Defects in Coagulation Encountered in Small Animal Critical Care

Benjamin M. Brainard, VMD[a,*],
Andrew J. Brown, MA VetMB, MRCVS[b]

KEYWORDS

• DIC • Disseminated intravascular coagulation • Antithrombin
• Plasma • Heparin

Patients with systemic inflammatory response may develop hemostatic abnormalities, ranging from subtle and subclinical activation of coagulation to fulminant disseminated intravascular coagulation (DIC). Inflammation in these patients may be secondary to infection, trauma, pancreatitis, immune-mediated disease, or neoplasia, among other pathologies. In addition, metabolic abnormalities in the critically ill patient can result in altered hemostasis.

INFLAMMATION AND COAGULATION

Inflammation is an appropriate host response to infection or tissue damage. Activation of coagulation and intravascular thrombosis occurs in concert with inflammatory responses and acts to prevent spread of microorganisms into the systemic circulation, limit bleeding, and promote tissue repair.[1] Infectious and noninfectious insults, such as trauma, pancreatitis, immune-mediated disease, or neoplasia, can result in the systemic inflammatory response syndrome (SIRS), when the appropriate localized inflammatory response becomes a generalized reaction. Severe systemic inflammatory cytokine release, activation of leukocytes and endothelial cells, and decreased tissue oxygen delivery can eventually result in multiple organ dysfunction syndrome (MODS) and ultimately organ failure and death. As the local inflammatory response becomes a systemic response, activation of coagulation occurs, resulting in extensive formation, and subsequent fibrinolysis, of microthrombi. This widespread activation of the hemostatic system is termed disseminated intravascular coagulation. Just as SIRS has a spectrum from mild to severe inflammation, DIC can be present on a scale from

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[a] Department of Small Animal Medicine and Surgery, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602, USA
[b] VetsNow Referral Hospital, 123-145 North Street, Glasgow, G3 7DA Scotland, UK
* Corresponding author.
E-mail address: brainard@uga.edu

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mild to severe intravascular coagulation. In its mildest form, DIC may be limited to mild intravascular thrombosis detected only by hemostatic markers. However, marked activation of coagulation and subsequent fibrinolysis can result in the development of fulminant DIC characterized by widespread microvascular thrombosis and profuse bleeding, a consumptive thrombohemorrhagic syndrome.[2] DIC has traditionally been considered a consequence of SIRS,[3–6] and, when present, is a strong predictor of the development of MODS and mortality.[7] However, there is now evidence that a bidirectional interaction between SIRS and DIC exists, which together play a significant role in the development of microvascular thrombosis, MODS, and poor prognosis in critically ill patients.[8]

INTRAVASCULAR THROMBOSIS AND COAGULOPATHY

Intravascular thrombosis is a result of activation of coagulation, inhibition of anticoagulation, and depression of fibrinolysis. A fine balance normally exists between hemostatic and fibrinolytic pathways. This delicate balance is altered by tissue injury and inflammation (both infectious and sterile). Initiation of inflammation-induced coagulation occurs by tissue factor (TF)-mediated thrombin generation.[9] Blood comes into contact with TF when blood vessel wall integrity is lost or activated endothelial cells and monocytes/macrophages express TF. Activation of platelets also results in the release of the alpha and dense granules from the platelet cytoplasm. Substances released from platelets serve to promote coagulation (TF, factors Va and VIIIa), alter vascular tone (serotonin), and activate or recruit additional platelets (ADP, P-selectin). In addition, a membrane shape change occurs and the platelets express the active form of the fibrinogen receptor (GPIIb/IIIa). Platelet activation may also contribute to TF expression, and the platelet membrane plays an important role in supporting the initiation of coagulation.[10] Following trauma, tissue injury leads to TF exposure and thrombin generation. In addition, exposed subendothelial collagen may result in platelet activation. In sepsis, initial expression of TF is mediated by proinflammatory cytokines, namely interleukin (IL)-6, tumor necrosis factor (TNF)-alpha, and IL-1 beta.[11–13] The TF-VIIa complex can also stimulate production of additional proinflammatory cytokines by upregulation of nF-kB.[14] TF is shuttled between endothelial and polymorphonuclear cells through microparticles (small, membrane-derived vesicles) released from activated mononuclear cells.[15] TF-bearing microparticles may contribute to the systemic activation of coagulation; both endothelial cells and platelets may also release microparticles into the general circulation.[16]

The endogenous anticoagulant pathways, antithrombin (AT), protein C/protein S system, and tissue-factor pathway inhibitor (TFPI) closely regulate procoagulant pathways and are all impaired during inflammation-induced coagulation. AT is the primary inhibitor of thrombin and factor Xa, and AT levels are markedly decreased during severe inflammation because of impaired synthesis, neutrophil-mediated degradation, and consumption secondary to ongoing thrombin generation.[17] Activated protein C (aPC) in concert with protein S degrades cofactors Va and VIIIa, which are essential cofactors for the intrinsic and common pathways. Similar to AT, plasma levels of zymogen protein C are decreased during severe inflammation.[18,19] Zymogen protein C is activated by thrombomodulin, which is in turn activated by thrombin. Although this balances the procoagulant response to mild inflammation, severe inflammation results in downregulation of the endothelial thrombomodulin-protein C receptor pathway[20] via activation of endothelial nF-kB.[21] Under normal conditions, the endothelial protein C receptor (EPCR) accelerates the activation of protein C (PC) and amplifies the anticoagulant and antiinflammatory effects of aPC.[22] EPCR expression is downregulated in sepsis, further

reducing the effects of the protein C system. The final endogenous anticoagulant system that is affected by inflammation is TFPI, which is released from endothelial cells in response to inflammation or damage. TFPI forms a quaternary complex with factors Xa and VIIa to inhibit coagulation induced by TF.[23] Levels of TFPI in patients with sepsis are initially low, and rabbits that are depleted of TFPI are prone to intravascular coagulation, although a major multicenter trial studying the infusion of recombinant human TFPI failed to show an effect on the mortality of a group of patients with septic shock.[24]

Plasminogen activators are released into the circulation from vascular endothelial cells in response to clot formation and inflammatory mediators, such as TNF-alpha and IL-1 beta.[25] Plasminogen is converted to plasmin by specific activators, such as tissue plasminogen activator (tPA). Plasmin is then able to break down the fibrin strands that create a thrombus. The fibrinolytic action of plasminogen is balanced by a natural inhibitor, plasminogen activator inhibitor type 1 (PAI-1), which is released by endothelial cells and prevents activation of plasmin. Plasmin may be directly inactivated by circulating alpha 2-antiplasmin. In severe inflammation, there is a delayed yet sustained increase in PAI-1that slows fibrinolysis and contributes to persistence of microvascular thrombi and thromboemboli.[26]

DIC can manifest as both diffuse intravascular thrombosis leading to organ dysfunction or a hemorrhagic phenotype characterized by excessive bleeding, which is one reason for the preference of the term "consumptive thrombohemorrhagic disorder" rather than DIC.[12] The consumptive phase of DIC uses platelets and coagulation factors (consumptive coagulopathy) to produce microthrombosis and occasionally macrothrombosis, and is associated with tPA-induced fibrino/fibrinogenolysis.[27] When platelets and hemostatic factors reach critically low levels caused by consumption, bleeding ensues.

TRAUMA AND HEMOSTASIS

Hemostatic abnormalities are common in critically ill patients following trauma, resulting in either a hypercoagulable or hypocoagulable state. Following trauma-induced tissue damage and exposure of the subendothelial layer of blood vessels, blood is exposed to TF, collagen, and von Willebrand factor (vWF). Clotting is rapidly initiated and amplified via the TF pathway following platelet activation and aggregation. The coagulation cascade is therefore an integral part of limiting hemorrhage in the trauma patient. However, the resultant hypercoagulable state after injury is thought to play a major role in the development of multiple organ dysfunction syndrome, primarily caused by microthrombosis and disruption of blood flow in end organs. MODS is the leading cause of death in people after the initial 48 hours following trauma.[28,29] MODS and DIC have also been documented in dogs secondary to trauma, and have been shown to be significantly associated with nonsurvival.[30]

Unlike DIC resulting from inflammatory conditions, the DIC-like syndrome following trauma is likely less associated with inflammation and upregulation of cellular receptors and more related to the activation of coagulation from widespread endothelial disruption. The hypovolemia and hypoperfusion that may accompany trauma also impair clearance of thrombin, allowing increased formation of thrombin-thrombomodulin complexes.[31] Subsequent activation of protein C and upregulation (decreased inhibition) of fibrinolysis can lead to a DIC-like syndrome.[31]

Subsequent to this early hypercoagulable state, blood loss, factor consumption, and fluid resuscitation may promote a systemic hypocoagulable state. The coagulopathy is initiated by consumption of factors and platelets following hemorrhage. The administration of crystalloids and colloids for resuscitation will result in a dilutional

coagulopathy, and colloids will also impede the interaction of factor VIII and vWF.[32] Shock and subsequent acidosis may alter coagulation protease function and may contribute to hemorrhagic diatheses. Trauma patients are commonly hypothermic when presented to the hospital, and this can worsen a coagulopathy. A lethal triad of coagulopathy, hypothermia, and acidemia is well described.[31] Resuscitation with refrigerated blood products or room-temperature fluids may further worsen patient hypothermia, and crystalloids with a high concentration of chloride can worsen the acidosis.

DEFECTS IN HEMOSTASIS SECONDARY TO METABOLIC DISEASE

In addition to the activation and consumption of platelets and coagulation factors, animals with severe organ dysfunction may experience impaired platelet activity, which can promote bleeding and complicate therapy. Moderate to severe uremia and renal disease can result in impaired platelet secretion of ADP, serotonin, and production of thromboxane A_2 (TXA_2), as well as changes in cytoplasmic calcium dynamics.[33] Impaired expression of fibrinogen receptors and von Willebrand activity may decrease the ability of platelets to adhere to sites of injury, especially under high shear conditions.[33] Studies in dogs have documented alterations in ex vivo platelet aggregation in the presence of uremia.[34] Decreased platelet function can complicate therapies that require additional anticoagulation, such as hemodialysis. In human patients with severe acute hepatitis and encephalopathy, abnormalities of platelet function have been noted, and a study of platelet aggregation in a group of dogs with various types of liver disease showed decreased whole blood platelet aggregation responses to collagen and arachidonic acid in some dogs.[35,36] Patients with chronic liver disease may exhibit decreased platelet TXA_2 production.[36] The bone marrow of patients with various types of hematopoietic diseases may produce abnormal platelets, with incomplete or abnormal granule contents (generally referred to as acquired storage pool disease [SPD]). SPD has been described in human patients with autoimmune disease, DIC, antiplatelet antibodies, and hemangioma.[37] Although the clinical implications of some of these abnormalities in platelet function are unclear in companion animals, platelet dysfunction may compound any coagulopathy and may complicate invasive diagnostic or therapeutic procedures.

DIAGNOSIS

Although DIC frequently occurs secondary to severe sepsis, polytrauma, and other inflammatory conditions, no single clinical sign or laboratory test has been identified that possesses sufficient accuracy to confirm or reject a diagnosis.[12,38] Because it is difficult to accurately identify animals with DIC, it is important to critically assess research evaluating animals with DIC for the specific criteria used to diagnose DIC. In veterinary medicine, DIC is commonly diagnosed based upon abnormalities in at least 3 of the following hemostatic parameters: activated partial thromboplastin time (aPTT), prothrombin time PT, fibrinogen, D-dimer (DD), platelet concentration, and erythrocyte morphology, together with evidence of a predisposing condition.[39–41] Although sensitive, this is a nonspecific approach.[39–41] Because no gold standard exists in the diagnosis of DIC in either human or veterinary medicine, expert evaluation of an extended hemostatic panel has been used to increase sensitivity and specificity of diagnosis in the research setting.[40,42] The subcommittee on DIC of the Scientific and Standardization of the International Society of Thrombosis and Haemostasis has proposed a scoring system in people, based on a combination of commonly measured hemostatic parameters.[43] This scoring system was prospectively validated and was deemed

sufficiently accurate to make or reject a diagnosis of DIC in intensive care patients with a clinical suspicion of DIC.[42] In addition, there was a strong correlation between DIC score and 28-day mortality. This scoring system was recently used as a template to develop a model-based scoring system for diagnosis of canine DIC.[39] The model included values for the PT, aPTT, fibrinogen, and DD concentrations, and was prospectively evaluated, with a reported sensitivity of 83.3% and specificity of 77.3% (based upon expert evaluation and diagnosis of DIC as the gold standard). In addition to those data that were included in the final model, this study evaluated platelet count, antithrombin, proteins C and S, alpha-1 antiplasmin, and plasminogen concentrations.[39]

PLATELET COUNT

Thrombocytopenia can occur secondary to increased consumption, destruction, dilution, sequestration, and decreased production. Dogs may develop thrombocytopenia secondary to sepsis, trauma, and immune-mediated hemolytic anemia (IMHA). Thrombocytopenia may also occur secondary to immune-mediated thrombocytopenia, but is beyond the scope of this review. Thrombocytopenia from DIC or other consumptive causes frequently results in a platelet count between 40 and 100 $\times$ 10^9 L^{-1}, whereas immune-mediated destruction of platelets usually results in a platelet count less than 20 $\times$ 10^9 L^{-1}. The true incidence of thrombocytopenia is unknown and in part depends on the definition. Although individual laboratories have reference intervals, it has been suggested that a threshold of 100 $\times$ 10^9 L^{-1} be termed thrombocytopenia in critically ill people[44,45] because of the high incidence and lack of significant bleeding in these patients. In critically ill people, platelet concentrations less than 100 $\times$ 10^9 L^{-1} are associated with a 10-fold increased risk of bleeding than a concentration between 100 and 150 $\times$ 10^9 L^{-1}.[46] Surgical bleeding is uncommon if platelet concentration is greater than 50 $\times$ 10^9 L^{-1} and data from human patients with cancer suggest that the risk of spontaneous bleeding does not increase until the concentration is less than 20 $\times$ 10^9 L^{-1}.[47,48] Platelet count may be estimated using many automated complete blood count machines, but it is always indicated to review a blood smear, especially in patients with severe inflammatory disease, as platelet clumping or changes in mean platelet volume can result in erroneous values. This factor is especially true in cats. When reviewing a blood smear, each platelet seen on a high power field (100X) represents approximately 15 $\times$ 10^9 L^{-1} circulating platelets.

Independent of the risk of bleeding, thrombocytopenia serves as a marker of morbidity and mortality, likely related to the severity of the underlying condition. Severity of thrombocytopenia is inversely correlated to survival in critically ill people, and sustained thrombocytopenia over 4 days is associated with a 4- to 6-fold increase in mortality.[46,49]

PROTHROMBIN AND ACTIVATED PARTIAL THROMBOPLASTIN TIME

The prothrombin time monitors the tissue factor (extrinsic) pathway and common portions of the coagulation cascade. Tissue factor and calcium are added to plasma, activating factor VII and in turn, factors X, V, and II (prothrombin). Fibrin formation from fibrinogen is the end point of the assay, measured using either optical or mechanical means, depending on the methodology. Activated partial thromboplastin time is measured in citrated plasma by adding thromboplastin or a similar source of lipoprotein, with calcium and other activators, again allowing coagulation to proceed to fibrin formation, in this case by the subsequent activation of the factors in the intrinsic pathway (factors XII, XI, IX, VIII, X, V, and II). Final fibrin formation is again monitored by optical or mechanical means. In one study, dogs with sepsis had significantly higher PT and

aPTT values than controls.[50] It appears in many animals with DIC that the aPTT is the first clotting time to become prolonged, and animals with early DIC may only display a moderate prolongation of aPTT and thrombocytopenia. This combination should alert the astute clinician to monitor patients closely for progression of the syndrome.

VISCOELASTIC COAGULATION MONITORING

Viscoelastic coagulation monitors, such as thromboelastography (TEG, Haemoscope/Haemonetics, Niles, IL, USA) or Sonoclot (Sienco Inc, Arveda, CO, USA), assess the viscoelastic changes in whole blood during clot formation and provide a global assessment of hemostatic capability, because whole blood analysis integrates both cellular and plasmatic contributions to coagulation. TEG has recently been used to identify prothrombotic states in dogs with IMHA, neoplasia, and DIC.[40,51,52] Unlike PT and aPTT, these machines can identify both hypercoagulable and hypocoagulable states.[53] One recent study evaluating TEG in dogs with DIC demonstrated a greater fatality rate in hypocoagulable dogs than those that were hypercoagulable.[40] However, because TEG assesses whole blood, it is potentially affected by all constituent components of blood, including platelet concentration and hematocrit.[54,55] It also may be affected by contact (intrinsic) pathway activation resulting from differences in sample collection method and quality of venipuncture.[56,57] As such, these factors should be considered during interpretation of TEG and when developing reference intervals. Although the TEG is generally run at a test temperature of 37°C, the temperature of the blood sample during the rest period does not appear to result in clinically relevant differences in contact activation or TEG parameters.[58]

TEG has potential for documenting a prothrombotic state in early DIC and may therefore identify those patients that require thromboprophylaxis before the development of overt DIC. In addition, viscoelastic coagulation testing has the potential to be used to monitor patient response to therapy, allowing treatments to be tailored accordingly. The addition of specific inhibitors of platelet function or contact activation also have promise for more specific diagnosis of the components of hypercoagulable or hypocoagulable states.[59,60]

MICROPARTICLES

Microparticles (MPs) may be released from cellular sources during inflammatory conditions and can circulate systemically. Circulating MPs may be detected by the use of flow cytometry.[61,62] They have also been identified using plate adherence techniques, as well as electron microscopy.[63] Because the MPs may be of varied cellular origin (platelet, endothelial cell, monocyte), it is important, in addition to evaluating these particles for expression of markers, such as CD62P (P-selectin) and TF, to include specific markers to determine the cellular origin (eg, CD41/61 for platelet MPs and CD104 for endothelial MPs).[62] Although elevated levels of circulating MPs have been identified in human patients with trauma, neoplasia, and DIC,[64,65] the prognostic significance of this elevation is not yet clear; in some cases, elevations of MPs may indicate an appropriate response to inflammation and the potential for successful resolution of the disease.[66]

PLATELET FUNCTION ASSAYS

Platelet function defects can be difficult to evaluate in the context of some whole blood coagulation testing (eg, TEG), and may require more specific diagnostics.[67] Optical aggregometry (OA) uses a spectrophotometric technique to evaluate platelets in

platelet rich plasma (PRP) for responses to various agonists and is considered the gold standard for assessment of platelet function.[68] Whole blood (impedance) aggregometry (WBA) uses an electrical probe and measures the resistance caused by platelets as they adhere to the probe after exposure to agonists, the impedance of the circuit being directly related to the degree of aggregation.[69] WBA is convenient and requires a smaller blood volume than OA, but may not be as accurate. With platelet counts less than 100 to 150 $\times 10^9\,L^{-1}$, however, the accuracy of both techniques is decreased; ideal analyses use PRP platelet counts around 300 $\times 10^9\,L^{-1}$. The platelet function analyzer (PFA-100 [Siemens Healthcare Diagnostics, Deerfield, IL, USA]) is a system that aspirates citrated whole blood through a small aperture primed with agonists for platelet aggregation (eg, collagen and ADP). The machine measures the time (up to 300 seconds) that it takes for a platelet plug to occlude the aperture (closure time).[70] The PFA-100 has been validated for use in small animal patients, and can be useful for diagnosing disorders of primary hemostasis, such as von Willebrand disease.[71,72] The accuracy of this machine is also decreased in patients with low platelet counts.[73] Other machines designed to evaluate platelet function (eg, the Impact system [Matis Medical, Beersel, Belgium]) are pending further assessment for utility in analysis of platelet function in veterinary patients. Activated platelets have been identified in many species using flow cytometry,[74–76] and as this modality becomes more available to veterinarians, may add additional diagnostic information to the state of circulating platelets in patients with DIC. Flow cytometry is not affected by patients' total platelet count, but analyses may take longer in patients who are thrombocytopenic.

ANTITHROMBIN AND PROTEIN C ACTIVITY

Antithrombin and PC are typically measured by functional activity assays compared with reference plasma. As important physiologic inhibitors of hemostasis, a decreased activity indicates a prothrombotic state. Low AT and PC activity have been identified in dogs with sepsis, IMHA and DIC, and low activity has been associated with an increased mortality risk.[40,41,50,77–80] Studies in people have suggested that serial measurements of AT and PC have prognostic utility.[7,81] A preliminary study in septic dogs was consistent with this; both PC and AT activities changed significantly over time and were associated with outcome.[77]

ASSESSMENT OF FIBRINOLYSIS

Fibrin degradation products (FDP) are the breakdown products of both fibrin and fibrinogen generated by the enzymatic action of plasmin, whereas DD result only from degradation of fibrin that is part of an intact clot (ie, has been cross-linked).[82] FDP assays lack specificity and are also insensitive for identifying thromboembolic disease[83]; as such, measuring FDP concentration is not useful in critically ill patients. Studies have demonstrated an increased DD concentration in dogs with clinical diagnoses of sepsis, IMHA, DIC, and thromboembolic disease.[50,79,83,84] The greatest utility for the DD assay is likely as an adjunct test to rule out thromboembolic disease. As DD concentrations are used primarily for negative predictive value, it is important that an ultrasensitive assay be used to avoid false-negative results.

THERAPY FOR DIC

Because DIC is a multifactorial syndrome, therapy is primarily supportive and directed toward resolution of the inciting cause. Supportive therapy should restore and

maintain tissue oxygen delivery, and consideration of the pathophysiology of DIC may define additional options for therapeutic intervention.

The first aspect of therapy is treatment of the primary underlying condition; this can include surgical (eg, to drain an abscess or debride tissue) or medical (eg, antibiotics to treat sepsis) approaches. The inflammation that drives DIC may continue even after appropriate therapy is initiated. Another important part of therapy is to treat shock and to maximize oxygen delivery to the tissues. Tissue hypoxia may promote additional inflammation that can worsen SIRS and lead to MODS.

Resuscitation from shock associated with trauma should include the use of both intravenous crystalloid and colloids, in addition to the transfusion of red blood cells to preserve oxygen carrying capacity. The addition of fresh frozen plasma (FFP) to this regime may help to reverse some of the early aberrations and ameliorate the developing coagulopathy.[31] This point is especially true when treating the coagulopathy associated with trauma. Crystalloid fluids low in chloride (eg, Normosol-R or lactated Ringer's solution) should be chosen to minimize the acidosis associated with iatrogenic hyperchloremia (that may result from the use of 0.9% sodium chloride). Recent studies of humans with trauma have advocated a 1:1 or 1:2 ratio of FFP to packed red blood cells (pRBC) in patients receiving massive transfusions, although the benefit of this concept for different patient populations and those not requiring massive transfusions remains to be determined.[85]

FRESH FROZEN PLASMA

Fresh frozen plasma administration is indicated in patients with coagulopathy caused by factor deficiency, especially if hemorrhage is ongoing. Patients with significant bleeding and thrombocytopenia may benefit from a transfusion of fresh whole blood or platelet-rich plasma. A recent study evaluating the administration of a single transfusion of FFP (median 16.5 mL/kg, range 4–30 mL/kg) to dogs with pancreatitis did not demonstrate a beneficial effect from the administration of FFP.[86] In this study, only 2 dogs of 77 had evidence of coagulopathy, and 17/38 met criteria for SIRS. Platelet counts were not reported, so it cannot be determined if a subset of the studied population was experiencing DIC (although given the low incidence of coagulopathy, only 2 patients might have met the criteria for DIC). The retrospective nature of this study and the small numbers merit additional prospective studies, as well as studies of patients with other inflammatory states that can lead to DIC. It is important to note the results of a 1991 study in humans with severe acute pancreatitis, however, where high-dose FFP (8 units daily for 3 days) did not result in a difference in mortality, although this regime did result in significant improvements (ie, maintenance in the normal range) in plasma antithrombin, and α2-macroglobulin.[87] The ability of this protocol to maintain levels of antiinflammatory proteins may be applicable to other inflammatory and consumptive diseases.

Few guidelines exist in the veterinary literature for dosages for FFP transfusion.[88,89] An initial dose of 6 to 10 mL/kg is indicated for correction of coagulopathy, but hemostatic parameters should be reevaluated after therapy, and additional FFP administered if necessary. In a 3-month survey of FFP transfusions in a veterinary teaching hospital , canine patients received an average FFP dose of 9 mL/kg (ranging from 2–30 mL/kg).[90] These dogs received FFP for indications ranging from coagulopathy to replacement of plasma proteins (eg, α-macroglobulin, albumin). Although 50% of the dogs in this study received a single FFP transfusion, 46% received a FFP transfusion either twice or 3 times daily. Patient outcomes were not reported in this study. A more recent article evaluating FFP transfusion in dogs noted median transfusion

volumes of 15 to 18 mL/kg.[91] This article demonstrated a significant shortening in patient PT and aPTT times in patients who were coagulopathic following plasma transfusion, although it was not able to determine whether these patients had evidence of hemorrhage or just prolonged clotting times. In a small cohort of cats (n = 46) with DIC, therapy included transfusion of a single unit (volume unspecified) of FFP in 21 cats (46%). Survival statistics were not different between cats who received FFP and those who did not.[92]

In patients with an ongoing consumptive coagulopathy, frequent redosing of FFP may help to replace coagulation factors and anticoagulant proteins. Some authors have advocated anticoagulation before FFP administration in human patients with DIC, although the implications for veterinary medicine are unclear.[93] In veterinary patients with severe inflammatory conditions and DIC, some clinicians advocate a dosing regimen of 10 mL/kg of FFP up to 3 times daily, if clinical and laboratory signs warrant. Extrapolation from human data indicates that this large amount of FFP may not be necessary, and suggests that FFP should be used only when clinically indicated by coagulopathy accompanied by hemorrhage (this is especially true in patients with sepsis). A retrospective human study has questioned even the utility of this practice, noting that patients who received FFP transfusion had a significantly higher incidence of acute lung injury, although other outcome measures were not different between groups.[94] Prospective studies of FFP use specifically in veterinary patients with inflammatory disease are indicated to better determine appropriate dosing regimens for these patients.

Although plasma or other blood product transfusions may benefit veterinary patients with DIC, the complications associated with plasma transfusions should be kept in mind. Although transfusion reactions are not frequently reported in recent veterinary studies,[86,90] all transfusions must be monitored closely. It seems logical that type-specific FFP should be administered when available, although this has not been specifically studied. Transfusion reactions can be mild, such as pruritus, facial swelling, or rash,[95] or more severe, such as fever,[96] anaphylaxis, or death. In other studies, the transfusion of perioperative blood products (FFP and pRBC) occurred more frequently in animals that developed postoperative pulmonary complications.[97] This finding raises the possibility of transfusion-related acute lung injury (TRALI), which is an acute respiratory distress syndrome (ARDS)-like event that occurs during or within 6 hours of a transfusion.[98] TRALI in humans has been associated with transfusion of both pRBCs and FFP, but has not been definitively described in the veterinary literature to date.

ANTITHROMBIN

Experimental models have suggested that the administration of antithrombin concentrate may attenuate the clinical course of DIC. AT, in combination with endogenous or exogenous heparinlike substances, promotes anticoagulation by inactivation of (primarily) factors IIa and Xa. Because thrombin (IIa) is a potent stimulus for additional coagulation, inactivation may help quell additional clot formation. In addition, AT is a potent antiinflammatory molecule, contributing to endothelial cell prostacyclin production,[99] as well as decreasing margination and leukocyte-endothelial cell interaction.[100] Prostacyclin production also inhibits platelet activation, resulting in less release of procoagulant and proinflammatory (eg, IL-1) factors.[99]

Dogs with inflammatory states and DIC have decreased AT activities, which may continue to decrease with continued inflammation and activation of coagulation.[40,77,101,102] Extensive studies have not been performed in cats with

inflammatory disease,[103–105] and AT levels in cats with cardiomyopathy were in the normal range in one study and increased in another.[106,107] Despite the fact that low AT levels are associated with a poor prognosis in both dogs[40] and humans[108] with DIC, a large human study failed to show a survival benefit of AT administration, except in a subgroup that did not receive concomitant heparin.[109] In fact, the use of heparin appears to negate the benefits of AT administration and may result in an increase in bleeding complications.[110] Subsequent studies evaluating the use of AT concentrate in patients with DIC caused by sepsis or burns, while avoiding concomitant heparin therapy, have been more promising.[111,112] The binding of AT to exogenous heparin likely inhibits AT binding to endothelial cell glycosaminoglycans,[113,114] resulting in a mitigation of the antiinflammatory effects of AT.[115]

There are no current recommendations on the use of AT concentrate in small animal patients. In humans, AT concentrate is dosed to elevate AT levels to 120%.[116] Doses are calculated based on an activity elevation of 1.4% per U/kg of AT. Human FFP contains about 1 unit of AT activity per milliliter, so 10 mL/kg of FFP would be expected to provide an elevation of 14% in AT activity.[116] One study in dogs with IMHA evaluated the change in AT activity after transfusion of a single 10 mL/kg dose of FFP. In this study, there were no significant changes in AT activity after this transfusion. There was also a variable AT activity in the transfused plasma, ranging from 55% to 96%.[117] These dogs were also receiving unfractionated heparin. Similar results on AT levels were found after transfusion of 15 mL/kg of FFP in dogs with IMHA.[118] A single study that evaluated the use of human AT concentrate (administered over 90 minutes at a dose of 1 U/mL of calculated circulating canine plasma; presumably 50–60 U/kg) in dogs with experimental DIC showed less glomerular fibrin deposition and a blunted rise in FDPs compared with control dogs.[119] Experimental studies using feline AT concentrate in cats showed a decrease in thrombin-induced neutrophil rolling after ischemia and reperfusion injury, but not during lipopolysaccharide challenge.[100,120] Because human and canine and feline AT are different, the possibility exists that infusion of human AT concentrate into veterinary species could result in the formation of antihuman AT antibodies or in hypersensitivity reactions.

ACTIVATED PROTEIN C

Human studies have evaluated the administration of activated protein C to patients with severe sepsis. Under normal circumstances, thrombin bound to thrombomodulin activates PC, which, with its cofactor protein S, acts to inactivate factors Va and VIIIa. When infused intravenously (IV), aPC binds thrombomodulin and acts as a potent anticoagulant protein. Activation of the endothelial protein C receptor modulates cytokine release by interfering with NF-κB translocation, resulting in a decreased expression of cytokines by endothelial cells and interference with thrombin binding to PAR-1 receptors.[99] aPC also has a binding site on monocytes and may decrease production of proinflammatory mediators.[121] A landmark human study (PROWESS) published in 2001 showed promise that aPC could reduce mortality and organ dysfunction in patients suffering from severe sepsis.[122,123] Of note, the subgroup of patients with DIC in this study experienced a greater relative benefit from the infusion of aPC.[124]

Despite the findings of the PROWESS trial, the use of aPC in human patients with severe sepsis and DIC remains controversial. Subsequent studies and meta-analyses, specifically in patients with sepsis, have failed to provide compelling evidence for its use.[125] There is only one published study of the use of aPC in dogs, which infused 1 and 2 mg/kg/h of aPC IV for 2 hours and demonstrated a dose-dependent prolongation of aPTT (2.0- and 3.7-fold prolongation, respectively) without

significant effect on platelet function or thrombin clot time.[126] These effects were gone by 60 minutes after cessation of the infusion. In dogs, however, aPC is antigenic and may result in anaphylaxis or in development of anti-aPC antibodies, which may predispose treated animals to thrombosis.[127] The required dose in dogs is also approximately 20-fold greater than humans to achieve the same anticoagulant effects.[126] These properties have hampered further investigation of aPC in dogs.

FFP contains PC and is the only available source of natural anticoagulant compounds for veterinary patients. Because of the extremely short half-life of aPC, there is unlikely to be any aPC contained in an FFP transfusion. FFP also contains molecules, such as protein C inhibitor, α2-macroglobulin, and alpha 1-antitrypsin, that can scavenge aPC.

PLATELET TRANSFUSION

Platelet transfusions are generally not considered in human patients with DIC without active hemorrhage until the platelet count drops lower than 20,000 platelets/μL.[128,129] The cut-off number for transfusion is 50,000 platelets/μL in patients with ongoing hemorrhage, or in at-risk patients who must undergo invasive procedures.[128] Transfusions are generally of fresh platelet concentrates. There is no evidence in humans to show that transfusion to a platelet count higher than 50,000 platelets/μL has additional benefits. These guidelines seem reasonable for companion animals. There are several platelet products available for use in dogs, but many have not been extensively studied, even in healthy dogs.[130] Platelets may be transfused to dogs and cats via fresh whole blood transfusions if the blood is kept at room temperature and given within 4 hours of collection. Fresh whole blood may also be used to prepare PRP if the red blood cells are not required. Fresh canine platelet concentrates prepared by plateletpheresis may be available from some animal blood banks, and platelet concentrates are also available as a frozen dimethyl sulfoxide-stabilized product.[130] The recent availability of lyophilized canine platelets may be another treatment option for dogs who are experiencing hemorrhage secondary to thrombocytopenia.[130]

HEPARIN

The use of unfractionated heparin (UFH) in human patients with DIC is controversial. Although intuitively logical for slowing the consumptive aspects of DIC and minimizing the formation of microthrombi, the heparin molecule may also mitigate some of the antiinflammatory effects of endogenous compounds, such as AT. If the initial hypercoagulable phase of DIC could be reliably identified, heparin administration might be indicated to decrease thrombin production at that point. Heparin exerts an anticoagulant effect primarily by binding to AT, resulting in a 1000-fold increase in activity of the complex to inactivate coagulation factors Xa and IIa (among others).[131] Heparin binding to AT may interfere with the antiinflammatory effects of AT. For this reason, heparin is not indicated in patients with pending or actual DIC. A study in dogs showed that administration of heparin to healthy dogs caused a decrease in AT activity, presumably caused by increased participation in neutralizing procoagulant proteins.[118]

The use of heparin in human patients with DIC is indicated in those with overt thromboembolic disease (macrovascular thrombosis) and those at risk of extensive fibrin formation that could result in end-organ dysfunction (eg, renal failure from glomerular fibrin plugging). In addition, the presence of dermal or acral necrosis is a strong indication for heparin therapy.[132] Because heparin works in concert with AT, the activity may be diminished in patients with low AT activity. There has been no evidence to

suggest that preincubation of FFP with plasma results in an increased heparin effect. Patients with thrombocytopenia or low levels of fibrinogen or those being treated concurrently with antiplatelet or other anticoagulant medications (eg, clopidogrel) may be at increased risk of bleeding with heparin therapy.[132]

Although the proinflammatory effects of unfractionated heparin are described, less information is available about whether the same can be expected of low molecular weight heparins (LMWH). There are few studies of LMWH as adjunctive therapy for DIC. One human study showed that dalteparin administration was more effective than unfractionated heparin at mitigating the thrombocytopenia and increase in FDP concentration associated with the development DIC in clinically ill humans.[133] Because LMWH also interacts with AT, similar proinflammatory effects may be seen, and the circulating AT levels will decrease over time with continued treatment at therapeutic levels. At least one study using dalteparin in rats showed attenuation of the inflammatory changes that occurred secondary to ischemia/reperfusion injury.[134] In this study, dalteparin did not affect endothelial prostacyclin production.

Human guidelines for dosing of heparin are based on anti-Xa activity (aXa) values, and vary depending on whether the drug is administered for prophylactic reasons or to treat a preexisting thrombus. The relationship between aPTT and aXa activity is variable, both with age as well as with laboratory equipment.[135] The recommendation for therapeutic UFH dosing in adults is target aXa levels between 0.35 and 0.7 U/mL. Patients with preexisting thrombi are more likely to benefit from intravenous dosing, rather than subcutaneous (SC) administration, at least when using UFH.[135] Prophylactic doses of UFH are 10% of the therapeutic levels and may be administered subcutaneously.[135] Guidelines for LMWH in both adult and pediatric humans target an aXa value of 0.5 to 1.0 U/mL for therapeutic uses and 0.1 to 0.3 U/mL for prophylaxis.[136] aXa activity is measured 4 to 6 hours after SC dosing.

In patients without an overt risk of hemorrhage, prophylactic doses of UFH or LMWH may be indicated for prevention of venous thromboembolism.[129] A low dose of UFH administered as an IV constant rate infusion (5–10 U/kg/h) for prophylaxis for venous thromboembolism has been advocated in human patients who have a concurrent bleeding risk, such as those with DIC.[129] Despite these recommendations, there are no clinical randomized, controlled trials in human medicine that demonstrate that the use of heparin in patients with DIC improves clinical outcome.[129] No studies have been done in veterinary species using these lower prophylactic doses of heparin.

Heparin has been administered to dogs using a wide variety of dosing regimens and is usually dose adjusted using the aPTT, with a goal of extending the aPTT 1.5 to 2.0 times the mean normal or baseline value.[101] These guidelines are derived from early human recommendations.[135] Just as in human medicine, the relationship of the aPTT to the actual aXa value is not easily predicted, and is likely variable based on the route of administration, individual patient, and clinical laboratory methodology.[137,138] The actual amount of circulating heparin is most accurately monitored by measuring anti-Xa activity levels in plasma,[139] although viscoelastic coagulation monitoring may provide another option if correlations with aXa values can be established.[140,141] In some canine studies, 200 U/kg of UFH administered subcutaneously to beagle dogs resulted in a peak mean aXa activity of 0.56 $\pm$ 0.2 U/mL approximately 4 hours after dosing. Another study using the same UFH dose in mixed-breed dogs achieved a peak mean aXa of 0.1 U/mL (range <0.1–0.5 U/mL) at 3 hours after a single dose. After 3 days of UFH administration to dogs at 200 U/kg subcutaneously every 8 hours, peak median aXa was 0.4 U/mL (range 0.2–0.65 U/mL).[137] A similar range was achieved in 6 healthy dogs given 300 U/kg of UFH subcutaneously every 8 hours for 3 days (0.4–0.6 U/mL, except for one dog who remained at 0.1 U/mL).[141] Studies in

dogs with IMHA have shown that the administration of UFH at a dose of 300 U/kg subcutaneously every 6 hours resulted in therapeutic (ie, >0.35 U/mL) aXa levels in 31% of dogs (5/16) after 14 hours of therapy.[101] In this study and others, a significant correlation between aXa levels and aPTT was noted,[101,142] whereas this has not been the case in others.[137] In animals with acute inflammation, higher doses of UFH may be necessary to allow for nonspecific binding of the heparin molecules.[101] Another study in dogs in an intensive care unit noted hemorrhage in 4 of 6 dogs exposed to a high-dose UFH (900 U/kg/d, IV) protocol, whereas a lower dose (300 U/kg/d, IV) failed to achieve consistent aXa values.[143] Dosage studies in cats have been limited, but a dose of 250 U/kg UFH subcutaneously every 6 hours for 5 days resulted in aXa values in the therapeutic range (0.35–0.7 U/mL) in most cats for the majority of the study.[144] There was also a significant correlation between aPTT times and aXa values in the cats receiving UFH.[144] It is unclear if the target anti-Xa recommendations from human medicine can be transferred directly to veterinary patients, and prospective studies are warranted.

LMWH has become more popular in recent years, however, the optimal dose and drug for use in veterinary species is still undecided. In dogs with thromboplastin-induced DIC, dalteparin given as an IV CRI targeted to achieve aXa concentrations of 0.6 to 0.9 U/mL attenuated the hematologic changes associated with DIC.[145] A recent study of enoxaparin administered to dogs at a dose of 0.8 mg/kg subcutaneously every 6 hours indicated reliable aXa values more than 0.5 U/mL for the 36-hour dosing period.[146] A group of dogs in an intensive care unit setting who received dalteparin (100 U/kg subcutaneously every 12 hours) failed to achieve aXa values greater than 0.5 U/mL.[143] Another study of dalteparin in dogs using 150 U/kg subcutaneously every 8 hours showed a more reliable dose response.[147] Dalteparin given at 100 U/kg subcutaneously every 12 hours to cats also failed to reliably achieve target aXa values.[148] These results are consistent with another pharmacologic study, which predicted an effective dose of dalteparin, 150 IU/kg SC every 4 hours or enoxaparin, 1.5 mg/kg SC every 6 hours, to reliably achieve target aXa levels in healthy cats.[144]

ADDITIONAL THERAPY

In patients with DIC subsequent to hepatic or gastrointestinal disease, where there may be an absolute deficiency of vitamin K, this vitamin may be supplemented parenterally. This therapy may be especially relevant in cats. Vitamin K may be given to these patients at a dosage of 1 to 2 mg/kg subcutaneously every 24 hours.

In general, there is no indication for the use of antifibrinolytic agents in the treatment of DIC. In rare cases where the inciting cause may be hyperfibrinolysis leading to further consumptive coagulopathy, drugs, such as α amino caproic acid or tranexamic acid, may be indicated. To the authors' knowledge, this is a rare event, although the postoperative quasi-DIC hemorrhagic syndrome recognized in some greyhounds may be a result of enhanced fibrinolysis and may merit treatment in this manner.[149]

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