between sepsis and the fibrinolytic system has long been recognized, as indicated earlier, and recent outcome studies confirm this. In the initial phases of sepsis there seems to be an activation of the fibrinolytic system, but this activation seems to be ultimately inhibited by release of PAI-1, explaining the observed association of elevated PAI-1 concentrations with fatal outcome. PAI-1 concentrations of >5 U/mL at onset of fever predicted lethal outcome in septic patients with a sensitivity of 96% and a specificity of 100%. PAI-1 in patients with septic shock is probably released from damaged endothelial cells, leucocytes, platelets and perhaps hepatocytes. This release seems to be mediated by endotoxin, interleukin-1 and -6, TNF-α and even thrombin. The concept of an inhibited fibrinolytic system in sepsis was recently supported by the observation that the simultaneous administration of tissue-type plasminogen activator and endotoxin reduced PAI-1 synthesis, fibrin deposition and mortality in rabbits.

Treatment of DIC

The management of patients with DIC, whether compensated or decompensated, has to follow three basic approaches: (i) elimination of the underlying disorder (trigger); (ii) arrest of the intravascular clotting process; and (iii) substitution of coagulation constituents lost to consumption. The first approach is a very important one. In some instances, such as patients with obstetric complications associated with DIC, the underlying cause can be eliminated fairly rapidly. In others, treatment takes time so that the trigger will persist. It must be remembered that DIC will persist as long as the underlying disorder is not controlled.

The second approach should attempt to arrest the intravascular clotting process and the third one to replenish the consumed coagulation constituents. Diffuse haemorrhage in patients with acute, decompensated DIC is due to low concentrations of clotting factors and thrombocytopenia. Attempts must be made to raise the plasma concentrations of these lost constituents so that normal physiological haemostasis can be achieved. Platelet concentrates and fresh frozen plasma contain all the procoagulant factors needed for haemostasis and both should be administered in large quantities, depending, of course, on platelet counts and factor concentrations measured by APTT and PT. Clotting factor concentrates may be used to substitute lost components more rapidly but contain only selected factors. In DIC virtually all factors are consumed. Cryoprecipitate can be administered as a source of fibrinogen and other non-vitamin K-dependent factors. Erythrocyte concentrates are indicated to raise the oxygen carrying potential.

The control of the intravascular clotting process is a more difficult and controversial problem. It varies from country to country and from one institution to another. If the underlying disorder can be managed, there is probably no need to control intravascular clotting and rigorous substitution therapy should suffice. Should the underlying disorder persist, an anticoagulant approach may be considered.

In patients with chronic, compensated DIC, such as some cancer patients, low dose heparin (5-7000 U bid or tid) may be considered. These patients usually have no serious haemorrhagic disorder and display no symptoms or signs. The diagnosis is therefore usually based on laboratory data. The rationale behind this low dose heparin
approach is that heparin activates antithrombin and thereby boosts the body's own clotting system. It is not known if this therapy is effective since no extensive studies have been performed. It is recommended that patients with compensated DIC should be carefully monitored, and repeated measurements of plasma antithrombin and protein C concentrations should give information on the patient's clotting status. A nitrothrombin concentrations should never decrease to <70% of normal values.

In some countries and institutions patients with acute, decompensated DIC are treated with full therapeutic doses of heparin or other treatment schemes that clearly prolong APTTs. This treatment is designed to interrupt the intravascular clotting process, i.e. to stop the thrombotic component of DIC. Unfortunately, heparin given to an already bleeding patient will exacerbate the haemorrhagic component of DIC. For this reason heparin therapy, in therapeutic doses, is rarely used in the United States and is not the standard of care for septic patients. The experience with heparin in sepsis and septic shock is controversial. Colman et al. reported a significant improvement in mortality in septic patients treated with heparin, while Corrigan's review of the literature found no significant differences in mortality. There are no well-controlled prospective studies on the use of heparin therapy in sepsis that support its use.

Low molecular weight heparin has also been used in a multicentre trial, but these patients were predominantly uninfected. Bleeding and multiple organ failure was reduced in the treated group.

Antithrombin III concentrates and DIC. The rationale for considering concentrates of antithrombin in the management of DIC is three-fold: (i) antithrombin is one of the key regulators of the clotting system; (ii) heparin exerts its anticoagulant effect through antithrombin; and (iii) antithrombin concentrations decrease during DIC, leading to the above described positive feedback loop.

The term 'antithrombin' is misleading since it seems to suggest that only thrombin is inhibited. A nitrothrombin is, in reality, an inhibitor of all serine protease enzymes, and all clotting related enzymes (factors Xa, IXa, VIIa, XIa, XIIa) fall into this category. This means that all enzymes that are produced during the activation of the clotting system are neutralized by antithrombin. A nitrothrombin forms irreversible equilomolar complexes with these enzymes, and these complexes are ultimately destroyed by the reticuloendothelial system. Antithrombin is also the substrate for heparin. The binding of one heparin molecule to one antithrombin molecule induces a profound change in the tertiary structure of the antithrombin molecule which increases its anticoagulant activity 1000- to 2000-fold. A nitrothrombin also binds to heparin-like molecules on the endothelial cell surface (dermatan sulphate, heparin sulphate and other glycosaminoglycans) and thus increases the physiological anticoagulant status of the endothelium.

In recent years it has become apparent that antithrombin also has an anti-inflammatory effect. This was hypothesized by Taylor et al. based on animal experiments. It was subsequently found that the binding of antithrombin to the glycosaminoglycans of endothelial cells released prostacyclin (PGI₂). In the microvasculature PGI₂ has three strong anti-inflammatory properties: it reduces the release of oxygen radicals from activated granulocytes, decreases the release of TNF-α from activated monocytes and reduces platelet aggregability. These properties of PGI₂ protect endothelial cells from damage and preserve blood flow through the microcirculation. If the heparin binding site on the antithrombin molecule is blocked, as for example by heparin, the PGI₂ release from endothelial cells is abolished. This must be considered when using heparin in the treatment of DIC. Antithrombin has also been shown to inhibit activation of granulocytes and to decrease pulmonary vascular permeability after endotoxin administration in a rodent sepsis model. These properties, together with the observation that high plasma concentrations of antithrombin do not cause bleeding, make the use of antithrombin concentrates an interesting potential treatment for DIC patients.

In animal models (rat, rabbit, dog, non-human primates) antithrombin III concentrate substitution in experimentally induced DIC not only suppressed the laboratory markers of DIC but also improved haemodynamics and prevented multiple organ failure, as summarized by Emerson. In many of these models DIC was triggered by the injection of endotoxin or Escherichia coli in lethal doses. There is thus little doubt that in animal models, antithrombin III concentrates are effective in combating DIC.

The experience with antithrombin concentrates in humans is more limited, especially for septic patients. There are a number of case reports and pilot studies, reviewed elsewhere, where DIC was controlled in patients with DIC caused by liver disorders, obstetrical complications, multiple trauma and severe infections. One of the first larger trials in humans by Blauhut et al. involved 15 patients with shock and DIC; three of them were in septic shock. A nitrothrombin was administered in fixed doses, simultaneously with heparin. No data were reported on mortality, the prime focus of the study being half-life of antithrombin. Patients in DIC had a considerably shorter half-life of antithrombin than patients in a steady state.

In 1985 Blauhut et al. reported the results of a trial where patients with shock and DIC were randomly assigned to receive heparin, antithrombin concentrate or heparin plus antithrombin concentrate. Fifty-one patients were treated. Of these, 16 had septic shock, 29 traumatic shock and six were in hepatic coma. Duration of the signs
and laboratory markers of DIC was significantly shorter (P < 0.001) in the two groups receiving antithrombin concentrate (compared with the heparin-only group). There were no differences in mortality between the groups. However, the need for blood replacement was significantly greater in the group treated with both heparin plus antithrombin concentrate (P < 0.01). The authors concluded that heparin should not be administered when antithrombin concentrates are given.

In a follow-up study involving 133 patients assigned to receive either heparin or antithrombin concentrate, mortality was significantly lower (P = 0.04) in the antithrombin-treated group. When a subgroup of patients in phase IV shock was analysed separately, mortality was again significantly lower (P = 0.001) in the antithrombin-treated group. The author concluded that antithrombin substitution in shock patients with DIC not only reduced the time of DIC, but significantly reduced mortality.

A second double-blind, placebo-controlled trial was conducted in Germany. It involved 35 patients with septic shock. Seventeen received antithrombin concentrate using a weight-based dose regimen over 4 days, 18 served as control. Fifty percent of the 18 placebo-treated patients and 41% of the 17 treated patients died. A re-analysis of the data excluded three patients from the treatment group, two because of intractable shock leading to death within 3 h after entering the study and one because of acute myeloid leukaemia. These exclusions reduced mortality to 29% for the treatment group. While this difference was not statistically significant, the comparative Kaplan–Meier survival plot showed a difference in survival over 28 days.

In 1995 a multicentre, double-blind, placebo-controlled septic shock trial was conducted in Germany. A nithrombin concentrate was administered in fixed doses every 4 h over 7 days, but heparin was given simultaneously. The placebo group only received heparin. Preliminary analysis revealed a 45% 30 day mortality for the placebo group (22 patients) and a 39% mortality for the treatment group (23 patients). The difference was not statistically significant. A plot of survival time after admission again suggested a better outcome for the treatment group.

At the same time a second multicentre, placebo-controlled septic shock trial was conducted in Northern and Western Europe (Belgium, The Netherlands, Scandinavia). A nithrombin concentrate was given twice daily in fixed doses over 5 days, but no heparin was administered. Preliminary analysis resulted in a 30 day mortality of 41% in the placebo group (22 patients) and 25% in the antithrombin-treated group. The difference was again not significant, but the survival plot revealed a better outcome for the treated patients.

When all the data of the last three studies were subjected to a meta-analysis, the overall mortality for the placebo group was 45% (62 patients), 35% of the 60 treated patients died; this difference was not significant.

**Perspective**

The differences between antithrombin concentrate substitution in animal models, where time of DIC, onset of multiple organ failure and overall mortality are markedly reduced and the limited experience in humans requires careful consideration. One likely explanation arises from the timing of onset of sepsis which is carefully controlled in the animal models. Considering the various stages of shock, which are: systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock, patients admitted because of infections potentially fall into any one of these stages, so the patient populations entering these trials are thus heterogeneous in the stage of development of their infection. The three studies listed above (in France, Germany and North-west Europe) enrolled only patients in septic shock, i.e. the end stage of the septic process. Septic shock is more than DIC. Considering Böde’s statement that, ‘Septic shock is a state of metabolic anarchy in which the body can no longer control what it has created,’ one has to wonder why antithrombin substitution should work. When comparing the Austrian studies with the other three, it is noteworthy that in the Austrian trials only a few patients had septic shock, the majority had DIC not related to sepsis. This could explain why the reduction in mortality in the Austrian studies was so significantly greater than in the trials involving septic shock only. A further possibility is that the lack of statistical significance in the three septic shock trials is due to the limited number of patients, and that a larger multicentre trial would demonstrate a difference; the Kaplan–Meier survival plots demonstrated benefit in each one of the three trials.

Since DIC plays an important role in the pathogenesis of sepsis and multiple organ failure, and since DIC develops early in sepsis (at the SIRS stage), use of antithrombin substitution should probably be considered early in this process and not for septic shock patients. It is conceivable that early blockade of DIC, by antithrombin concentrates long before ‘metabolic anarchy’ is reached, would improve outcome. Investigators in this area believe that: (i) only patients with SIRS plus one or two failed organs should be considered for treatment with antithrombin concentrates, i.e. patients with sepsis or severe sepsis; (ii) patients in septic shock would probably not benefit from this treatment; (iii) in such trials plasma antithrombin concentrations should be kept between 100 and 150% of normal values; and (iv) heparin in therapeutic doses should not be administered simultaneously. Such a study has been started in the USA, but results have not yet been reported.

It is also conceivable that a ‘multicomponent’ treatment approach may be considered in the future to interrupt the key steps in the ‘septic cascade’ of which DIC is clearly only one.
Hematological manifestations of sepsis

References


