Laboratory Diagnosis of Disseminated Intravascular Coagulation in Dogs and Cats: The Past, the Present, and the Future

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KEYWORDS

- Hemostasis • Thromboelastography • Laboratory tests
- Disseminated intravascular coagulation • Diagnosis
- Review

The hemostatic system is an intricate co-operative network of proteins and cells that produces then dissolves fibrin clots. Disruptions in the balance between stimulatory and inhibitory forces that drive clotting and fibrinolysis cause hemorrhagic or thrombotic disorders, the most severe of which is disseminated intravascular coagulation (DIC). DIC is a secondary complication of infections, inflammation and neoplasia. It contributes to morbidity and mortality through systemic microvascular thrombosis. Since clinical signs and imaging techniques are insensitive to thrombosis, laboratory testing is essential for DIC detection. Early diagnosis and mitigation can potentially improve survival and decrease hospitalization costs of affected animals.

PATHOPHYSIOLOGY OF DIC

Our view of physiologic hemostasis has evolved from the concept of a series of sequential cascading "waterfall" enzymatic reactions to more complex interrelated reactions that are grounded on cell surfaces, primarily provided by activated platelets. For a more in-depth appreciation of this "cell-based model" of hemostasis, the reader is referred to a recent review.[1] DIC essentially represents this normal hemostatic process gone viral—instead of being localized to a site of vessel injury and to platelet surfaces, hemostasis becomes unrestricted, uncontrolled, and systemic in DIC. Bacterial sepsis is one of the main causes of DIC in humans and animals.[2,3] As such,

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animal models of sepsis have provided substantial insight into how hemostasis becomes sufficiently perturbed to manifest in DIC.[4–6] There is some redundancy in the ways in which the hemostatic system responds to triggering stimuli; however, it is unlikely that "one rule fits all." The type of stimulus and the resulting interplay between the different cellular and enzymatic hemostatic components and between hemostasis and inflammation likely dictate the mechanisms by which DIC is initiated and progresses in different disorders and the interventions required to limit or halt this process.[7–9] Below is a summary of current concepts on the pathophysiology of DIC.

Two central themes of DIC are that thrombin generation is excessive and uncontrolled and that thrombin generation occurs and is amplified and disseminated on cell surfaces.[2,10] This indicates that the coagulation cascade must be excessively activated in DIC and that cell surfaces must be available to propagate and disseminate thrombin generation. Tissue factor (coagulation factor III, tissue thromboplastin) has been designated the main culprit in activation of coagulation in DIC.[2,10,11] Exposure of large amounts of tissue factor in the extravascular space or intravascular expression of tissue factor on circulating cells or cell membrane–derived microparticles begins the process of DIC (**Fig. 1**). Tissue factor activates the coagulation cascade (in health and in DIC) by binding to and activating its circulating enzymatic partner, coagulation factor VII (FVII), in the extrinsic pathway of coagulation. When complexed with tissue factor, activated FVII efficiently activates surface-bound factor X (FX) and factor IX. Once the extrinsic pathway generates thrombin via activated FX, thrombin amplifies its own production by activating other coagulation factor enzymes (factor XI) and cofactors (factors VIII and V) of the intrinsic and common pathways. This eventually terminates in fibrin production (and thrombus formation).[1,2] When tissue factor is exposed intravascularly or in large amounts, the normal spatial restriction of coagulation is lost and inhibitory mechanisms are disrupted, leading to excessive thrombin generation. Although tissue factor is important in initiation of DIC, there are other ways in which the coagulation cascade can be activated in disease states. For example, snake venom components and cancer proteases can directly activate other coagulation factors, including FX, inducing a DIC-like syndrome.[12,13]

Tissue factor fuels the fire of DIC, but alone is insufficient to result in dissemination of coagulation. Rather, thrombin generation is facilitated and propagated systemically through phospholipid-containing microparticles, lack of appropriate inhibition and a feedback cycle that is initiated (at least in inflammatory causes of DIC) between coagulation and inflammation.[2,10] Microparticles are small ($<1\ \mu m$) vesicles that are shed from the surface membrane of many different cell types (platelets, monocytes, granulocytes, erythrocytes, and endothelial cells), particularly after activation.[14] Membrane-derived microparticles are enriched in phosphatidylserine, a negatively charged phospholipid that is normally found on the inner leaflet of cell membranes, but is flipped to the outer membrane when cells become activated. Phosphatidylserine is the binding site for Gla-domain–containing coagulation factors and enables the assembly of the tenase (FX activating) and prothrombinase (thrombin activating) complexes on cell surfaces. The formation of coagulation factor complexes on phosphatidylserine-bearing surfaces produces high local factor concentrations, protects factors from inhibition, and amplifies their activity over 1000-fold.[14,15] Due to their small size, microparticles are not restricted to an injured site and persist in the circulation, thus providing a large functional surface area on which coagulation can propagate systemically.[14] Under physiologic conditions, activated platelets are the main source of phosphatidylserine membranes (both the intact cell and shed microparticles); however, in DIC, additional procoagulant membranes are provided by

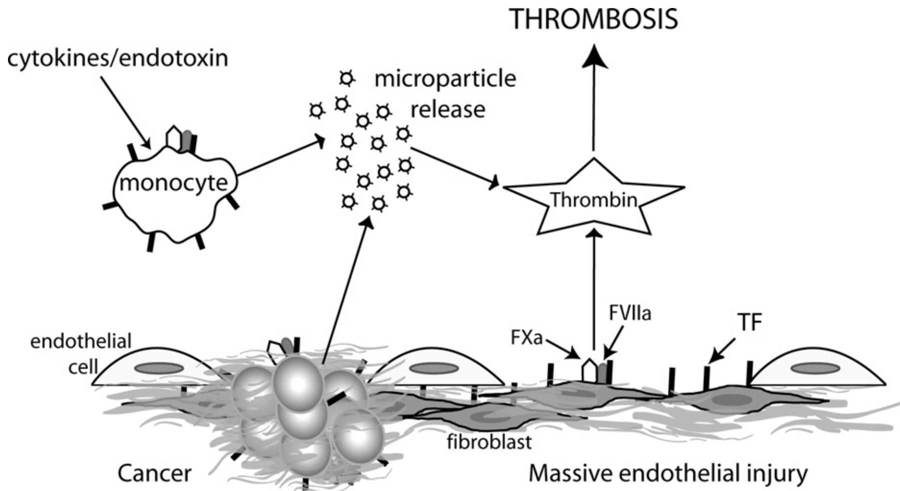

Fig. 1. Sources of tissue factor in DIC. Tissue factor is constitutively expressed on subendothelial fibroblasts and smooth muscle cells, where it is sequestered from its plasma ligand, FVII. Under physiologic conditions, this localizes activation of coagulation to a site of blood vessel injury. With massive endothelial or tissue injury, large amounts of this extravascular tissue factor are exposed to FVII, which subsequently binds to tissue factor and becomes activated (FVIIa). The tissue factor–FVIIa complex then binds to and activates factor IX (not shown) and factor X (FXa), the latter of which cleaves prothrombin to thrombin, initiating coagulation. In inflammatory states or sepsis, tissue factor expression is induced in circulating monocytes by endotoxin or inflammatory cytokines (interleukin-1β and tumor necrosis factor-α), which also shed tissue factor–bearing microparticles from their membranes.[16,63] This mechanism is also likely operative in other inflammatory states that initiate DIC, such as pancreatitis and heatstroke. Cancer cells can constitutively express high concentrations of tissue factor and also release tissue factor–enriched microparticles.[12,42,64] Release of large amounts of tissue factor generates excessive thrombin, which overwhelms inhibitory mechanisms and disseminates, resulting in widespread thrombosis. Although it is possible that other cells (granulocytes, platelets, and endothelial cells) can express tissue factor in disease states, it is now thought that these cells derive tissue factor from fusion of monocyte-derived microparticles versus synthesizing this protein de novo.[63]

other cell types, notably tissue factor–expressing monocytes, cancer cells, and apoptotic cells.[6,14,16,17] Thus, the driving force for coagulation—tissue factor—and the membrane support—phosphatidylserine—are colocalized to a greater degree in DIC than under physiologic conditions. Studies in humans demonstrate that oxidized lipoproteins can also provide phospholipid membrane support for coagulation and may have a role in dissemination of coagulation in DIC associated with sepsis[18]; however, the extent to which this occurs in domestic species is unknown.

Concurrent with thrombin generation is activation of fibrinolysis, resulting in the release of fibrin split products. However, fibrinolysis may be inhibited to some degree in DIC, particularly in later stages of sepsis and trauma. Inhibition is mediated through thrombin itself, high concentrations of which result in the production of thick strands of lysis-resistant fibrin and activation of a carboxypeptidase that inhibits fibrinolysis (thrombin-activatable thrombolysis inhibitor).[1,19] The concomitant release of polyphosphates from activated platelets[20] and tissue plasminogen activator inhibitors from the endothelium contributes to inhibition of fibrinolysis, particularly in sepsis.[2]

Inhibition of fibrinolysis would favor the development of thrombi, which is characteristic of DIC. However, since increased concentrations of fibrin split products are a characteristic and early laboratory finding in DIC with tests for these products having high negative predictive values,[21,22] fibrinolysis is always occurring in DIC to some degree.

Thrombin generation is normally balanced by inhibitors, specifically antithrombin (AT), activated protein C, and tissue factor pathway inhibitor.[1] Inhibitory function can be defective in DIC, either as a direct consequence of this process or secondary to the underlying disease. During DIC, inhibitors (AT, activated protein C) are consumed as they complex with their activated targets and are cleared from the circulation. In inflammation-induced DIC, inflammatory cytokines can downregulate production of inhibitors or their cofactors or receptors, resulting in decreased inhibitor activity. Inhibitors or their cofactors can also be degraded by neutrophil proteases.[2,10,23,24] Thus, inhibitors no longer constrain coagulation, which is excessive to begin with, resulting in the progression and dissemination of DIC.

It is now well accepted that coagulation and inflammation are intertwined.[2,24] Inflammation is one of the most common instigators of DIC, with inflammatory cytokines and complement components upregulating tissue factor, downregulating inhibitors, and activating various cells inducing vesiculation and phosphatidylserine exposure.[24,25] Conversely, activated coagulation factors (notably thrombin, FX, and the tissue factor–FVII complex) can potentiate the inflammatory response by binding to and activating protease-activated receptors (PARs) on platelets, leukocytes, and endothelial cells.[24,26] Activated PAR induce G protein–coupled cell signaling in these cells, resulting in upregulation of adhesion molecules (eg, intracellular adhesion molecule-1) and secretion of inflammatory mediators (eg, interleukin-6 and interleukin-8). Coagulation-induced inflammation is potentiated by the loss of hemostasis inhibitors, which can elicit anti-inflammatory and cell-protective responses, particularly activated protein C.[24,27] The extent to which this positive feedback loop is initiated in DIC may depend on the underlying cause of DIC and the precise balance between coagulation activating and opposing forces. The recognition of these intimate links between inflammation and coagulation has advanced the use of activated protein C concentrates for treating DIC,[28] with the intent on capitalizing on the inflammatory versus the anticoagulant properties of this inhibitor.

This summary of the pathophysiology of DIC has focused on the coagulation cascade, cell surfaces, and inhibitors, with little emphasis being placed on platelets. Yet platelets are important in perpetuating and disseminating coagulation and their contribution cannot be underestimated. Whether platelets are activated directly by the primary disease or as a consequence of generation of activated coagulation factor proteases, activated platelets are still likely the main structural scaffold on which DIC proceeds and are a rich source of phosphatidylserine-expressing microparticles, platelet and inflammatory agonists, and coagulation factors. However, it is unlikely that platelet activation or phosphatidylserine exposure alone will result in DIC without concurrent direct activation of the coagulation cascade.

THE DIC CONTINUUM

In 2001, a Scientific Subcommittee of the International Society on Thrombosis and Haemostasis (ISTH) on DIC produced a series of recommendations aimed at standardizing diagnostic criteria for DIC to improve clinical outcomes.[29] This subcommittee defined DIC as “an acquired syndrome characterized by intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe,

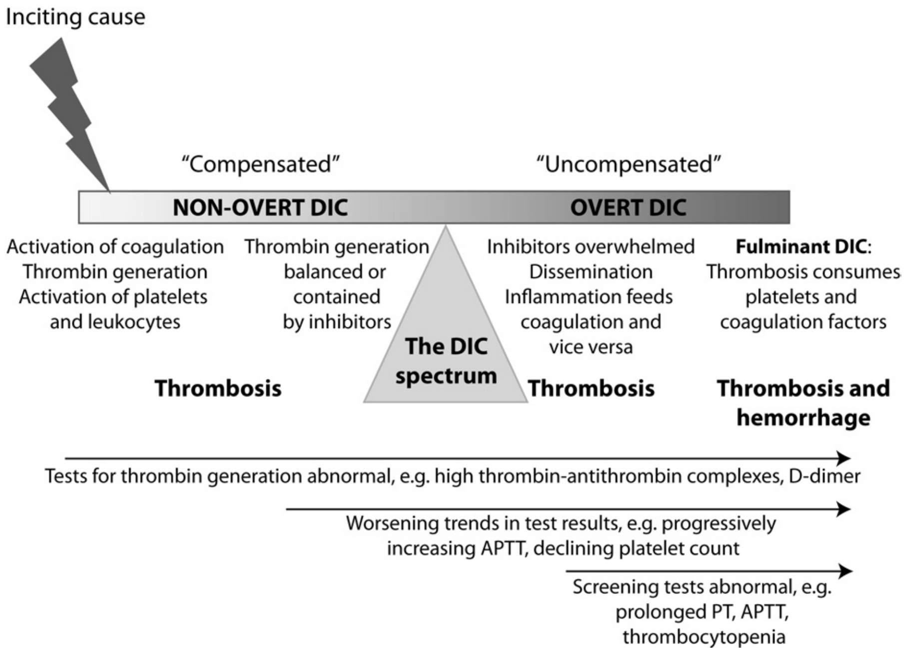

Fig. 2. The DIC continuum. A primary disease (inflammation, neoplasia, infection, trauma) activates the coagulation cascade, resulting in generation of thrombin, which subsequently amplifies its own production. Simultaneously, platelets and leukocytes are activated (by the underlying disease or activated coagulation factors) and release microparticles and exteriorize phosphatidylserine, providing a supportive enhancing framework for thrombin production. Thrombin generation is initially restrained by several inhibitors in the "compensated" or nonovert phase of DIC. Some degree of thrombosis is likely occurring and abnormalities in tests that detect thrombin generation, such as thrombin-antithrombin complexes and D-dimer, may be observed. As the inciting stimulus continues to activate coagulation, inhibitors become overwhelmed and inflammation is exacerbated by the hyperactive coagulation system. An "uncompensated" phase or overt DIC then ensues, in which thrombin generation becomes uncontrolled and systemic. This results in widespread thrombosis with abnormal hemostatic test results that are typical of DIC. Platelets and coagulation factors eventually become deficient manifesting as hemorrhage. Deficiencies have been mostly attributed to consumption with thrombosis; however, other mechanisms are likely operative (such as enhanced hepatic clearance of platelets[65] and cleavage of coagulation factors by proteases). Serial monitoring of hemostatic test results may detect progressive disruption of hemostasis as it becomes more and more unrestrained in non-overt DIC, eventually transforming into overt DIC. Note that these phases of DIC do not always naturally segue into one another. For instance, massive head trauma may immediately result in overt DIC from release of tissue factor in the brain, whereas low-grade inflammation may incite a more slow-burning controlled process (nonovert DIC) that may not progress.

can produce organ dysfunction." The subcommittee advanced the concept that DIC is an evolving process that can be separated into different stages: nonovert DIC, "controlled" overt DIC, and "uncontrolled" overt DIC (**Fig. 2**). In nonovert DIC, coagulation has been activated (usually through tissue factor exposure); however, thrombin generation is constrained by inhibitors and there is minimal incitement of inflammation. The subcommittee also refers to this stage as "a stressed but

compensated" hemostatic system, and this stage likely encompasses what has been previously referred to as "low-grade or chronic DIC."[30] Although not clearly stated, it is likely that microvascular thrombosis is occurring to some extent in this nonovert stage of DIC. This stage of DIC is difficult to diagnose because it is characterized primarily by thrombin generation, which cannot be reliably detected by routine coagulation screening assays, such as the prothrombin time (PT) and activated partial thromboplastin time (APTT).[21] Overt DIC reflects an activated and uncompensated hemostatic system, where inhibitors are overwhelmed, thrombin generation proceeds unopposed, the inflammation-hemostasis feedback loop is operative, and thrombosis is causing organ dysfunction. The subcommittee defines "controlled" overt DIC as a temporary condition, which can be ceased by intervention (eg, ruptured placenta), whereas "uncontrolled" overt DIC cannot be reversed by sole removal of the underlying cause. As thrombosis proceeds in overt DIC, platelets and coagulation factors are consumed, cleared, or cleaved, resulting in abnormalities in screening hemostasis assays, which are designed to pick up deficiencies in these cells and proteins. With time, these deficiencies may dominate the clinical syndrome, resulting in hemorrhage, which is more clinically discernable than thrombosis. This is the most severe manifestation of DIC and has been referred to as "fulminant" or "end-stage" DIC. Since this is the most clinically apparent and readily diagnosed stage of DIC, the moniker "death is coming" was coined for this hemostatic disorder. Although there is some evidence that nonovert DIC can progress to overt DIC in some patients,[31] this progression is not inevitable and likely depends on many factors, including the nature of the underlying disease, inherent differences in susceptibility to DIC, and disease- or DIC-related variables that influence activation or containment of coagulation.

LABORATORY TESTING OF DIC IN DOGS AND CATS

The Past

In veterinary medicine, DIC has been traditionally diagnosed on a combination of clinical and laboratory criteria (**Box 1**). To diagnose DIC, animals must fulfill both clinical criteria (a primary disease and clinical symptoms) and have two or more abnormal laboratory tests, reflecting abnormalities in all pathways of hemostasis.[3,32–36] The rationale for using a combination of test results for DIC diagnosis is sound, because none of these tests alone are specific for DIC. Although detection of fibrin thrombi in the microvasculature on histopathologic examination is considered the "gold standard" for diagnosis of DIC, it is a poor standard because fibrin thrombi can lyse rapidly after death[37]; thus, DIC is essentially an antemortem clinical and laboratory diagnosis. Nevertheless, this traditional method for diagnosing DIC has major shortcomings. Hemorrhage manifests late in the course of DIC and may not be evident at all in cats with this syndrome.[35,36,38] Unfortunately, thrombosis is far more difficult to recognize in affected patients and yet is the more important abnormality, being the major factor responsible for the high morbidity and mortality associated with DIC. Thus, clinical signs of hemorrhage or thrombosis cannot be relied upon to facilitate the diagnosis of DIC and these symptoms are not included in currently recommended DIC scoring systems in human medicine.[29] Since we rely heavily on clinical assessment (which depends on the index of suspicion harbored by the attending clinician), this means that the presence of DIC is likely frequently missed in animals, removing a potential opportunity for altering patient outcome. There is no standardization regarding the tests (type, number, or cut-offs) used to diagnose DIC, which makes it difficult to compare across published studies. Coagulation screening assays (PT, APTT, thrombin clot time, and fibrinogen concentration) are optimized for detection of factor deficiencies and are thus geared toward detection of the most

Box 1
Traditional criteria for diagnosis of disseminated intravascular coagulation

Clinical findings

Presence of a primary disease initiating DIC

Excessive hemorrhage or thrombosis

Laboratory tests

Consumption of platelets

- Thrombocytopenia

Consumption of coagulation factors

- Prolonged PT
- Prolonged APTT
- Prolonged thrombin clot time
- Hypofibrinogenemia

Consumption of inhibitors

- Low AT

Evidence of fibrinolysis

- Increased fibrin(ogen) degration products
- Increased D-dimer

Evidence of a microangiopathy

- Red blood cell fragments in a blood smear (schistocytes, keratocytes, acanthocytes)

advanced stages of DIC. These tests are insensitive to an activated but contained hemostatic system (nonovert DIC) or earlier stages of overt DIC where coagulation activation dominates over consumption.[21,39,40] Furthermore, test sensitivity to DIC differs between species and criteria used in dogs are not necessarily applicable to cats. DIC is an infrequent diagnosis in cats, likely because cats rarely demonstrate clinical signs of excessive hemorrhage and routine hemostatic assays are insensitive to DIC in this species. Reduced AT activity and high fibrin(ogen) degradation product (FDP) or D-dimer concentrations are two of the more sensitive tests for diagnosis of DIC in dogs,[32,33,41] but low AT activity is uncommon and FDP or D-dimer concentrations are not as reliably increased in cats with DIC (personal observations, 2011).[35,38] It is difficult to diagnose overt DIC in the face of a normal platelet count in dogs, yet cats with DIC may not be thrombocytopenic.[38] As indicated earlier, none of the laboratory tests are specific for DIC (even when used in combination) and some of the tests are so nonspecific (such as red blood cell fragments in cats), that they are not useful for DIC diagnosis.

The Present

To overcome the inherent limitations of traditional criteria, the diagnostic emphasis in humans has shifted toward the application of scoring systems for diagnosis of nonovert and overt DIC and new tools for detection of thrombin activation or hypercoagulability.[21,40] Although several assays for thrombin activation (eg, thrombin–AT complexes, fibrinopeptides, prothrombin fragments) and microparticle detection

have been evaluated in human patients,[21,40,42] little information exists on these assays in veterinary medicine and none have been tested in animals with DIC. Rather there is enormous interest in viscoelastographic-based coagulation testing for the detection of hypercoagulability (including that due to DIC) in animals. Thus, this section will focus on scoring systems and viscoelastographic testing.

In order to standardize DIC diagnosis for clinical use and outcome assessment, the DIC Scientific Subcommittee of the ISTH proposed a scoring system for nonovert and overt DIC.[29] The scheme was deliberately based on widely available standard diagnostic assays in human medicine, such as the PT and platelet count, without the need for specialized tests, such as AT or protein C activity, fibrinopeptides, or thrombin–AT complexes. For overt DIC, the scoring system can only be applied to patients that have a predisposing disorder and no points are given for the underlying disease. Since overt DIC was defined as an excessively activated and uncontrolled hemostatic system that is characterized by widespread and ongoing thrombosis, results of routine assays that detect platelet and coagulation factor depletion and release of fibrinolytic products should be abnormal in this stage of DIC. Therefore, the overt DIC score was based a moderate thrombocytopenia, a prolonged PT, low fibrinogen, increased fibrinolytic products (FDP, D-dimer), or soluble fibrin monomer (which is produced from the action of thrombin on fibrinogen). In essence, the overt DIC score represents a standardized format for traditional DIC testing (without a requirement for relevant clinical signs). In contrast, laboratory detection of nonovert DIC is far more difficult because this stage is characterized by an activated but contained hemostatic system (i.e., thrombin generation) and most of the routine screening assays of coagulation are insensitive to thrombin generation.[39] Thus, for the nonovert score, points were given for the underlying disease (i.e., the patient is at risk of DIC) and trends in data from routine assays (PT, platelet count, fibrinolytic products, or soluble fibrin monomer) were included in order to detect a progressively activated hemostatic system with deteriorating inhibitory control. Specialized tests that reflect thrombin activation (thrombin–AT complexes, low activity of inhibitors) could be done and were included in the scheme but were not necessary for attaining a score consistent with nonovert DIC. The recommended testing interval for evaluating dynamic trends was 24 to 48 hours. Weaknesses in the ISTH scoring system were a relatively low platelet cut-off ($<100 \times 10^6$/L) and no defined test or cut-offs for fibrinolytic products or soluble fibrin monomer (which could be used interchangeably with FDP or D-dimer). Other scoring systems for overt DIC have been used for several years in Japan.[21] Prospective validation of the ISTH scoring system, particularly that for overt DIC, has been accomplished and the scheme has been subjected to a 5-year review.[21] The performed studies support the continued use of the ISTH scoring system for overt DIC because patients classified with overt DIC have higher mortality rates and incidence of organ dysfunction than those not in overt DIC in most studies. Clinical treatment trials also show that the overt DIC score is useful in evaluating beneficial responses to therapy with regard to standardized patient outcomes.[28,43] The studies also illustrated that the overt DIC score is largely dependent on the PT and platelet count, that fibrinogen could be eliminated from the scoring scheme, and that fibrinolytic products or soluble fibrin monomer do not contribute substantially to the score due to their high sensitivity. There is still uncertainty over which test and test cut-off for fibrin generation or lysis should be used for ISTH scoring and there is a substantial lack of concordance between the ISTH and Japanese schemes (the Japanese scoring systems, particularly that from the Japanese Association for Acute Medicine, appear to perform better overall, perhaps due to regional differences in disease demographics or because they ascribe points for the primary disorder). Less

validation of nonovert DIC has been done; however, one study demonstrated that patients in nonovert DIC have higher mortality rates than patients without DIC and some progress to overt DIC.[31] The latter study also showed that serial monitoring of screening assays (PT, platelet count, fibrinolytic products, or soluble fibrin monomer) were useful for the diagnosis of nonovert DIC, with inhibitor assays for AT and activated protein C activity not contributing substantially to the score.

There has been one preliminary study on application of a modified ISTH scoring system for diagnosis of nonovert DIC in dogs.[44] In this study, the authors used total hospitalization days and 28-day mortality rates as outcome measures in 24 dogs with diseases associated with DIC. They combined selected nonovert and overt DIC criteria (points were given for underlying disease, a prolonged PT, thrombocytopenia, high D-dimer, and low AT activity) for diagnosing DIC and compared it to traditional assessment (three or more abnormal tests including thrombocytopenia and prolonged coagulation times) on daily blood samples, selecting the highest values for comparison. They found that more dogs were diagnosed in DIC with traditional criteria but mortality rates were higher in the dogs that were classified in DIC by modified ISTH criteria. Total days of hospitalization were no different between groups. This study suggests that ISTH criteria are potentially applicable to dogs. However, the tests and cutoffs selected for the canine ISTH scoring scheme may not have been optimized for the dog, resulting in the reduced sensitivity of the scheme compared to traditional assessment (which has since been corroborated by subsequent studies by these authors in larger numbers of dogs[45]). For example, the APTT is more sensitive than the PT as an indicator of DIC in dogs.[32,33]

The same authors have recently published a diagnostic scoring algorithm for DIC based on multiple logistic regression analysis of hemostatic assays. To develop the model, dogs admitted to a single academic veterinary hospital were tested daily for various hemostatic test results (PT, APTT, D-dimer, platelet count, fibrinogen, AT, and protein C) and results were scored per ISTH overt DIC criteria. Using data from the day of the highest ISTH DIC score, a diagnosis of DIC was made by a simple majority opinion of three experts based on traditional DIC criteria (abnormal test results reflecting coagulation factor and platelet consumption, inhibitor depletion, and fibrinolytic activity). The final model was based on results from the PT, APTT, fibrinogen, and dichotomized D-dimer (>0.5 mg/L for a latex agglutination-based card assay) from 63 dogs (37% of which had DIC per expert opinion). A logistic value of $P>.40$ was designated as the optimal diagnostic cut-off for the model. The model was tested on a different population of dogs from another academic institution, with the assays presumably being performed at the first institution. Dogs with P values greater than the cut-off had a higher relative risk of death (the latter was not defined). This same study also revealed that the sensitivity of ISTH overt criteria for diagnosis of DIC in dogs was lower than expert opinion. This proposed model may be a promising way to standardize diagnosis of DIC in later stages of overt disease (although is no better than expert opinion), but the model still awaits verification from additional independent studies, particularly using different methods and reagents since these assay variables may markedly influence usefulness of the model.[46] For instance, the latex agglutination D-dimer assay used in the model is not commercially available in the United States and a previous study has shown a lack of concordance between diagnostic assays for this marker.[47]

Viscoelastographic testing is a source of tremendous interest in veterinary medicine, yielding a plethora of recent publications, particularly with respect to the use of these assays for diagnosing hypercoagulability in animals, such as nonovert DIC. Viscoelastograph-based testing is referred to as a “global” hemostatic assay because

it is performed in whole blood, thus assessing all soluble factors and cellular constituents involved in hemostasis, with the exception of the endothelium and tissue-derived proteins. Interestingly, these assays are used to dictate or assess response to transfusion therapy in humans undergoing surgery and are not generally used for diagnosis of specific hemostatic disorders.[10,48] There have been several recent substantive reviews on the use of viscoelastographic coagulation testing in veterinary medicine[49,50]; hence, only a few points will be emphasized here. Important factors that influence coagulation results from these analyzers are the type and concentration of the activator (if one is used), the timing of analysis, and hematocrit.[49–53] Tissue factor and kaolin are two commonly used activators, with tissue factor activation being analogous to a whole blood PT and kaolin activation with a whole blood APTT. In support of this, tissue factor-activated thromboelastographic (TEG, performed with the TEG 5000 analyzer from Haemoscope Corporation) results display no abnormalities (similar to the PT) in blood from dogs with hemophilia A (M. Brooks, personal communication, 2011). There is a trend toward hypercoagulable tracings with storage of blood and lower hematocrits (which may be an artifact of the technique, since it is unlikely all anemic dogs are truly hypercoagulable).[49,50] Thus, studies using different viscoelastic techniques, activators, and time of analysis cannot be directly compared and hematocrit is clearly a confounding variable that must be taken into consideration. Furthermore, there is no consensus on the definition of hypercoagulability, with some authors using the G or global clot strength[54,55] and others using one or more abnormal TEG results, including mathematical formulae that combines most of the major tests, the coagulation index, or total thrombin generation (area under the TEG curve).[56–60]

Only one viscoelastographic study has been performed in dogs with DIC.[55] In this study, tissue factor–activated TEG was performed 30 minutes after blood was collected from 50 dogs admitted to the intensive care unit of two university hospitals. Similar to that done previously by these authors,[45] the diagnosis of DIC was based on expert opinion. Overall coagulation state (hyper-, normo-, and hypocoagulability) from the TEG was defined on global clot strength or G, a direct mathematical derivation of the maximum amplitude (MA) of clot formation, using data from the highest ISTH overt DIC score. Outcome was based on 28-day mortality rates. The authors found that hypercoagulability was the most common coagulation state in dogs with DIC but that hypocoagulable dogs had a higher risk of mortality than dogs with normal or hypercoagulable status. The influence of hematocrit on test results was not evaluated. This study and others by the same and other authors[22,54,59] also illustrated the dependence of the G (or MA) on the platelet count and fibrinogen concentration, with blood from dogs with platelet counts less than 30×10^6/L being hypocoagulable and high fibrinogen being associated with hypercoagulability. These data suggest that TEG results defined by G (or MA) may provide no information on overall coagulation status in severely thrombocytopenic dogs or dogs with hyperfibrinogenemia. Indeed, hyperfibrinogenemia may be an independent risk factor for thrombosis[61] and measuring fibrinogen in dogs may yield similar information on hypercoagulability than in vogue viscoelastographic techniques.

The Future

It is likely that we will continue to use traditional criteria to diagnose DIC in animals, with DIC remaining a rare diagnosis in cats. Time and additional studies will tell whether viscoelastographic coagulation testing will live up to the hoped for promise for detecting earlier nonovert or thrombotic phases of overt DIC. However, a recent study suggests TEG is not a good screening tool for detection of early hemostatic

abnormalities in experimental endotoxemia in dogs.[22] It is also unlikely that a single sensitive and specific diagnostic assay for DIC will be discovered in the next few years, although new prognostic tests may become available.[21,62] The search for molecular markers of thrombin activation will no doubt continue and potentially become more targeted as our understanding of the complex hemostatic system evolves. In the interim, we will continue to rely upon clinical acumen and a battery of affordable and readily available imaging and laboratory assays to diagnose DIC and detect thrombosis. Although costly, daily or alternate-day testing to monitor for trends in hemostatic test results (such as a normal but declining platelet count, progressively increasing D-dimer concentrations, or decreasing AT activity) may prove the best means to identify dogs in early or thrombotic phases of DIC.[21,40] To effectively evaluate risk factors and new diagnostic tests and treatment strategies, it is imperative that we reach consensus and standardize, to the best of our ability, the definition of DIC, the best tests and test cut offs used to diagnose DIC, and outcome assessment for clinical trials or research studies in dogs and cats. The multiple logistic regression model proposed by Wiinberg and colleagues[45] may be a good starting point to reach this consensus, once it has been independently verified and applied across clot detection methods and reagents.

REFERENCES

1. Smith S. Overview of hemostasis. In: Weiss DJ, Wardrop KJ, editors. Schalm's veterinary hematology. 6th edition. Ames (IA): Blackwell Publishing; 2010. p. 635–53.
2. Levi M, Schultz M, van der Poll T. Disseminated intravascular coagulation in infectious disease. Semin Thromb Hemost 2010;36:367–77.
3. de Laforcade AM, Freeman LM, Shaw SP, et al. Hemostatic changes in dogs with naturally occurring sepsis. J Vet Intern Med 2003;17:674–9.
4. Taylor FB Jr, Chang A, Ruf W, et al. Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ Shock 1991;33:127–34.
5. Warr TA, Rao LV, Rapaport SI. Disseminated intravascular coagulation in rabbits induced by administration of endotoxin or tissue factor: effect of anti-tissue factor antibodies and measurement of plasma extrinsic pathway inhibitor activity. Blood 1990;75:1481–9.
6. Pawlinski R, Wang JG, Owens AP 3rd, et al. Hematopoietic and non-hematopoietic cell tissue factor activates the coagulation cascade in endotoxemic mice. Blood 2010;116:806–14.
7. Asakura H, Suga Y, Yoshida T, et al. Pathophysiology of disseminated intravascular coagulation (DIC) progresses at a different rate in tissue factor-induced and lipopolysaccharide-induced DIC models in rats. Blood Coagul Fibrinolysis 2003;14:221–8.
8. Ontachi Y, Asakura H, Takahashi Y, et al. No interplay between the pathways mediating coagulation and inflammation in tissue factor-induced disseminated intravascular coagulation in rats. Crit Care Med 2006;34:2646–50.
9. Patel KN, Soubra SH, Lam FW, et al. Polymicrobial sepsis and endotoxemia promote microvascular thrombosis via distinct mechanisms. J Thromb Haemost 2010;8:1403–9.
10. Toh CH, Downey C. Back to the future: testing in disseminated intravascular coagulation. Blood Coagul Fibrinolysis 2005;16:535–42.
11. Berthelsen LO, Kristensen AT, Tranholm M. Purified thromboplastin causes haemostatic abnormalities but not overt DIC in an experimental rabbit model. Thromb Res 2010;126:337–44.
12. Zwicker JI, Furie BC, Furie B. Cancer-associated thrombosis. Crit Rev Oncol Hematol 2007;62:126–36.

13. Isbister GK. Snakebite doesn't cause disseminated intravascular coagulation: coagulopathy and thrombotic microangiopathy in snake envenoming. Semin Thromb Hemost 2010;36:444–51.
14. Freyssinet JM. Cellular microparticles: what are they bad or good for? J Thromb Haemost 2003;1:1655–62.
15. Sinauridze EI, Kireev DA, Popenko NY, et al. Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. Thromb Haemost 2007;97:425–34.
16. Satta N, Toti F, Feugeas O, et al. Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharide. J Immunol 1994;153:3245–55.
17. Wang JG, Manly D, Kirchhofer D, et al. Levels of microparticle tissue factor activity correlate with coagulation activation in endotoxemic mice. J Thromb Haemost 2009;7:1092–8.
18. Dennis MW, Downey C, Brufatto N, et al. Prothrombinase enhancement through quantitative and qualitative changes affecting very low density lipoprotein in complex with C-reactive protein. Thromb Haemost 2004;91:522–30.
19. Weisel JW. Structure of fibrin: impact on clot stability. J Thromb Haemost 2007; 5(Suppl 1):116–24.
20. Smith SA, Morrissey JH. Polyphosphate enhances fibrin clot structure. Blood 2008; 112:2810–6.
21. Favaloro EJ. Laboratory testing in disseminated intravascular coagulation. Semin Thromb Hemost 2010;36:458–67.
22. Eralp O, Yilmaz Z, Failing K, et al. Effect of experimental endotoxemia on thrombelastography parameters, secondary and tertiary hemostasis in dogs. J Vet Intern Med 2011;25:524–31.
23. Massberg S, Grahl L, von Bruehl ML, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat Med 2010;16:887–96.
24. van der Poll T, Boer JD, Levi M. The effect of inflammation on coagulation and vice versa. Curr Opin Infect Dis 2011;24:273–8.
25. Laudes IJ, Chu JC, Sikranth S, et al. Anti-c5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. Am J Pathol 2002;160:1867–75.
26. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000; 407(6801):258–64.
27. Danese S, Vetrano S, Zhang L, et al. The protein C pathway in tissue inflammation and injury: pathogenic role and therapeutic implications. Blood 2010;115:112111–30.
28. Dhainaut JF, Yan SB, Joyce DE, et al. Treatment effects of drotrecogin alfa (activated) in patients with severe sepsis with or without overt disseminated intravascular coagulation. J Thromb Haemost 2004;2:1924–33.
29. Taylor FB Jr, Toh CH, Hoots WK, et al. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost 2001;86:1327–30.
30. Bick RL. Disseminated intravascular coagulation: a review of etiology, pathophysiology, diagnosis, and management: guidelines for care. Clin Appl Thromb Hemost 2002;8:1–31.
31. Toh CH, Downey C. Performance and prognostic importance of a new clinical and laboratory scoring system for identifying non-overt disseminated intravascular coagulation. Blood Coagul Fibrinolysis 2005;16:69–74.
32. Feldman BF, Madewell BR, O'Neill S. Disseminated intravascular coagulation: antithrombin, plasminogen, and coagulation abnormalities in 41 dogs. J Am Vet Med Assoc 1981;179:151–4.

33. Stokol T, Brooks M, Erb H, et al. Evaluation of kits for the detection of fibrin(ogen) degradation products in dogs. J Vet Intern Med 1999;13:478–84.
34. Bateman SW, Mathews KA, Abrams-Ogg ACG, et al. Diagnosis of disseminated intravascular coagulation in dogs admitted to an intensive care unit. J Am Vet Med Assoc 1999;215:798–804.
35. Peterson JL, Couto CG, Wellman ML. Hemostatic disorders in cats: a retrospective study and review of the literature. J Vet Intern Med 1995;9:298–303.
36. Thomas JS, Green RA. Clotting times and antithrombin III activity in cats with naturally developing diseases: 85 cases (1984–1994). J Am Vet Med Assoc 1998;213:1290–5.
37. Moser KM, Guisan M, Bartimmo EE, et al. In vivo and post mortem dissolution rates of pulmonary emboli and venous thrombi in the dog. Circulation 1973;48:170–8.
38. Estrin MA, Wehausen CE, Jessen CR, et al. Disseminated intravascular coagulation in cats. J Vet Intern Med 2006;20:1334–9.
39. Taylor FB Jr, Wada H, Kinasewitz G. Description of compensated and uncompensated disseminated intravascular coagulation (DIC) responses (non-overt and overt DIC) in baboon models of intravenous and intraperitoneal Escherichia coli sepsis and in the human model of endotoxemia: toward a better definition of DIC. Crit Care Med 2000;28(9 Suppl):S12–9.
40. Levi M, Meijers JC. DIC: which laboratory tests are most useful. Blood Rev 2011;25:33–7.
41. Stokol T, Brooks MB, Erb HN, et al. D-dimer concentrations in healthy dogs and dogs with disseminated intravascular coagulation. Am J Vet Res 2000;61:393–8.
42. Langer F, Spath B, Haubold K, et al. Tissue factor procoagulant activity of plasma microparticles in patients with cancer-associated disseminated intravascular coagulation. Ann Hematol 2008;87:451–7.
43. Kienast J, Juers M, Wiedermann CJ, et al. Treatment effects of high-dose antithrombin without concomitant heparin in patients with severe sepsis with or without disseminated intravascular coagulation. J Thromb Haemost 2006;4:90–7.
44. Wiinberg B, Jensen AL, Rojkjaer R, et al. Prospective pilot study on performance and prognostic value of a new human, ISTH-based scoring system for identifying non-overt disseminated intravascular coagulation in dogs [abstract]. J Vet Intern Med 2006;20:765.
45. Wiinberg B, Jensen AL, Johansson PI, et al. Development of a model based scoring system for diagnosis of canine disseminated intravascular coagulation with independent assessment of sensitivity and specificity. Vet J 2010;185:292–8.
46. Stokol T, Brooks MB, Erb HN. Effect of citrate concentration on coagulation test results in dogs. J Am Vet Med Assoc 2000;217:1672–7.
47. Stokol T, Erb HN, De Wilde L, et al. Evaluation of latex agglutination kits for detection of fibrin(ogen) degradation products and D-dimer in healthy horses and horses with severe colic. Vet Clin Pathol 2005;34:375–82.
48. Luddington RJ. Thrombelastography/thromboelastometry. Clin Lab Haematol 2005;27:81–90.
49. McMichael MA, Smith SA. Viscoelastic coagulation testing: technology, applications, and limitations. Vet Clin Pathol 2011;40:140–53.
50. Kol A, Borjesson DL. Application of thrombelastography/thromboelastometry to veterinary medicine. Vet Clin Pathol 2010;39:405–16.
51. Wiinberg B, Jensen AL, Rojkjaer R, et al. Validation of human recombinant tissue factor-activated thromboelastography on citrated whole blood from clinically healthy dogs. Vet Clin Pathol 2005;34:389–93.

52. Marschner CB, Bjornvad CR, Kristensen AT, et al. Thromboelastography results on citrated whole blood from clinically healthy cats depend on modes of activation. Acta Vet Scand 2010;52:38.
53. Smith SA, McMichael M, Galligan A, et al. Clot formation in canine whole blood as measured by rotational thromboelastometry is influenced by sample handling and coagulation activator. Blood Coagul Fibrinolysis 2010;21:692–702.
54. Wiinberg B, Jensen AL, Rozanski E, et al. Tissue factor activated thromboelastography correlates to clinical signs of bleeding in dogs. Vet J 2009;179:121–9.
55. Wiinberg B, Jensen AL, Johansson PI, et al. Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. J Vet Intern Med 2008;22:357–65.
56. Sinnott VB, Otto CM. Use of thromboelastography in dogs with immune-mediated hemolytic anemia: 39 cases (2000–2008). J Vet Emerg Crit Care (San Antonio 2009;19:484–8.
57. Fenty RK, Delaforcade AM, Shaw SE, et al. Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia by means of thromboelastography. J Am Vet Med Assoc 2011;238:463–7.
58. Donahue SM, Otto CM. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. J Vet Emerg Crit Care 2005;15:9–16.
59. Wagg CR, Boysen SR, Bedard C. Thrombelastography in dogs admitted to an intensive care unit. Vet Clin Pathol 2009;38:453–61.
60. Vilar Saavedra P, Lara Garcia A, Zaldivar Lopez S, et al. Hemostatic abnormalities in dogs with carcinoma: a thromboelastographic characterization of hypercoagulability. Vet J 2011.
61. Machlus KR, Cardenas JC, Church FC, et al. Causal relationship between hyperfibrinogenemia, thrombosis, and resistance to thrombolysis in mice. Blood 2011;117: 4953–63.
62. Hwang SM, Kim JE, Han KS, et al. Thrombomodulin phenotype of a distinct monocyte subtype is an independent prognostic marker for disseminated intravascular coagulation. Crit Care 2011;15:R113.
63. Pawlinski R, Mackman N. Cellular sources of tissue factor in endotoxemia and sepsis. Thromb Res 2010;125(Suppl 1):S70–3.
64. Stokol T, Daddona J, Mubayed L, et al. Tissue factor expression in canine tumor cell lines. Am J Vet Res 2011;72:1097–106.
65. Grozovsky R, Hoffmeister KM, Falet H. Novel clearance mechanisms of platelets. Curr Opin Hematol 2010;17:585–9.