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The Link Between Inflammation and Coagulation: Influence on the Interpretation of Diagnostic Laboratory Tests

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Abstract: *This article provides an overview of the complex relationship between inflammation and coagulation and a review of routinely available laboratory and point-of-care tests for the detection of inflammation and coagulopathies. In the management of cases requiring ongoing laboratory and clinical evaluation, examination of these two major pathologic processes may assist with diagnosis and improve outcome. Early identification of a pathologic inflammatory process may allow prevention of its progression to syndromes carrying a poorer prognosis, such as disseminated intravascular coagulation and multiple organ dysfunction syndrome.*

The inflammatory response is an important local defense mechanism against infection and injury.[1] However, when this response becomes systemic and severe, pathologic damage can ensue. Conditions that may result in severe inflammation include pancreatitis, sepsis, trauma, immune-mediated diseases, and neoplasia. Because the inflammatory response is inseparable from the coagulation process, coagulation disorders are often associated with severe inflammatory disease.

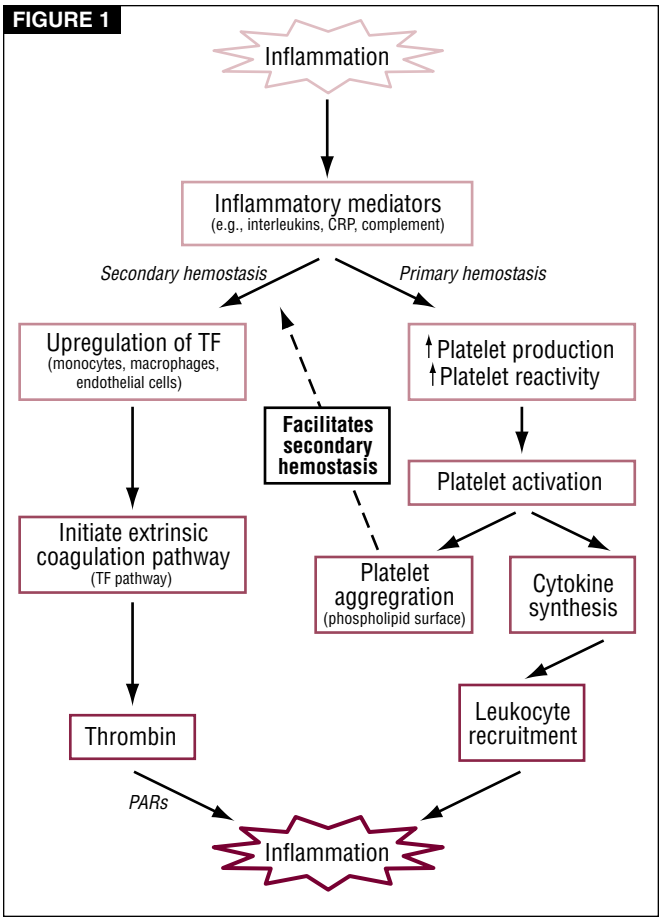
Normally, a fine balance between coagulation and fibrinolysis is maintained to prevent hemorrhage and disseminated thrombosis. This homeostatic state is disrupted by inflammation, which tends to shift the balance toward a procoagulant, antifibrinolytic environment.[2,3] The outcome of imbalance between thrombin formation and fibrin degradation is pathologic thrombosis, resulting in microvascular failure with development of multiple organ dysfunction syndrome (MODS).[4,5] Inflammation is known to directly affect coagulation through three main mechanisms: (1) activation of coagulation, (2) downregulation of endogenous anticoagulants, and (3) inhibition of fibrinolysis.[6–9]

Activation of Coagulation

Inflammation can initiate coagulation at several points in primary and secondary hemostatic systems (**FIGURE 1**). Overviews of normal hemostasis have been published elsewhere.[10,11] Inflammatory cytokines are fundamental mediators of the immune system, and among them, interleukin (IL)-6 has been shown to stimulate platelet production.[12,13] Platelets produced in response to inflammation are more thrombogenic, with an increased sensitivity to platelet agonists.[12,13] Infectious agents and several inflammatory mediators have been found to be platelet activators, including bacterial endotoxin, thromboxane A_2, platelet activating factor, and cathepsin G (an enzyme released by neutrophils).[1,14,15]

Inflammation-induced activation of platelets can further perpetuate the inflammatory response by two means. First, the activated platelets aggregate to provide the negatively charged phospholipid surface necessary for secondary hemostasis to occur. The end result of secondary hemostasis is the formation of thrombin. Thrombin is traditionally regarded as the catalyst for the conversion of fibrinogen to fibrin; however, thrombin is itself a strong platelet agonist and inflam-

FIGURE 1



The relationship between inflammation and activation of coagulation. *CRP* = C-reactive protein; *PARs* = protease-activated receptors; *TF* = tissue factor.

matory mediator.[9,11] Thrombin modulates the inflammatory system through binding to a specific group of cell surface receptors known as *protease-activated receptors*.[6] Second, activated platelets interact with underlying endothelial cells to stimulate the adhesion and recruitment of inflammatory leukocytes.[16] Activated platelets can synthesize IL-1β, which targets endothelial cells to enhance their adhesive properties.[17]

Several mediators and products of inflammation produce a procoagulant effect on secondary hemostasis, including tumor necrosis factor-α (TNF-α) and other inflammatory cytokines, lipoproteins, C-reactive protein (CRP), and bacterial endotoxins, as does complement activation.[7,8,18–21] These mediators initiate coagulation by upregulating tissue factor expression on endothelial cells, circulating monocytes, and macrophages[7,8,18,19,21–23] (**FIGURE 1**). The induction of tissue factor promotes coagulation through the extrinsic (tissue factor) pathway; once initiated, coagulation progresses through the common coagulation pathway (conversion of factor X to factor Xa), resulting in the formation of thrombin.[7,8,18] Clots develop subsequent to thrombin formation. Inflammation has also been found to mediate coagulation through a mechanism that is independent of the tissue factor pathway: monocytes activated by inflammation can directly activate factor X to catalyze the conversion of prothrombin to thrombin.[24] **BOX 1** summarizes the procoagulant properties of inflammation.

BOX 1

Procoagulant Properties of Inflammation

Cytokines

- TNF-α
  - —Suppresses tPA
  - —Stimulates release of PAI-1
  - —Inhibits expression of EPCR and thrombomodulin
  - —Induces tissue factor expression on monocytes and endothelial cells
- IL-1
  - —Inhibits expression of EPCR and thrombomodulin
- IL-6
  - —Induces tissue factor expression on monocytes and endothelial cells
  - —Increases platelet count
  - —Increases platelet thrombogenicity

Leukocytes

- Neutrophils
  - —Elastase destroys antithrombin and C1 inhibitor
  - —Elastase cleaves TFPI and thrombomodulin
- Monocytes
  - —Release microvesicles (source of tissue factor)
  - —Activate factor X

Acute-phase proteins

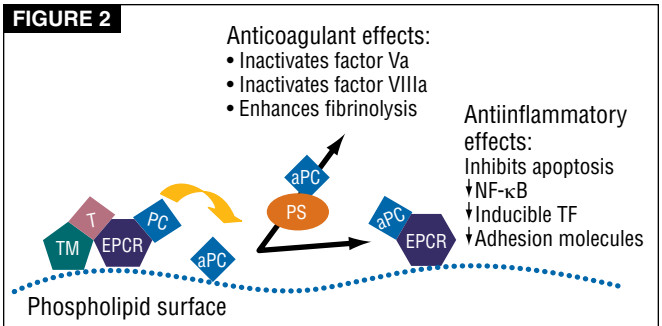
- CRP
  - —Induces tissue factor expression
  - —Promotes complement activation to:
    - –activate neutrophils
    - –promote neutrophil chemotaxis
    - –promote cytokines
    - –expose phospholipid on cell surfaces
    - –inactivate protein S
- α_1–Antitrypsin
  - —Inhibits aPC
  - —Cleaves TFPI

aPC = activated protein C; *CRP* = C-reactive protein; *EPCR* = endothelial cell protein C receptor; *PAI-1* = plasminogen activator inhibitor-1; *TFPI* = tissue factor pathway inhibitor; *TNF-α* = tumor necrosis factor α; *tPA* = tissue plasminogen activator

Downregulation of Endogenous Anticoagulants

The three main endogenous anticoagulants are tissue factor pathway inhibitor (TFPI), antithrombin, and protein C (pro-

FIGURE 2



Functions of activated protein C (aPC). Activation of protein C (PC) is initiated by the binding of thrombin (T) to thrombomodulin (TM); however, this reaction is more efficient when PC forms a complex with endothelial cell protein C receptor (EPCR). Subsequent function of aPC depends on its associations. aPC that remains bound to EPCR exhibits antiinflammatory properties. Free aPC can complex with protein S (PS) to exert anticoagulant properties. *NF-κB* = nuclear factor κB; *TF* = tissue factor.

tein C activation and functions are reviewed in **FIGURE 2**). Inflammatory conditions cause a decrease in the expression and function of antithrombin and activated protein C (aPC), while the concentration of TFPI is not consistently altered.[4,25] With inflammatory injuries, antithrombin is consumed and inactivated by proteolysis; in severe illness, its functional activity is reduced to <50% of normal.[21] The endogenous heparin-like endothelial glycosaminoglycans that enhance antithrombin activity are reduced by neutrophil release products and inflammatory cytokines,[21] leading to a further decline of antithrombin activity. Inflammation downregulates the protein C pathway primarily through inhibition of thrombomodulin and endothelial cell protein C receptor (EPCR) transcription, resulting in a reduced ability to generate aPC.[7,21] Neutrophil elastases cleave thrombomodulin from endothelial cells, thereby greatly reducing thrombomodulin's activity. Other causes of decreased protein C concentrations in severe inflammation include increased consumption and a compromised ability to synthesize the protein due to hepatic dysfunction.[7,21]

Additional evidence supporting the connection between inflammation and coagulation is seen from the antiinflammatory effects demonstrated by natural anticoagulants (**BOX 2**). Although the mechanism is unknown, the antiinflammatory effects of TFPI include reducing leukocyte activation and dampening the expression of TNF-α.[26] When antithrombin binds to endogenous endothelial cell glycosaminoglycans like heparan sulfate and dermatan sulfate, its inherent antiinflammatory effects include increased prostacyclin formation, decreased nuclear factor κB (NF-κB) activation, and decreased leukocyte activation and adhesion to endothelial cells.[7,8] This antiinflammatory effect is eliminated when antithrombin is bound to exogenously administered heparins,[8] which contributes to the controversy of administering heparin during systemic inflammatory conditions resulting in disseminated intravascular coagulation (DIC). aPC possesses both anticoagulant and antiinflammatory properties, depending on its association with EPCR. If aPC dissociates from EPCR, it can form a complex with the cofactor protein S to proteolytically inactivate cofactors Va and VIIIa to exert an anticoagulant effect.[7] When aPC remains bound with EPCR, intracellular signals are generated that inhibit apoptosis, decrease the expression of NF-κB, and decrease

BOX 2

Proinflammatory and Antiinflammatory Properties of Coagulation

Proinflammatory

- Thrombin
  - —Promotes cytokine synthesis
  - —Expresses endothelial cell P-selectin
  - —Chemotactic for polymorphonucleocytes
  - —Stimulates production of platelet activating factor
  - —Promotes neutrophil-monocyte adhesion
- Tissue factor–factor VIIa complex
  - —Promotes cytokine synthesis
  - —Induces expression of macrophage cell adhesion molecules
  - —Stimulates neutrophil infiltration
- Fibrinogen/fibrin
  - —Increases neutrophil and monocyte adhesion
  - —Promotes cytokine synthesis and monocyte chemoattractant protein 1

Antiinflammatory

- Antithrombin
  - —Blocks NF-κB expression
  - —Increases prostacyclin formation
  - —Decreases leukocyte activation/adhesion
- TFPI
  - —Decreases expression of cytokines
  - —Decreases leukocyte activation
- aPC
  - —Decreases NF-κB mRNA levels, which:
    - –decreases cytokine formation
    - –decreases tissue factor expression
    - –decreases cellular adhesion molecule expression
    - –prevents apoptosis
- Thrombomodulin
  - —Inhibits leukocyte adhesion to endothelial cells
  - —Blocks cytokine synthesis

aPC = activated protein C; *NF-κB* = nuclear factor κB; *TFPI* = tissue factor pathway inhibitor

BOX 3

Proinflammatory and Antiinflammatory Properties of Fibrinolytic Proteins

Proinflammatory

- uPA
  - —Enhances TNF-α secretion
  - —Potentiates neutrophil activation and migration
- uPAR
  - —Facilitates leukocyte adhesion
  - —Stimulates cytokine and growth factor production

Antiinflammatory

- TAFI
  - —Inactivates vasoactive complement peptide C5a
- PAI-1
  - —Competes with uPAR
  - —Inhibits leukocyte adhesion and migration
  - —Inhibits TNF-α production

PAI-1 = plasminogen activator inhibitor 1; *TAFI* = thrombin activatable fibrinolysis inhibitor; *TNF-α* = tumor necrosis factor α; *uPA* = urokinase plasminogen activator; *uPAR* = urokinase plasminogen activator receptor

BOX 4

Effect of Inflammation on Traditional Coagulation Assays

- Activated clotting time: Prolonged
- Antithrombin: Decreased
- D-dimers: Increased
- Fibrin degradation products: Increased
- Fibrinogen: Increased

expression of adhesion molecules and inducible tissue factor[7] (**FIGURE 2**). These cellular responses serve to directly minimize the inflammatory process.

Inhibition of Fibrinolysis

Early in the course of inflammation, fibrinolysis is enhanced through increased release of stored plasminogen activators from epithelial and endothelial cells, monocytes, and neutrophils.[18,27,28] Tissue plasminogen activator (tPA) converts plasminogen to plasmin, which is responsible for dissolving intravascular fibrin clots.[29] Within the extracellular matrix, urokinase plasminogen activator (uPA) and its receptor also function to initiate fibrinolysis through the activation of plasminogen to plasmin.[28] Inflammation subsequently impairs the fibrinolytic system through significantly enhanced production of plasminogen activator inhibitor-1 (PAI-1), which acts as a potent inhibitor of tPA and uPA. PAI-1 upregulation is mediated by inflammatory cytokines (e.g., TNF-α) and CRP.[7,30] Fibrinolysis is further inhibited through the generation of the zymogen thrombin activatable fibrinolysis inhibitor (TAFI). The activation of TAFI depends on generation of large amounts of thrombin and thrombin's ability to complex with thrombomodulin. Activated TAFI inhibits fibrinolysis by indirectly decreasing plasminogen activation, resulting in decreased generation of plasmin.[30] TAFI levels were significantly decreased in human sepsis patients in one study.[30] However, these low levels are speculated to reflect consumption of TAFI. An alternative explanation might be the significant reduction in thrombomodulin, which is required to activate TAFI, during systemic inflammation.[30] The proinflammatory and antiinflammatory properties of fibrinolytic proteins are summarized in **BOX 3**.

In summary, the effects of inflammation on the coagulation system culminate in the creation of a systemic procoagulant state that increases a patient's susceptibility to thrombosis, DIC, and MODS. This has overwhelming clinical significance because patients with MODS frequently have a poor outcome. Vigilance is required for early detection of unregulated inflammation in an effort to prevent its escalation to a clinical coagulopathy. Understanding the various points at which inflammation and coagulation interact will allow future research to focus on novel therapeutics that can target the pathologic autoamplification observed with uncontrolled inflammation and coagulation activation.

The following is a review of assays available to veterinary practitioners for the evaluation of inflammation and coagulation. The mechanism of each test is provided to highlight its primary diagnostic function and to elucidate how the assay may be influenced by the many interconnections between inflammation and coagulation. **BOX 4** summarizes the known effects of inflammation on coagulation assays.

Laboratory Evaluation of Inflammation

Complete Blood Count

The complete blood count (CBC) evaluates the three hematopoietic cell lines: erythrocytes, leukocytes, and platelets. Although erythrocyte and platelet changes can accompany an inflammatory response, alterations in leukocyte quantity and type primarily determine the presence or absence of inflammation. The platelet changes induced by inflammation have been briefly reviewed above. Inflammatory leukograms vary, especially with the superimposition of stress-induced cortisol and physiologic epinephrine responses. The most common manifestation of inflammation is a leukocytosis characterized by a mature neutrophilia and a regenerative left shift.[31] A regenerative left shift is an increase in band neutrophils that is less than the accompanying increase in mature neutrophils. The more severe the leukocyte abnormality, the more likely the cause

is to be a pathologic process than a physiologic or drug-induced response. A neutrophilia caused by glucocorticoids is expected to be less than two to three times the upper reference limit.[32] Extreme elevations in leukocytes to >70 × 10^9/L in cats and >65 × 10^9/L in dogs have been associated with a poor prognosis independent of etiology.[33,34]

Conversely, acute severe inflammatory diseases can result in leukopenia that may be accompanied by a degenerative left shift. In this instance, the number of band neutrophils is equal to or greater than that of segmented neutrophils, a situation that also carries a poor prognosis.[31] The primary differential diagnosis for a marked leukocytosis includes infectious, neoplastic, or immune-mediated diseases,[33,34] while the causes of leukopenia include decreased bone marrow production or a peracute, overwhelming infection.[31] Evaluation of neutrophils for evidence of toxic changes may assist in distinguishing an underlying inflammatory process from other causes of leukocytosis. Toxic changes represent defects in neutrophil maturation and include vacuolated cytoplasm, diffuse cytoplasmic basophilia, Döhle bodies, and, rarely, azurophilic (toxic) granules.[32,35] Toxic changes can precede abnormalities in absolute neutrophil counts and may closely reflect the progression of inflammation.[36] Toxic changes may be absent despite overt infectious disease if appropriate antibiotics have been administered.

Interpretation of a single CBC can be influenced by simultaneous effects of glucocorticoids, catecholamines, neoplasia, or antibiotic therapy. Leukograms are also poorly sensitive for inflammation and can be unremarkable despite an active inflammatory process.[37,38] Although a leukogram is considered an essential component of a minimum database for any critically ill patient and can provide useful information when applied to the clinical situation, its use as a gold standard for the evaluation of inflammation is minimized by its variability.

Fibrinogen

Fibrinogen is an acute-phase protein that increases in response to inflammation. In companion animals, the interpretive value of fibrinogen alone as a marker of inflammation is restricted by its lack of specificity and modest response to stimulation.[39] However, hyperfibrinogenemia in conjunction with an inflammatory leukogram is useful for identifying inflammation secondary to bacterial infections in many animal species, most notably ruminants and horses.[37,40] Plasma viscosity can increase with minor elevations in fibrinogen concentrations, altering blood rheology and predisposing patients to thromboembolism.[41] Several human studies have reported that elevated fibrinogen levels serve as predictors of stroke and myocardial infarction, among other arterial thrombotic disorders.[42]

The recommended method for fibrinogen determination is the Clauss assay, which relies on clot formation as an end point.[41] This assay is precise; however, it tends to be labor intensive and requires specialized equipment.[41] Reagent variability is a common source of inconsistent results among most coagulation tests, and the use of a single reagent with standardized procedures within each laboratory is highly recommended. The tensile strength of a clot may be altered by heparin therapy; therefore, samples for fibrinogen evaluation should not be collected within 4 hours after heparin administration or from heparin-contaminated lines.[41] Another problem is the variability in fibrinogen levels within an individual patient, which can create difficulties when interpreting results.[43] Human studies have shown that fibrinogen levels display seasonal differences[44]; however, this phenomenon has not been reported in veterinary patients. Because fibrinogen can increase with inflammation yet concomitantly decrease with a consumptive disorder, the fibrinogen concentration may be within the reference limits despite overt pathology.[45]

C-Reactive Protein

CRP is another acute-phase protein, but unlike fibrinogen, it can dramatically increase in dogs when induced by an inflammatory focus.[40] Elevated CRP levels have been reported in dogs after surgical trauma[46,47]; during infections, including pyometra,[48] leishmaniasis,[49,50] babesiosis,[51] ehrlichiosis,[52] bordetellosis,[53] leptospirosis,[47] and parvovirus infection[47]; and with noninfectious conditions, including neoplasia,[47,54] acute pancreatitis,[55] autoimmune disorders,[54] inflammatory bowel disease,[56] and cardiac valvular disease.[57] A commercially available ELISA for canine serum CRP has been validated as a useful diagnostic indicator for inflammation in dogs.[58] At this time, the main limitations of this assay are its lack of wide availability, requirement for specialized equipment, and relative labor intensiveness. CRP is not a major acute-phase protein in cats and is, therefore, considered a poor marker of inflammation in this species.[59]

Activated Clotting Time

The activated clotting time (ACT) assay was initially introduced in 1958 as a test of coagulation (see discussion under Laboratory Evaluation of Coagulation, below).[60] However, studies in human medicine have determined that inflammation affects ACT, suggesting an alternative interpretation of this test: that an increased ACT may be reflecting inflammation and not purely a coagulopathy.[61] Support for this novel interpretation in veterinary medicine is limited; however, we have recently found a strong correlation between inflammation (as measured by CRP) and ACT. Our study found that with greater CRP concentrations, the ACT tends to be prolonged despite the absence of a coagulopathy.[62] Given the laborious process to determine canine CRP at this time, this result allows ACT, as a point-of-care assay, to provide additional information regarding the inflammatory component of a canine patient's condition, providing there

is no concurrent coagulopathy. The mechanism by which inflammation prolongs the ACT remains unknown.[62]

Future Tests

In veterinary medicine, the ability to objectively detect inflammation is limited and is largely based on clinical suspicion drawn from a combination of our understanding of relevant pathologic processes and accompanying laboratory data. An additional marker of inflammation predominantly used in human medicine is procalcitonin. This assay is currently restricted to the research setting in veterinary medicine.

Laboratory Evaluation of Coagulation

Sample Collection

Poor sample collection remains an important source of error in hemostatic assays, and proper technique is imperative. Atraumatic blood collection from a fasted patient is ideal; hemolyzed and lipemic samples are not acceptable. The recommended anticoagulant-to-blood ratio is 1:9. Routine coagulation assays can be prolonged with underfilled (i.e., overcitrated) samples, leading to inaccurate results.[63] Hematocrit influences the anticoagulant-to-blood ratio; therefore, samples from patients with abnormally high or low hematocrit values are not recommended for hemostatic tests.[63]

Prothrombin Time

The prothrombin time (PT) assay provides information about the extrinsic (tissue factor) and common coagulation pathways. Prolonged PT values indicate a possible deficiency of factor II, V, VII, or X or fibrinogen.[64,65] The PT assay is frequently conducted to detect acquired clotting disorders, including vitamin K factor deficiency or antagonism, hepatic disease, and DIC.[66] This assay adds a high concentration of thromboplastin and calcium to a citrated plasma sample. The end point for the assay is formation of a clot.

The limitation of this test largely results from the marked variability between thromboplastin reagents used by different laboratories. Different reagents contain different concentrations and sources of thromboplastin and phospholipids, resulting in a variable sensitivity for detecting factor deficiencies.[66] Generally, it has been reported that factors must be depleted by 70% before the PT is prolonged; therefore, this measure is insensitive to mild factor deficiencies.[67] Other drawbacks to consider with this assay include an inability to reliably detect hypercoagulability, prolongation by heparin or high doses of low-molecular-weight heparin, and interference by lupus anticoagulants, which are not associated with bleeding tendencies.[64,66] The impracticality of the PT assay in urgent situations is no longer an issue with the availability of point-of-care coagulation analyzers.[68] In human medicine, standardized reporting of PT despite variability between different thromboplastin reagents can be overcome by the international normalized ratio (INR): INR = (patient PT/mean normal PT)^{ISI}. The ISI is the international sensitivity index, which is unique to each thromboplastin reagent. INRs are not widely used in veterinary medicine at this time.

Activated Partial Thromboplastin Time

The activated partial thromboplastin time (aPTT) assay screens for deficiencies and inhibition of the intrinsic (contact activation) and common coagulation pathways. Prolonged values indicate a possible deficiency of factor II, V, VIII, IX, X, XI, or XII; prekallikrein; high-molecular-weight kininogen (HMWK); or fibrinogen.[64,65] The aPTT assay should detect all factor-dependent coagulopathies with the exception of factor VII deficiency. The process to determine aPTT is similar to that for PT, with clot formation as an end point. However, two important differences distinguish specificity for the intrinsic pathway. The first is the addition of a particulate activator, usually either celite or kaolin, which provides the large surface area required for rapid initiation of intrinsic coagulation. The second difference is the lack of a thromboplastin reagent, which excludes factor VII involvement in this assay.[64]

The limitations of the PT assay also affect the aPTT assay. Limitations specific to the aPTT assay include variable outcomes resulting from use of different types and concentrations of particulate activators.[64] A problem commonly encountered with this assay in emergency medicine is the considerable increase in factor VIII resulting from trauma, including surgical trauma; physical stress and pregnancy may also induce this phenomenon. Stress-induced increases in factor VIII may conceal a true coagulopathy like mild hemophilia A.[66] Prolonged aPTT values due to deficiencies in factor XII, prekallikrein, and HMWK are of little clinical consequence; however, they may prompt further unnecessary diagnostic testing and supportive care.[65]

Activated Clotting Time

As a single-reagent assay, the ACT has the advantages of simplicity and rapidity. In human medicine, this assay is used extensively for anticoagulation monitoring during cardiac bypass surgery, coronary angioplasty, dialysis, and therapy for thromboembolic diseases.[61,69,70] In veterinary medicine, a common indication for its use is suspected vitamin K anticoagulant toxicosis. The ACT assay evaluates the function of the intrinsic and common coagulation pathways, with clot formation signifying the end point. However, unlike the aPTT assay, ACT does not require the addition of multiple exogenous reagents, allowing it to be an indicator of a patient's overall state of coagulation.[70] PT and aPTT assays are routinely performed on platelet-poor plasma and do not consider the significant interaction of in vivo platelets in clot formation.[71] The ACT is sufficiently sensitive to distinguish hypercoagulable disorders,[70] and its rapid results

BOX 5

Ontario Veterinary College Activated Clotting Time Protocol

Collect whole blood sample:

- Atraumatically collect blood from a vein or venous catheter. Immediately remove needle, deposit the sample in the tube, and start timing.
- Keep the tube upright.

For 0.5-mL tubes[a]:

- Swirl gently for 10 revolutions.
- Axillary, water bath, or heating block method:
  —Place tube in human axilla (tucked up high with only a shirt interface, keeping tube as upright as possible), a 37°C water bath, or heating block.
  —Examine the tube at 60 seconds and every 10 seconds thereafter for clot formation. Tip the tube no more than 45° to avoid contact with the tube wall, where the blood will stick. The blood must remain in the bottom of the tube with the activator and magnet.
- Incubator method[b]: Tap the tube two or three times before placing it in the incubator to prevent the magnet from sticking prematurely and producing invalid results. The incubator should be set at 38.4°C.

For 2-mL tubes[c]:

- Tip the tube back and forth to mix the activator with the blood.
- Place the tube horizontally in the axilla or incubator or vertically in a 37°C water bath or heating block.
- Examine the tube according to same schedule as for 0.5-mL tubes.

Reported Activated Clotting Time Reference Ranges for MAX-ACT[a] and C-ACT[c] Tubes[d]

Species (number of animals in study)	Method	Range (sec)	Collection site
MAX-ACT			
Feline (32 normal cats[e])	37°C water bath	55–85	Jugular
Canine (47 normal dogs[e])	37°C water bath	55–80	Jugular
Canine (9 normal dogs[f])	37°C heating block	65–90	Jugular
Canine (9 normal dogs[f])	Axilla	70–105	Jugular
Canine (9 normal dogs[f])	Incubator[b]	78–110	Jugular
Canine (20 normal dogs[g])	Incubator[b]	93–108	Saphenous vein catheter
C-ACT			
Canine (20 normal dogs[g])	Axilla	85–105	Saphenous vein catheter
Canine (20 normal dogs[g])	Axilla	80–100	Jugular
Canine (20 normal dogs[g])	Incubator[b]	95–115	Saphenous vein catheter
Canine (20 normal dogs[g])	Incubator[b]	94–112	Jugular

[a]Helena Laboratories, Beaumont, Texas. These tubes contain kaolin, celite, and glass beads.
[b]Actalyke incubator, Helena Laboratories.
[c]Helena Laboratories. These tubes contain celite.
[d]Each institution should establish its own reference range specific to the ACT protocol and instrumentation used.
[e]See AM, Swindells KL, Sharman MJ, et al. Activated coagulation times in normal cats and dogs using MAX-ACT tubes. *Aust Vet J* 2009;87:292-295.
[f]Normal range established at the Ontario Veterinary College, University of Guelph, Ontario, Canada. Blood from a single sample was used for three incubation techniques.
[g]Normal range established at the Ontario Veterinary College.

and wide availability make it adaptable to any emergency situation.

Coagulation is highly dependent on the temperature at which fibrin polymerization and platelet aggregation and adhesion occur[71–73]; therefore, ACT results can vary with temperature. It has been recommended to prewarm the collection tubes and to maintain the testing procedure at 37°C via a constant heating source.[74,75] Acceptable heat sources include heat blocks, water baths, and the human axilla.[76] It is also recommended to establish a specific hospital proto-

col with reference ranges to reduce variability caused by the use of different contact activators and heat sources.[77] Celite within tubes is known to be inconsistent within lots and may also change reactivity over time.[77]

Both automated (Actalyke, Helena Laboratories, Beaumont, Texas) and axilla methods of ACT testing are used at the Ontario Veterinary College and are summarized in **BOX 5**. The sample tubes, which can be used for axilla, automated, or water bath incubation, contain a magnet. When these tubes are used in the incubator, the magnet spins and stops when a clot is formed. The incubator then displays the ACT. With methods requiring visual clot formation, the ACT reported is the time of initial clot observation. This time usually corresponds to complete clot formation; however, it may occasionally represent partial clotting. When a partial clot is observed, the time should be noted and the sample rechecked in 5 seconds for a complete clot. Rarely, full clotting does not occur. In these cases, the time to formation of the partial clot should be reported. The subjectivity of time to visual clot formation can be a source of error, with the inability to identify partial clots consequently yielding erroneously prolonged coagulation times.[74]

Caution is required when interpreting the ACT after surgery, which induces thromboplastin release that can accelerate clotting.[78] Greatly prolonged coagulation times also increase the variability of ACT results. A cutoff of 300 seconds has been suggested as the time beyond which the accuracy of ACT as a guide for therapeutic anticoagulation dosing is reduced.[79] This limitation may be of little clinical consequence, as 300 seconds is well above the normal reported ranges in veterinary and human patients.

The ACT requires a phospholipid surface to mediate the process of coagulation. This surface is provided by the patient's own platelets. Although moderate thrombocytopenia (50×10^9/L) has not been shown to prolong ACT, severe thrombocytopenia ($<10 \times 10^9$/L) and qualitative platelet abnormalities are thought to influence ACT outcome.[80] Newer point-of-care analyzers require the addition of phospholipid reagents, stabilizers, and buffers. These multireagent ACT assays produce results that offer no additional information from aPTT assays.[68]

Antithrombin

Antithrombin is synthesized by the liver and has a molecular weight of 70,000 daltons and a half-life of 2 days (in dogs).[81] Human and animal studies have shown that antithrombin behaves as a negative acute-phase protein.[82,83] Hereditary and acquired antithrombin deficiencies can promote thrombosis. In veterinary medicine, acquired antithrombin deficiencies due to impaired synthesis or diseases increasing antithrombin loss are more common. Impaired synthesis primarily results from hepatic diseases or (less commonly) malnutrition. Antithrombin loss may be caused by renal disease, gastrointestinal disorders, extensive burns, malignancy, or consumption during the process of dysfunctional coagulation (i.e., DIC, sepsis) and acute thrombotic events.[25,84] Anticoagulation therapy with heparin has also been associated with decreased antithrombin levels as thrombin–antithrombin complexes are inactivated and removed by the mononuclear phagocytic system.[8,84] Several assays are available for antithrombin evaluation; however, functional chromogenic assays provide more dependable results.[84]

Limitations of antithrombin testing include the inability to detect an antithrombin deficiency in some patients on oral anticoagulant therapy, which can elevate the antithrombin level.[85] The timing of antithrombin evaluation also warrants consideration, as antithrombin consumption in acute thrombosis or decreased antithrombin levels resulting from heparin therapy may not reflect a true antithrombin deficiency. It is recommended to delay antithrombin testing for 5 days after heparin therapy and 3 months after an acute thrombotic event.[84] These delays can limit the practicality of the antithrombin assay in the emergency setting.

Thromboelastography

Thromboelastography (TEG) is regaining considerable attention as a hemostatic assay. Originally described in 1948, TEG is unique in its ability to provide information about all phases of coagulation and fibrinolysis.[86,87] As such, this assay is a potential tool for measurement of a patient's global coagulation status, including hypercoagulation and platelet dysfunction. The advantages of TEG in the emergency setting include its point-of-care capability and rapid results (approximately 30 minutes).[88] Reviews of this diagnostic tool have been published[87,89]; therefore, the principles are only highlighted here.

TEG graphically captures the viscoelastic changes that occur during clot formation and subsequent lysis.[86,88] Transmitted rotational forces generated between a cup and pin during clot formation result in a trace called a *thromboelastogram*.[87,88] Hypocoagulable, hypercoagulable and hyperfibrinolytic conditions produce characteristic thromboelastograms (**FIGURE 3**). Several variables can then be evaluated to derive specific information relating to fibrin formation (coagulation), fibrinogen turnover (speed of clot formation), and platelet–fibrin interactions (strength of clot).[87,90] The analysis of these variables has driven TEG-guided blood component replacement therapy in human medicine, allowing cardiac surgery patients to receive blood from fewer donors.[88] The limitations of TEG include the significant equipment expense and instrumental sensitivity to physical vibrations or jolts.[86] The latter shortcoming has been mitigated through modified instrumentation used in rotational thromboelastometry.[86] Whole blood or citrated plasma can be used for analysis; however, the results generated from each are not comparable.[88] Thus, reference intervals must be obtained from similar samples.[88] Although TEG can help detect platelet dysfunction, its sensitivity in identifying von Willebrand's dis-

ease is limited, and its ability to therapeutically monitor the use of platelet-inhibiting drugs (e.g., aspirin) requires further investigation.[89,91] Variation in results due to patient age and sex differences has also been reported.[87,88] TEG results cannot be standardized between laboratories because of the variety of reagents and activators in use.[88]

Fibrin Degradation Products

Thromboembolic disorders cause considerable patient morbidity and are being increasingly recognized in veterinary medicine.[92] However, the ability to diagnose thromboembolic disease remains elusive because most available laboratory tests lack specificity. Assays evaluating fibrin degradation products (FDPs) generally provide supportive evidence for a diagnosis of thromboembolism or coagulopathy (e.g., DIC), but they have many limitations that restrict their clinical utility. Although FDPs are generated from the degradation of fibrinogen, soluble fibrin, or cross-linked fibrin, fibrinogen degradation is rare in human patients and not yet documented in dogs.[93] The presence of FDPs reliably indicates plasmin activation but not the presence of cross-linked fibrin, which limits the ability of FDP assays to reliably detect thromboembolic disorders.[94] In addition to thromboembolic events and DIC, conditions that may increase the concentration of FDPs include inflammation, neoplasia, trauma, and internal hemorrhage.[92,93] Liver and renal dysfunction may also cause elevations in FDPs secondary to defective clearance mechanisms[95] or associated systemic inflammation.[96] Laboratory results must always be interpreted within the context of clinical presentation.

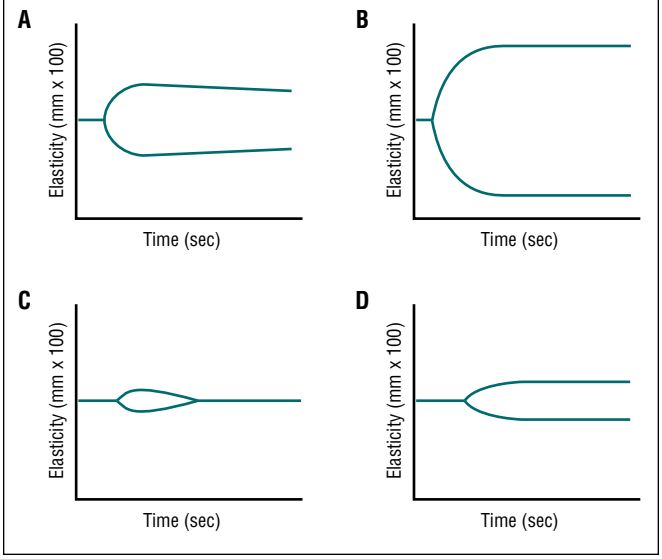
Several commercially available FDP latex agglutination kits use either serum or citrated plasma. Stokol et al[97] evaluated several FDP kits in dogs with DIC and reported comparable sensitivity for all kits tested.

D-dimers

D-dimers, like FDPs, are generated through the activation of plasmin; however, plasmin's substrate confers a unique specificity of D-dimers for fibrinolysis. When thrombin cleaves fibrinogen to form fibrin, the resulting soluble fibrin monomers form noncovalent bonds with each other to create an insoluble polymer. Thrombin-activated factor XIII and calcium are required to stabilize this mesh-like polymer by covalently binding adjacent fibrin molecules.[94] When insoluble fibrin polymers become the substrate for plasmin-mediated degradation, the cross-linked products are of varying sizes; the smallest product is the D-dimer.

Assays available for D-dimer detection include latex agglutination, immunoturbidimetric, and ELISA methods. Latex agglutination assays are most frequently used and contain beads coated with antibodies against human D-dimers. Citrated plasma from the patient is combined with the beads, and the D-dimers within the sample complex with the antibodies. Agglutination is the visible end point of these assays. The limitations of agglutination assays include difficult interpretation of weak reactions and slightly lower sensitivities compared with ELISA or immunoturbidimetric techniques.[94] Regardless of the assay employed, it should be validated for use in the intended species because most assays are manufactured with anti-human D-dimer antibodies and the degree of cross-reactivity with other species is unknown.[94] Fibrinolysis with subsequent elevations in D-dimer concentrations is not confined to thromboembolic diseases and also occurs after surgical procedures; with neoplastic, cardiac, hepatic, and renal diseases; with immune-mediated hemolytic anemia; and secondary to any coagulopathy, including DIC. Also, as with FDPs, nonspecific inflammatory processes can cause neutrophil elastase–mediated fibrinolysis.[94] Studies have shown that higher D-dimer concentrations may more likely reflect a thromboembolic disorder, so quantitative or semiquantitative results are preferred to qualitative results[92]; however, D-dimer assay results are qualitative or semiquantitative at best when samples are serially diluted. Although canine-specific D-dimer point-of-care tests are available and simple to use, their qualitative results are a considerable limitation.[93] Because false-negative results are rare with D-dimer assays, these assays may be used to confidently rule out a thromboembolic event or DIC.[92,93]

FIGURE 3



Thromboelastograms. (a) Normal coagulation. (b) Hypercoagulation. (c) Hyperfibrinolysis. (d) Hypocoagulation.

Fibrinogen

Fibrinogen assays are often incorporated into coagulation screening profiles because decreased fibrinogen levels indicate an increased hemorrhagic risk. Genetic disorders affecting normal hepatic fibrinogen synthesis (afibrinogen-

emia, hypofibrinogenemia, dysfibrinogenemia) can also be a source of abnormally low fibrinogen levels.[41] Acquired fibrinogen disorders are often related to liver disease causing excessive post-translational fibrinogen glycosylation.[41] Overt hepatic failure reduces fibrinogen levels simply from a lack of synthesis; other syndromes, including DIC, consume fibrinogen as widespread microthrombi are produced and subsequently broken down by an active fibrinolytic system.[41,98] The increased concentration of FDPs secondary to DIC also impairs fibrinogen function.[41,98] Thrombolytic agents significantly reduce fibrinogen levels, making fibrinogen assays useful for monitoring patients receiving this therapy.[41] The mechanism of the fibrinogen assay is discussed under Laboratory Evaluation of Inflammation, above.

Treatment

From a clinical perspective, the treatment options for inflammatory disorders with the potential to result in hypercoagulable conditions remain limited. The basic principles include control of the underlying disorder, supportive care to improve perfusion, replacement therapy with blood products such as fresh frozen plasma, and anticoagulant or antiplatelet therapy when indicated. In human medicine, recombinant human activated protein C has been found to be beneficial in patients with severe sepsis; this serves as additional supportive evidence of the close association between inflammation and coagulation.[99,100]

Conclusion

Given the evidence supporting the high degree of integration between the inflammatory and coagulation systems, it may be naïve to presume that diagnostic tests traditionally relied on to provide information about one system are not affected by the other. Fibrinogen concentration serves as a classic example of how one assay can be influenced by both systems. ACT has also been found to correlate with a patient's degree of inflammation.[62] These examples suggest that traditional coagulation assays may be influenced by inflammation and that their interpretation should be reevaluated.

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1. **Inflammation and coagulation are associated through**
   - **a.** downregulation of anticoagulants.
   - **b.** inhibition of fibrinolysis.
   - **c.** activation of coagulation.
   - **d.** all of the above

2. **What is the effect of inflammatory cytokines on platelets?**
   - **a.** They decrease platelet reactivity.
   - **b.** They accelerate platelet destruction.
   - **c.** They increase platelet count.
   - **d.** They decrease platelet count.

3. **Inflammatory mediators initiate coagulation by**
   - **a.** upregulating tissue factor expression.
   - **b.** decreasing expression of antithrombin.
   - **c.** increasing release of tPA.
   - **d.** activating cofactors V and VIII.

4. **Inflammatory cytokines downregulate the protein C pathway by inhibiting**
   - **a.** protein S.
   - **b.** protease-activated receptors.
   - **c.** EPCR.
   - **d.** heparan sulfate.

5. **Inflammation impairs fibrinolysis through**
   - **a.** enhanced PAI-1 production.
   - **b.** increased tPA release.
   - **c.** inhibition of TAFI.
   - **d.** increased plasminogen concentration.

6. **_____ samples are unacceptable for use in hemostatic tests.**
   - **a.** Overcitrated
   - **b.** Hemolytic
   - **c.** Lipemic
   - **d.** all of the above

7. **Recent research suggests that the ACT may be able to indicate disorders of the _____ in addition to its established utility.**
   - **a.** intrinsic coagulation pathway
   - **b.** extrinsic coagulation pathway
   - **c.** inflammatory system
   - **d.** a and c

8. **Acquired antithrombin deficiencies can occur with**
   - **a.** bone marrow suppression.
   - **b.** sepsis.
   - **c.** heart failure.
   - **d.** hypoadrenocortical crisis.

9. **Elevated D-dimer concentrations can be caused by**
   - **a.** rodenticide toxicosis.
   - **b.** thrombocytopenia.
   - **c.** hepatic disease.
   - **d.** a and c

10. **Elevated fibrinogen concentrations in animals may be attributed to**
    - **a.** season.
    - **b.** inflammation.
    - **c.** late-stage DIC.
    - **d.** tPA administration.