Abstract
The diagnosis of septic disseminated intravascular coagulation (DIC) relies on clinical signs and symptoms, identification of the underlying disease and results of laboratory testing. Since no single test result alone can definitely establish or rule out the diagnosis, the laboratory diagnostics of septic DIC encompass a combination of tests for which simple diagnostic algorithms are now available. Global tests of haemostasis provide evidence of activation of blood coagulation and, ultimately, consumption of coagulation factors, but their diagnostic efficiency is as yet questionable. Fibrinolytic markers, namely D-dimer, reflect the extent of activation of both coagulation and fibrinolysis, so a normal value can be used in a ruling-out strategy. Decreased levels of the natural inhibitors are frequently observed in patients with septic DIC, but antithrombin and protein C measurements are not incorporated in any of the widely used diagnostic algorithms. Among the inflammatory biomarkers, procalcitonin is currently regarded as the gold standard to differentiate the type of infection and guide antibiotic therapy, but its clinical usefulness in identifying and predicting the outcome of patients with septic DIC is still circumstantial.

Keywords
Disseminated intravascular coagulation (DIC), diagnosis, haemostasis, infection, sepsis

Disseminated intravascular coagulation (DIC) is a systemic, life-threatening disease characterised by excess thrombin generation and simultaneous activation of the fibrinolytic system. The clinical condition that follows is characterised by the presence of various degrees of both intravascular coagulation and haemorrhage resulting from consumption of coagulation factors. According to the Subcommittee (SSC) on DIC of the International Society on Thrombosis and Haemostasis (ISTH), the pathology can be defined as an acquired syndrome characterised by the intravascular activation of coagulation with loss of localisation arising from different causes that can originate from and cause damage to the microvasculature and that, if sufficiently severe, can lead to organ dysfunction.

Contrary to widespread belief, DIC should not be considered as a primary disease, but rather as one of the worst complications of several underlying illnesses. As such, DIC can arise from a variety of systemic or localised pathologies, involving most (if not all) organs and tissues. Systemic and persistent activation of blood coagulation may occur in patients with sepsis, severe infections, malignancies (solid tumours and myeloproliferative/lymphoproliferative malignancies), obstetric complications (amniotic fluid embolism, abruptio placentae, placenta previa, retained dead foetus syndrome), vascular disorders (vascular aneurysms, Kasabach-Merritt syndrome), severe organ injury (acute pancreatitis, hepatic failure), massive trauma, extensive burns, surgery and severe toxic (snake bites, drugs) or immunological reactions (haemolytic transfusion reaction, transplant rejection). Although the clinical spectrum of DIC is often heterogeneous and the balance between thrombosis (micro- or macrothrombosis) and bleeding (petechiae, ecchymoses, mucosal, skin and catheter haemorrhages) varies widely on an individual basis and according to the underlying cause, all bodily organs can be affected, such that multiorgan failure (MOF) is often a catastrophic end-point. In its most severe form, the combination of thrombosis and haemorrhage may trigger Waterhouse-Friderichsen syndrome (septicaemia and acute adrenal insufficiency), which is frequently observed in patients with fulminant meningococcal septicaemia.

The magnitude of the mutual clinical relationship between sepsis and DIC is reflected by epidemiological data attesting that DIC is a rather frequent complication in patients with sepsis (DIC is observed in up to 50% of patients with sepsis and is a strong predictor of mortality) and, on the other side of the coin, sepsis is by far the most frequent underlying condition identified in patients with DIC. Although lipopolysaccharide (LPS), conventionally referred to as endotoxin, is recognised as the most potent microbial mediator implicated in the pathogenesis of sepsis and septic shock, it is not alone as a cause of the worst overall complications (MOF and DIC). Cell membrane components of several micro-organisms (LPS or endotoxin) or bacterial exotoxins, such as staphylococcal α-haemolysin, can in fact induce a generalised inflammatory response through the activation of pro-inflammatory cytokines and thrombin generation by activation of the tissue factor/factor Vila pathway (see Figure 1).
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Figure 1: Pathogenesis of Disseminated Intravascular Coagulation in Sepsis

Table 1: Laboratory Tests in Diagnostics of Disseminated Intravascular Coagulation

Global Haemostatic Tests
- Prolongation of prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT)
- Thrombocytopenia
- Elevated fibrin/fibrinogen degradation products (FDP, D-dimer)
- Abnormalities on the peripheral smear (schistocytes, large platelets)
- Presence of biphasic waveform

Specific Tests Suggestive of Hypercoagulability
- Elevated prothrombin fragment 1 + 2 (F1+2)
- Elevated fibrinopeptide A (FPA) and B (FPB)
- Elevated thrombin–antithrombin complexes (TAT)
- Elevated soluble fibrin monomer (sFM)

Specific Tests Suggestive of Natural Inhibitor Consumption
- Decreased antithrombin
- Decreased protein C and S
- Decreased plasmin-antiplasmin (PAP) complexes

Inflammatory Biomarkers
- C-reactive protein (CRP)
- Procalcitonin (PCT)
- Interleukins 1, 6 and 8
- Tumour necrosis factor-α (TNF-α)
- LPS-binding protein (LBP)
- White blood cell and differential counting

A second important mechanism triggering sepsis-related DIC is thought to be the translocation of cell membrane aminophospholipids from the inner to the outer leaflet, or their release into the bloodstream as a consequence of cell breakdown. This is commonplace in several forms of sepsis, since host cells may be injured or broken directly by the microorganisms, whereas bacterial cells may be destroyed by heat, antibodies or antibiotics; as such, it can be concluded that ‘all bugs bite equally’. In fact, although there is a widespread perception that septic DIC is most commonly associated with Gram-negative bacteria infection, Gram-positive bacteria – as well as other micro-organisms, including viruses and parasites (such as malaria) – may cause dramatic activation of blood coagulation, causing severe forms of DIC.

Overall, the pathogenesis of DIC is characterised by simultaneous activation of haemostasis and fibrinolysis, resulting in extensive and persistent generation of thrombin and plasmin. However, studies in experimental models of sepsis have put forward the concept that thrombin generation might largely exceed that of plasmin. Convincing evidence also indicated that such an imbalance between coagulation and fibrinolysis might be due to increased levels of plasminogen activator inhibitor type 1 (PAI-1). A constellation of biological mechanisms has been advocated to trigger and/or boost DIC in patients with sepsis, including:

- increased thrombin generation mediated predominantly by activation of tissue factor/factor VIIa pathway and impaired function of physiological anticoagulant pathway (e.g. reduction of antithrombin levels, depression of the protein C pathway, inhibition of tissue factor pathway inhibitor);
- impaired fibrinolysis mediated by release of plasminogen activators from endothelial cells immediately followed by an increase in the plasma levels of PAI-1, and
- activation of the inflammatory pathway mediated by activated coagulation proteins and depression of the protein C pathway.

While cytokines and inflammatory mediators can induce coagulation, thrombin and other serine proteases interact with protease-activated receptors on cell surfaces to boost inflammation. Furthermore, since activated protein C has an anti-inflammatory effect through its inhibition of endotoxin-induced production of tumour necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6 and IL-8, depression of the protein C system may also result in a pro-inflammatory state. Taken together, this biological evidence is consistent with the hypothesis that septic DIC should be considered as a unique pathophysiological, biochemical and clinical entity in which inflammatory and coagulation pathways interplay in a sort of vicious circle (see Figure 1).

Laboratory Diagnostics in Septic Disseminated Intravascular Coagulation

The diagnosis of septic DIC is possible using a combination of laboratory tests for which simple diagnostic algorithms have now become available. However, these tests can be used for diagnosing and monitoring DIC only when at least two main requisites are fulfilled: identification of DIC at the earliest possible phase and availability in routine and, especially, urgent clinical settings. Patients with DIC have a low or rapidly decreasing platelet count, prolonged coagulation tests, low plasma levels of coagulation factors and inhibitors and increased markers of fibrin formation and/or degradation, such as D-dimer or fibrin degradation products (see Table 1). Therefore, a reliable approach to the diagnosis of DIC is the use of a simple scoring system based on the combination of routinely available coagulation tests.

Scoring Cards for Disseminated Intravascular Coagulation

The SSC on DIC of the ISTH has developed a four-parameter scoring card for DIC of ‘overt’ and ‘non-overt’ DIC. The scoring for overt DIC is as follows:

- platelet count: >100x10⁹/l = 0, <100x10⁹/l = 1, <50x10⁹/l = 2;
- elevated fibrin degradation products: no increase = 0, moderate increase = 2, strong increase = 3;
- prothrombin time (PT) upper limit of reference range: <3 seconds = 0, >3 seconds = 1, >6 seconds = 2; and
- fibrinogen level: >100mg/dl = 0, <100mg/dl = 1.
An overall score of 5 is compatible with overt DIC. A five-year overview by the ISTH SSC on DIC has recently confirmed that a score of 5 or greater can reliably identify overt DIC with a sensitivity of 91% and a specificity of 97%. It was also shown that patients with overt DIC diagnosed according to the ISTH DIC score had a significantly higher risk of death and of developing septic shock. However, since more than 95% of sepsis patients have elevated fibrin-related markers, the DIC score is strongly dependent on prolongation of PT and platelet count.

The Japanese Ministry of Health and Welfare (JMHW) established the diagnostic criteria for DIC in 1987. These criteria are also based on a scoring system relying on the presence of underlying disease, bleeding, organ failure and the results of global coagulation tests, and include:

- platelet count: ≤80x10⁹/l = 1, ≤50x10⁹/l = 2, ≤30x10⁹/l = 3;
- elevated fibrin degradation products: ≥10µg/ml = 1, ≥20µg/ml = 2, ≥40µg/ml = 3;
- PT: ≥1.25 = 1, ≥1.67 = 2, and
- fibrinogen levels: ≤150mg/dl = 1, ≤100 mg/dl = 2.

An overall score of ≤4 is compatible with DIC in patients with or without haematological malignancies, respectively. The rate of agreement in the diagnosis of DIC by ISTH and JMHW criteria was 67%; only 2% of non-DIC patients by JMHW criteria were diagnosed with overt DIC by ISTH criteria, suggesting that the ISTH criteria for overt DIC may include typical cases of DIC.

The Japanese Association for Acute Medicine (JAAM) DIC Study Group recently announced new DIC diagnostic criteria for critically ill patients, reaffirming the reliability of using a four-parameter scoring card that includes:

- inflammatory response syndrome criteria: ≥3 = 1, 1–2 = 0;
- platelet count: ≥80x10⁹/l or 50% decrease within 24 hours = 3, ≤80x10⁹/l, ≤120x10⁹/l or ≥30% decrease within 24 hours = 1 and ≤120x10⁹/l = 0;
- PT: ≥1.2 = 1, ≥1.2 = 0, 0–1 = 0;
- fibrin/fibrinogen degradation products: ≥25 mg/l = 3, ≥10 and <25 mg/dl = 1, <10 = 0.

An overall score of ≥4 is compatible with DIC. Two subsequent prospective surveys demonstrated the natural history of DIC patients diagnosed by the JAAM DIC diagnostic criteria in a critical care setting, highlighting progression from the JAAM DIC to the ISTH overt DIC, especially in patients with sepsis, thus enabling them to receive early treatment. More interestingly, a recent study by the JAAM DIC Study Group also showed that more than 50% of JAAM DIC patients with sepsis who died within 28 days could not be detected by ISTH DIC criteria during the initial three days.

**Global Tests of Haemostasis**

The PT is a simple, rapid and relatively inexpensive haemostatic test that explores the extrinsic and common pathways of blood coagulation. Although its values have been reported to be prolonged in the vast majority of patients with DIC (up to 70%), the use of PT as a diagnostic test is questionable because elevations often occur in a later stage of the disease when the outcome of the patients may already be compromised. The activated partial thromboplastin time (APTT) is a global clotting test sensitive to several plasma inhibitors and acquired or inherited deficiencies of coagulation factors of the intrinsic and common pathway. Prolonged APTT values have been reported in up to 50% of patients with DIC, but in the other 50% they can be normal or even shortened (mirroring the acute-phase-induced increase of von Willebrand factor). Accordingly, its clinical usefulness is limited in the diagnostics of DIC; it certainly cannot be used to rule out the diagnosis, and overall it is even more unreliable than the PT.

Fibrinogen levels often decline in patients with DIC due to massive consumption of the protein entrapped within the thrombi. However, the overall sensitivity of plasma fibrinogen levels for the diagnosis of DIC is low, since the protein is an additional well-recognised acute-phase reactant and its levels are often within the normal range for a long period of time, especially in patients with severe infections. Thus, hypofibrinogenaemia is frequently detected only in very severe cases of DIC, or in the advanced stages of the disease. More important is the longitudinal comparison of fibrinogen values of the patient, because a sudden and dramatic drop often underlies massive consumption, as is commonplace in patients with DIC.

In contrast to fibrinogen, platelet count is considered a very sensitive test in the diagnosis of DIC. Platelet count is strongly correlated with markers of thrombin generation, since thrombin-induced platelet aggregation is to a large extent responsible for platelet consumption. Moreover, platelet count is not influenced by the acute-phase response, so overall it is more reliable than fibrinogen testing. However, since the normal platelet count varies between 150 and 450 x10⁹/l, a single determination might be misleading; the longitudinal comparison of data may provide more valuable information in this setting. Many of the underlying conditions that are associated with DIC may in fact cause a low platelet count in the absence of DIC, such as severe local infections, malignancy or anticancer therapy. Accordingly, a continuous drop in the platelet count, mainly determined at intervals between one and four hours, underlies thrombin generation and the subsequent intravascular platelet aggregation. Conversely, a stable platelet count suggests that thrombin formation has stopped.

**Consumption of Natural Inhibitors**

The natural anticoagulants, especially antithrombin and protein C, are frequently reduced in patients with DIC, and they also have prognostic significance. A decrease in plasma antithrombin levels below 50% of normal is strongly associated with increased mortality in patients with DIC. Wilson et al. observed that in surgical patients with sepsis, antithrombin levels below 70 and 65% are associated with 90 and 100% mortality, respectively. Accordingly, baseline antithrombin levels have the best prognostic value for prediction of subsequent death in patients affected by septic shock. A significant prognostic value of protein C concentrations in neutropenic patients affected by septic shock...
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A hallmark of critically ill patients with DIC. Although the normal sigmoidal waveform pattern is characterised by an initial 100% light transmittance phase before clot formation, such biphasic patterns are instead sustained by an immediate, progressive decrease in light transmittance that occurs even in the pre-clotting phase. The definitive mechanisms underlying the biphasic waveform have not been definitively elucidated. However, it has been suggested that C-reactive protein (CRP), whose levels are persistently elevated in sepsis, may interact with very-low-density lipoproteins or intermediate-density lipoproteins to rapidly form a macroscopic precipitate upon recalcification of the plasma. These CRP–lipoprotein complexes can also be induced by incubating acute-phase serum containing elevated levels of CRP with serum from type III hyperlipidaemic patients. Whether CRP–lipoprotein complexes are an epiphenomenon of DIC, or whether they contribute to pathogenesis, is still controversial.

In 2002, it was reported that the biphasic waveform appears early in samples from patients who are later diagnosed with DIC by more conventional criteria. Further evidence corroborated this finding, also suggesting that this new APTT-derived parameter correlates with both the presence of DIC and overall mortality in patients with DIC. Matsumoto et al. showed that the prevalence of the biphasic waveform is as high as 87% (odds ratio 29.9) and 75% (odds ratio 19.0) in patients with septic DIC diagnosed by the ISTH and the JMWH scores, respectively. These findings led the authors to conclude that biphasic waveform is characterised by modest sensitivity (59.2% for the ISTH score; 47.9% for the JMWH score) but high specificity (95.4% for both scores). Similar diagnostic performances were reported in a further investigation, where biphasic waveform was observed in one-third of patients with a clinical suspicion of DIC and correlated well with the presence of disease (sensitivity 88%, specificity 97%). Remarkably, in nearly 20% of the patients, the APTT-biphasic-waveform-based diagnosis of DIC preceded that based on the traditional ISTH scoring system. Compared with other conventional parameters, biphasic waveform is characterised by superior prediction compared with CRP alone, whereas its combination with procalcitonin significantly increases the specificity of the latter test. Taken together, this evidence suggests that APTT waveform analysis may be a promising, inexpensive and rapid test in the diagnosis of severe sepsis (with both sensitivity and negative predictive value >90%), as well as for gathering useful information for the prognosis of septic patients, that can be used much earlier than traditional score systems provided that the MDA-180, the only analyser where this function has been detected, is available.

Inflammatory Biomarkers
For a better understanding of the immunological dysregulation in sepsis, several biomarkers are now available for routine and urgent diagnostics, including CRP, differential blood counting, the cytokines IL-6, IL-8, TNF-α, procalcitonin and the LPS-binding protein (LBP). However, only a few of these have a real impact throughout the managed care of patients with sepsis and therefore fulfil the clinical requirements.

Procalcitonin is a 116 amino acid protein with a sequence identical to that of the prohormone of calcitonin (32 amino acids). Under physiological conditions, hormonally active calcitonin is produced and secreted in the C cells of the thyroid gland after specific intracellular proteolytic procession of the prohormone procalcitonin. However, in severe bacterial infections and sepsis, extrathyroidal production of
It should be noted that two recent meta-analyses of pooled data both concluded that procalcitonin cannot reliably differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome in critically ill adult patients, thus arguing against the widespread use of the procalcitonin test in critical care settings.34,35 Giamarellos-Bourboulis et al. earlier reported that procalcitonin can be regarded as an early prognostic marker of the advent of DIC and MOF, so its daily monitoring may be helpful in the follow-up of critically ill patients.36 Boussekey et al. also observed that procalcitonin levels were increased in patients with community-acquired pneumonia who developed infection-related DIC during their intensive care unit stay.37 Interestingly, Ucar et al. did not find any significant increase in the levels of TNF-α and serum amyloid A (SAA) in newborns with neonatal late-onset sepsis who developed DIC, but procalcitonin was markedly increased in all of these patients on days zero, four and eight.38

LBP is an acute-phase protein involved in the endotoxin-mediated immune response. Although the overall diagnostic performance of this test is comparable to that of procalcitonin, LBP has slow kinetics of induction and elimination, and the severity of the inflammatory response is not well diagnosed.39

IL-1β and IL-6, are biomarkers indicating the severity of the inflammatory response, but they are not specific for bacterial infection since they can be induced after surgery, autoimmune disorders, transplant rejection and viral infection. Immunosuppression also decreases the IL-1β response. The kinetics of most ILs is also very fast; concentrations increase only briefly or intermittently and decline very quickly in sepsis, so their measurement is not superior to that of procalcitonin for the diagnosis of sepsis and may be unsuitable to monitor the clinical course of the disease.42,43 Finally, the measurements of most ILs is still challenging for a variety of pre-analytical/analytical reasons, and several instruments in the statistics laboratory are not equipped to deal with these tests.

The routine use of other traditional laboratory tests (e.g. leukocyte count, blood differential counting and CRP) is prompted by low cost, easy availability and historical practice rather than strong evidence. However, their reliability is hampered by a protracted response with late peak levels, low specificity compared with procalcitonin, especially in patients with MODS,44,45 and a reduced increase in patients undergoing steroid or other immunosuppressive therapies. Moreover, unlike procalcitonin, the dynamics of CRP and blood differential counting have limited prognostic implication.46

**Conclusions**

Although the contribution of laboratory diagnostics to the diagnosis of DIC is unquestionable, no single test result alone can definitely establish or rule out the disease. When approaching patients with suspected DIC it is therefore essential to consider a constellation of parameters, including clinical signs and symptoms, the identification of a potential underlying disease and, last but not least, the results of laboratory testing. When choosing among the armamentarium of tests that can be used to assist in the diagnosis, one should first consider that these may ultimately mirror changes in haemostatic function and keep pace with the critical nature of this condition.46 Global tests of haemostasis such as PT, APTT, fibrinogen and platelet count provide important evidence of activation of blood coagulation and, ultimately, consumption of coagulation factors, but their diagnostic efficiency is as yet questionable. Fibrinolytic markers (namely D-dimer) reliably reflect the extent of activation of both coagulation and fibrinolysis, so evidence of normal values can be reliably used to rule out the disease. The contribution of other tests of haemostasis, such as antithrombin and protein C, is as yet questionable. Decreased levels of these natural inhibitors are a marker of consumption coagulopathy and are frequently observed in patients with septic DIC, but they are not currently incorporated in any of the three widely used diagnostic algorithms. Among the inflammatory biomarkers, procalcitonin is currently regarded as the ideal candidate to differentiate the type of the infection and guide antibiotic therapy due to several advantages over other inflammatory markers, including earlier increase that is preserved in the presence of immunosuppressive medication, a better negative predictive value and a better correlation with outcome (e.g. mortality).47 However, its clinical usefulness in identifying and predicting the outcome of patients with DIC is circumstantial; therefore, larger studies in which this marker is evaluated against clinical outcome are needed.


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