DIC: Which laboratory tests are most useful

Marcel Levi *, Joost C. Meijers

Department of Vascular Medicine and Internal Medicine, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

**Abstract**

In patients with disseminated intravascular coagulation (DIC) a variety of altered coagulation parameters may be detectable, such as thrombocytopenia, prolonged global coagulation times, reduced levels of coagulation inhibitors, or high levels of fibrin split products. In addition, more sophisticated tests for activation of individual factors or pathways of coagulation may point to specific involvement of these components in the pathogenesis of the disorder. There is not a single test, however, that is sufficiently accurate to establish or reject a diagnosis of DIC. Nevertheless, a combination of widely available tests may be helpful in making the diagnosis of DIC and can also be helpful to guide in the selection of DIC patients that require specific, often expensive, interventions in the coagulation system. More recently developed dynamic algorithms, assessing changes in coagulation parameters over sequential days, could further increase the diagnostic accuracy for DIC and may be helpful to detect early stages of coagulopathy potentially evolving into DIC.

© 2010 Elsevier Ltd. All rights reserved.

**Keywords:**

Disseminated intravascular coagulation
Platelets
Coagulation factors
Coagulation inhibitors
Fibrin degradation products
Scoring systems

1. Introduction

Disseminated intravascular coagulation (DIC) is an extreme form of coagulation activation that may complicate a myriad of clinical situations, most of which are characterized by some form of local or systemic inflammation. Intravascular activation of coagulation, inadequately balanced by physiologic anticoagulant systems and aggravated by impaired endogenous fibrinolysis, may contribute to (micro)vascular fibrin deposition and thrombotic microangiopathy. The sometimes massive and ongoing activation of coagulation may lead to exhaustion of coagulation factors and platelets, thereby predisposing the patient with DIC for severe bleeding complications, which in some cases may dominate the clinical picture. In recent years, the insights into contributory pathogenetic pathways in DIC have been largely increased, which could result in more precise diagnostic tests for this condition. However, the clinical and laboratory diagnosis of DIC may remain difficult, since most tests focus on the consumption of coagulation factors or platelet, whereas molecular markers that are more sensitive for coagulation activation are usually insufficiently specific and are often not available in most settings for daily clinical care. In this review manuscript we focus on both routinely available and more sophisticated laboratory tests that may be useful in the diagnosis of DIC.

2. Platelet count

Thrombocytopenia or a rapidly declining platelet count is an important diagnostic hallmark of DIC. As the incidence of thrombocytopenia (platelet count <150 × 10^9/l) in critically ill medical patients is 35–44% and the specificity of thrombocytopenia for the diagnosis of DIC is limited. A platelet count of <100 × 10^9/l is seen in 50–60% of DIC patients, whereas 10–15% of patients have a platelet count of <50 × 10^9/l. In surgical and trauma patients with DIC the incidence of thrombocytopenia is higher with >80% of patients having less than 100 × 10^9/l platelets. The relevance of thrombocytopenia in patients with DIC is indeed related to an increased risk of bleeding. In particular patients with a platelet count of <50 × 10^9/l have a 4- to 5-fold higher risk for bleeding as compared to patients with a higher platelet count, in particular when anticoagulants are used. The risk of intracerebral bleeding in patients with DIC is relatively low (0.3–0.5%), but in 88% of patients with this complication the platelet count is less than 100 × 10^9/l. Regardless of the cause, thrombocytopenia is an independent predictor of ICU mortality in multivariate analyses with a relative risk of 1.9 to 4.2 in various studies. In particular, a sustained thrombocytopenia during more than 4 days after ICU admission or a drop in platelet count of >50% during ICU stay is related to a 4- to 6-fold increase in mortality. The platelet count was shown to be a stronger predictor for ICU mortality than composite scoring systems, such as the Acute Physiology and Chronic Evaluation (APACHE) II score or the Multiple Organ Dysfunction Score (MODS). A platelet count of <100 × 10^9/l is also related to a longer ICU stay but not the total duration of hospital admission.

3. Global clotting times and coagulation factors

Consumption of coagulation factors leads to low levels of coagulation factors in patients with DIC. 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 in DIC as well. 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 the DIC. The low level of coagulation factors is reflected by prolonged coagulation screening tests, such as the prothrombin time (PT) or the activated partial thromboplastin time (aPTT). A prolonged PT or aPTT occurs in 14 to 28% of intensive care patients but is present in more than 95% of patients with DIC. A PT or aPTT ratio > 1.5 was found to predict excessive bleeding. It is important to emphasize that global coagulation tests, such as the PT and aPTT, poorly reflect in vivo hemostasis. However, these tests are a convenient method to quickly estimate the concentration of one or at times multiple coagulation factors for which each test is sensitive. In general, coagulation tests will prolong if the level of coagulation factors is below 50%. This is relevant since the levels of coagulation factors, that are needed for adequate hemostasis, are somewhere between 25 and 50%. The normal values and the sensitivity of these tests for deficiencies of coagulation factors may vary markedly between tests, dependent on the reagents used. Therefore, an increasing number of laboratories use the International Normalized Ratio (INR) instead of the prothrombin time. While this may carry the advantage of increased standardization between centers, it should be mentioned that the INR has only been validated for control of the intensity of vitamin K antagonist therapy and has never been developed for the use as a screening test for coagulation abnormalities.

Plasma levels of factor VIII are paradoxically increased in most patients with DIC, probably due to massive release of the von Willebrand factor from the endothelium in combination with the acute phase behaviour of factor VIII. Recent studies have pointed to a relative insufficiency of the von Willebrand cleaving protease ADAMTS-13, thereby causing high concentrations of ultralarge von Willebrand multimers in plasma, which may facilitate platelet–vessel wall interaction and the subsequent development of thrombotic microangiopathy, which may contribute to organ dysfunction.

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. Fibrinogen acts as an acute phase reactant and despite ongoing consumption plasma levels can remain well within the normal range for a long period of 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.

4. Fibrin-related markers

Theoretically, measurement of soluble fibrin or fibrin monomers in plasma could be helpful to diagnose intravascular fibrin formation in DIC. 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. The only problem so far is that a reliable test is not available for quantitating soluble fibrin in plasma. Since soluble fibrin in plasma can only be generated intravascularly, this test will not be influenced by extravascular fibrin formation, which for example may occur during local inflammation or trauma.

Other more frequently used tests include elevated fibrin split products. Fibrin split products are detectable in 42% of a consecutive series of intensive care patients, in 80% of trauma patients and in 99% of patients with sepsis and DIC. Fibrin degradation products (FDPs) may be detected by specific ELISA’s or by latex agglutination assays, allowing rapid and bedside determination in emergency cases. None of the available assays for fibrin degradation products discriminates between degradation products of cross-linked fibrin and fibrinogen degradation, which may cause spuriously high results.

The specificity of high levels of fibrin degradation products is therefore limited and many other conditions, such as trauma, recent surgery, inflammation or venous thromboembolism, are associated with elevated FDPs. Because FDP’s are metabolized by the liver and secreted by the kidneys, FDP levels are influenced by liver and kidney functions. Other tests are specifically aimed at the detection of neo-antigens 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. D-dimer levels are high in patients with DIC, but also poorly distinguish patients with DIC from patients with venous thromboembolism, recent surgery or inflammatory conditions.

5. Natural coagulation inhibitors

Plasma levels of physiological coagulation inhibitors, such as antithrombin III or protein C, may be useful indicators of ongoing coagulation activation. Low levels of these coagulation inhibitors are found in 40–60% of critically ill patients and in 90% of DIC patients.

Antithrombin is the principal inhibitor of thrombin and may be readily exhausted during continuous thrombin generation. Plasma levels of antithrombin have been shown to be potent predictors for survival in patients with sepsis and DIC. During severe inflammatory responses, antithrombin levels are markedly decreased not only due to consumption but also due to impaired synthesis (as a result of a negative acute phase response) and degradation by elastase from activated neutrophils. A reduction in glycosaminoglycan availability at the endothelial surface (due to the influence of pro-inflammatory cytokines on endothelial synthesis) will also contribute to reduced antithrombin function, since glycosaminoglycans act as physiological heparin-like cofactors of antithrombin. Binding of glycosaminoglycans to antithrombin induces a conformational change at the reactive center of the antithrombin molecule, thereby rendering this protease inhibitor from a slow to a very efficient inhibitor of thrombin and other active coagulation factors.

Levels of protein C may also indicate the severity of the DIC. In patients with meningococcal septicemia, very low plasma levels of protein C are observed and this may play a pivotal role in the occurrence of purpura fulminans in these patients. In fact, also the plasma level of protein C may be regarded as a strong predictor for the outcome in DIC patients. Endothelial dysfunction is even more important in the impairment of the protein C system during DIC. Under physiologic conditions protein C is activated by thrombin bound to the endothelial cell membrane-associated thrombomodulin. Thrombomodulin is a membrane protein with several domains, including a lectin-like domain, six epidermal growth factor (EGF)-like repeats, a transmembrane domain and a short cytoplasmatic tail. The binding of thrombin to thrombomodulin occurs at the site of the EGF-repeats. This binding not only results in an about 100-fold increase in the activation of protein C, but also blocks the thrombin-mediated conversion of fibrinogen into fibrin and inhibits the binding of thrombin to other cellular receptors on platelets and inflammatory cells. In addition, thrombomodulin accelerates the activation of the plasma carboxypeptidase thrombin-activatable fibrinolysis inhibitor (TAFI), an important inhibitor of fibrinolysis.

Binding of protein C to the endothelial protein C receptor (EPCR) results in a 5-fold augmentation of the activation of protein C by the thrombomodulin–thrombin complex. However, during severe inflammation and DIC the protein C system is defective due to downregulation of thrombomodulin at the endothelial surface, mediated by the pro-inflammatory cytokines TNF-α and IL-1β. Observations in patients with severe Gram-negative septicemia indeed confirmed the downregulation of thrombomodulin in vivo and impaired activation of protein C. Low levels of free protein S (the cofactor of activated protein C) may further compromise an adequate function of the protein C system. In plasma, 60% of protein S is complexed to a complement regulatory protein, C4b binding protein (C4bBP). Increased plasma levels of C4bBP as a consequence of the acute phase reaction in inflammatory diseases may result in a relative free protein S deficiency.
Although it has been shown that the β-chain of C4bBP (which mainly governs the binding to protein S) in not very much affected during the acute phase response, support from this hypothesis comes from studies showing that infusion of C4bBP increases organ dysfunction and mortality in septic baboons.

A third inhibitory mechanism of thrombin generation involves TFPI, the main inhibitor of the tissue factor–factor VIIa complex. TFPI is a complex multi-domain Kunitz-type protease inhibitor, which binds to the tissue factor–factor VIIa complex and factor Xa. The TFPI–factor Xa complex may bind to negatively charged membrane surfaces, which may increase the local concentration of TFPI at cellular sites and facilitate inhibition of membrane-bound tissue factor–factor VIIa complex. The role of TFPI in the regulation of inflammation-induced coagulation activation is not completely clear. Plasma levels of TFPI in DIC are usually moderately decreased. However, the endogenous concentration of TFPI is presumably insufficiently capable of regulating coagulation activation and downstream consequences during systemic inflammation, as has been confirmed in a clinical study of patients with sepsis.

6. Markers of fibrinolysis

The acute fibrinolytic response in DIC 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). In patients with severe DIC, enhanced fibrinolytic activity may be demonstrated by various tests. Nevertheless, activation of the fibrinolytic system is in most instances insufficient to counteract the ongoing systemic activation of coagulation and subsequent intravascular fibrin formation. Plasma levels of fibrin degradation products, as discussed here above, may theoretically be seen as an indicator of fibrinolytic activity but appear to correlate more strongly with fibrin formation. Fibrinolytic activation may be monitored by measurement of plasma levels of plasminogen and α2-antiplasmin. Low levels may indicate consumption of these proteins. Due to the relatively low plasma concentration of α2-antiplasmin, the determination of this protease inhibitor is a helpful test for judging the dynamics of fibrinolysis.

Plasmin generation may be best judged by measurement of plasmin–α2-antiplasmin (PAP) complexes, which are indeed moderately elevated in patients with DIC. However, because the concentration of α2-antiplasmin is relatively low and therefore sensitive to relatively rapid exhaustion, this test may underestimate total fibrinolytic activity. At low concentrations of α2-antiplasmin, other protease inhibitors, such as antithrombin, α2-macroglobulin, α1-antitrypsin, and C1-inhibitor may act as a plasmin inhibitor as well. The apparent insufficient fibrinolytic activity in patients with DIC is attributed to high levels of the inhibitor of plasminogen activation, plasminogen activator inhibitor type 1 (PAI-1). Indeed, plasma levels of PAI-1 are elevated in patients with DIC and various underlying conditions, and are strongly correlated with an unfavorable outcome. 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 was also linked to clinical outcome of meningococcal septicemia and associated DIC. Patients with the 4G/4G genotype had significantly higher PAI-1 concentrations in plasma and an increased risk of death.

7. Molecular markers of DIC

The dynamics of the coagulopathy in critically ill patients can be judged by measuring activation markers that are released upon the conversion of a coagulation factor zymogen to an active protease. Examples of such markers are prothrombin activation fragment F1 + 2 (F1 + 2), and the activation peptides of factors IX and X. Indeed, these markers are markedly elevated in critically ill patients. Elevated plasma concentrations of thrombin–antithrombin complexes may well reflect the increased generation of thrombin and thrombin-mediated fibrinogen to fibrin conversion can be monitored by increased levels of fibrinogen activation peptide fibrinopeptide-A (FPA). All these markers are increased in patients with critical illness and their high sensitivity may be helpful in detecting even low-grade activation of coagulation. The specificity of high levels of markers for coagulation factor activation is probably limited, since many other conditions may lead to elevated plasma levels. Another drawback may be that these assays are very much dependent on optimal venous puncture, which may be difficult in sick patients and during routine (intensive) care. The most important disadvantage of these tests may be that their use is limited to specialized coagulation laboratories and that they are not available for routine use in most clinical centers. Thus, although these tests are very relevant for research in critically ill patients with DIC and the effect of specific interventions in the coagulation cascade, their practical use in clinical medicine is very limited so far.

8. Point of care tests

Thrombelastography (TEG) is a method that has been developed decades ago and provides an overall picture of ex vivo coagulation. Modern techniques, such as rotational thrombelastography (ROTEM) enable bedside performance of this test and have again become popular recently in acute care settings. The theoretical advantage of TEG over conventional coagulation assays is that 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. Although its superiority over conventional tests has not unequivocally been established. 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.

A new method that has proved sensitive and specific for hypercoagulability in critically ill patients is the partial thromboplastin test (aPTT) biphasic waveform analysis. This test, which requires specific instrumentation, detects the presence of precipitates of a complex of very-low-density lipoprotein and C-reactive protein that appears very early in DIC. When such complexes first appear in the plasma of individuals with diseases known to predispose to hypercoagulability, they confer a greater than 90% sensitivity and specificity for subsequent development of DIC and fatal outcome.

9. Composite scoring systems for DIC

For the diagnosis of overt DIC a simple scoring system has been developed by the subcommittee on DIC of the International Society on Thrombosis and Hemostasis (ISTH). The score can be calculated based on routinely available laboratory tests, i.e. platelet count, prothrombin time, a fibrin-related marker (usually D-dimer), and fibrinogen. Tentatively, a score of 5 or more is compatible with DIC. For non-overt DIC more refined scoring systems have been developed, which are currently being evaluated. A recent study showed that the INR can be used (instead of PT prolongation), further facilitating international exchange and standardization. By using receiver–operating characteristics curves, an optimal cut-off for a quantitative D-dimer assay was determined, thereby optimizing sensitivity and the negative predictive value of the system. Prospective studies show that the sensitivity of the DIC score is 93%, and the specificity is 98%. In series of patients with specific underlying disorders causing DIC (e.g. cancer patients or patients with
obstetric complications) show similar results.\textsuperscript{71,72} The severity of DIC according to this scoring system is related to the mortality in patients with sepsis.\textsuperscript{73} 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 and extensively evaluated in Japan.\textsuperscript{74} The major difference between the international and Japanese scoring systems seems a slightly higher sensitivity of the Japanese algorithm, although this may be due to different patient populations (Japanese series typically include relatively large numbers of patients with haematological malignancies).\textsuperscript{75}

10. Dynamic monitoring

The scoring systems discussed above are mostly based on a static assessment on a given moment. However, a more dynamic approach might be useful to further increase the accuracy of composite systems with laboratory parameters for the diagnosis of DIC. In the algorithm for non-overt DIC as proposed by the ISTH, some tests should be serially repeated, whereas improvement in any laboratory test confers a negative score (rather than a zero or neutral score). This “trend” scoring allows longitudinal assessment of the patient’s coagulopathy and, when therapy has been instituted, inference on whether the therapy has improved the course of the disease.\textsuperscript{76} In a prospective study in 840 patients continuation of coagulopathy during the first calendar day correlated with development of new organ failure and 28-day mortality in patients with severe sepsis.\textsuperscript{77} Coagulopathy risk points (based on sustained abnormalities in prothrombin time and platelet count) were related to progression from single to multiple organ failure, time to resolution of organ failure, and 28-day mortality (p < .001). Adding the scoring system to APACHE II improved ability to predict which patients may progress from single to multiple organ failure.

11. Conclusion

Until recently, a diagnosis of DIC was hampered by the limited availability of reliable and simple tools with sufficient diagnostic accuracy. Many tests are available, both in a routine setting or in a more specialized or research laboratory, but most of these tests are not sufficient to diagnose DIC with adequate certainty. Indeed, there is no single clinical or laboratory test that has an adequate sensitivity and specificity on its own to confirm or reject a diagnosis of DIC. However, combinations of several readily available coagulation tests may be helpful to establish this diagnosis. The simple scoring algorithm for DIC as proposed by the ISTH, and using the platelet count, the prothrombin time and INR, and plasma levels of a fibrin-related marker, such as D-dimer or other fibrin degradation products was shown to be a relatively accurate system to establish or reject a diagnosis of DIC.\textsuperscript{88} Sequential monitoring of coagulation parameters may further refine this approach, possibly enabling to make an earlier diagnosis of deranged coagulation, predisposing for full blown DIC.

Conflict of interest statement

No conflicts of interest to declare.

References


