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Review Article

Point of Care Assessment of Coagulation

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Disorders of hemostasis can be difficult to fully elucidate but can severely affect patient outcome. The optimal therapy for coagulopathies is also not always clear. Point of care (POC) testing in veterinary medicine can assist in the diagnosis of hemostatic disorders and also direct treatment. Advantages of POC testing include rapid turnaround times, ease of use, and proximity to the patient. Disadvantages include differences in analytic performance compared with reference laboratory devices, the potential for operator error, and limited test options per device. Conventional coagulation tests such as prothrombin time, activated partial thromboplastin time, and activated clotting time can be measured by POC devices and can accurately diagnose hypocoagulability, but they cannot detect hypercoagulability or disorders of fibrinolysis. Viscoelastic POC coagulation testing more accurately evaluates in vivo coagulation, and can detect hypocoagulability, hypercoagulability, and alterations in fibrinolysis. POC platelet function testing methodologies can detect platelet adhesion abnormalities including von Willebrand disease, and can be used to monitor the efficacy of antiplatelet drugs. It is unlikely that a single test would be ideal for assessing the complete coagulation status of all patients; therefore, the ideal combination of tests for a specific patient needs to be determined based on an understanding of the underlying disease, and protocols must be standardized to minimize interoperator and interinstitutional variability.

Introduction

Point of care (POC) testing methodologies are designed to provide rapid results, using relatively self-contained or cartridge-based systems. By definition, a POC test should be available cage side, be a hand-held or benchtop analyzer, and be easy to perform by nonlaboratory personnel.[1] Advantages of POC testing include rapid turnaround time and round-the-clock availability, which can result in more rapid treatment decisions, and provide results outside of normal business hours or without a long delay for shipping. Most POC tests have comparable costs to reference laboratory analysis. Disadvantages of POC devices include the monetary investment for acquisition and maintenance of the machines and reagents, lack of research studies to aid in interpretation of results, quality control (QC) of noncartridge-based methodologies, inability to directly compare results across methodologies, and the limited menu of tests offered by each device. Some testing equipment is supplied with QC reagents or samples to ensure that the machinery is functioning properly, but the end users must regularly run these tests. For some POC devices, operator training and experience can influence results,[2,3] whereas others have features such as automated pipetting, which may minimize operator error. Adequate training, experience, with standardized protocols for running samples with performing regular QC, if indicated, can help minimize some of the variability in POC testing.

Overview of Hemostasis

Hemostasis can be divided into 2 phases, each distinct, but with significant overlap. Primary hemostasis begins immediately following disruption of the vascular endothelium, and results in the formation of a platelet plug. Secondary hemostasis involves soluble factors, and results in formation of a fibrin mesh to stabilize the platelet plug into a clot. Fibrinolysis is the process by which the clot is subsequently dissolved.[4] Separate POC devices exist for evaluating primary and secondary hemostasis alone, whereas the viscoelastic testing methodologies incorporate an evaluation of primary and secondary hemostasis, as well as fibrinolysis.

Testing methods for secondary hemostasis are best understood using the cascade or waterfall model wherein enzymatic activation of 1 factor leads to activation of subsequent factors, resulting in the defined intrinsic, extrinsic, and common pathways. The intrinsic pathway, as described in the cascade model, involves sequential activation of coagulation factors XII, XI, IX, and VIII, and terminates in the common pathway (factors X, V, II, and I), whereas the extrinsic pathway contains coagulation factor VII and tissue factor (TF). Although important for an understanding of coagulation, the cascade model does not completely account for the complexities of in vivo coagulation.[4]

The cell-based model of coagulation (Fig 1) accounts for the presence of the vascular endothelium and platelets and their roles in coagulation. It consists of 3 phases: initiation, amplification, and propagation. Coagulation is initiated when damage allows contact between TF-bearing cells and the plasma, where circulating factor VIIa binds TF and is activated. This leads to the generation of small amounts of thrombin, which acts as a positive stimulus on the production of additional thrombin, and also activates platelets. During the propagation phase, coagulation complexes are assembled on the surface of activated platelets, and thrombin is generated on a large scale, producing large amounts of fibrin from fibrinogen. The fibrin monomers are cross-linked to each other by activated factor XIII, forming fibrin polymers, and a stable clot. Once the initial vascular damage has healed, the degradation of the clot occurs as fibrinolysis. Plasminogen is enzymatically activated to plasmin by tissue plasminogen activator. Plasmin

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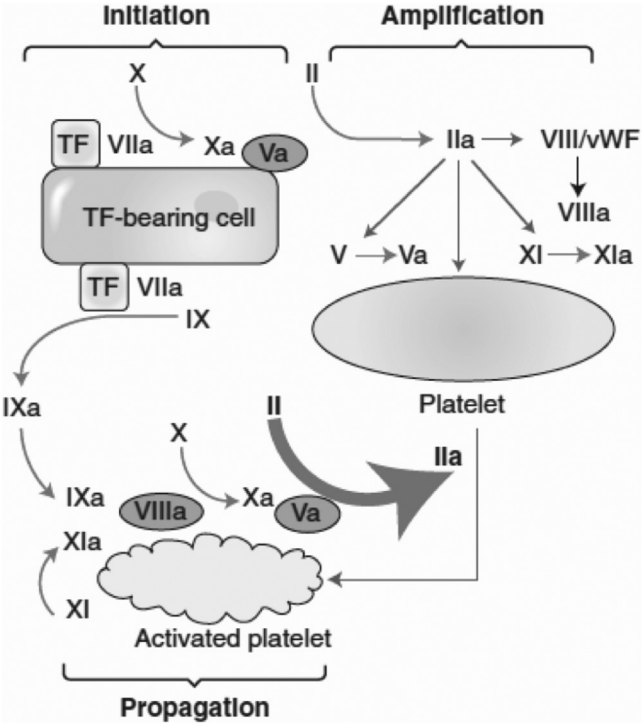

Fig. 1. Cell-based model of coagulation. *Initiation phase*: Exposure of tissue factor (TF) on damaged cells initiates coagulation and leads to the generation of small amounts of thrombin (IIa) from thrombin (II). *Amplification phase*: Thrombin activates platelets and cofactors such as Va and VIIIa on the platelet surfaces. *Propagation phase*: Large quantities of thrombin is produced on the surface of activated platelets. (Reprinted with permission from Hackner and White.[52])

degrades fibrin, leading to dissolution of the clot and resulting in the release of fibrin degradation products into the circulation.[4-6]

Conventional Coagulation Testing

Prothrombin Time, Activated Partial Thromboplastin Time, and Activated Coagulation Time

The prothrombin time (PT) measures the activity of the extrinsic and common coagulation pathways. To isolate the initiation of coagulation to the extrinsic pathway, reagents including TF, calcium, and a phospholipid are added to a warmed (37°C) citrated blood or plasma sample. The phospholipid provides support for assembly of the tenase complex and further thrombin generation. When whole blood is tested, activation of platelets by thrombin generated by coagulation itself helps to speed clot formation. In plasma-based tests (and in many whole blood assays as well), the phospholipid plays the same role as the activated platelet, so the results of these tests are independent of platelet number or function. The end point of this test is the formation of a clot as detected by the instrument. The POC devices vary in clot detection methodology (discussed later). The reference intervals for coagulation tests are thus dependent on not only the machine used, but also on the specific reagents.[7] Citrated whole blood is useful for this testing, because samples can be analyzed within an hour of acquisition (providing some flexibility vs. blood collected without anticoagulant, which would require immediate processing). In addition, the same sample can be used for both PT and activated partial thromboplastin time (aPTT) testing without the need for either 2 analyzers or 2 venipunctures; and if needed, the remainder of the sample can be centrifuged to obtain plasma that may be frozen for further analysis.[8]

Human medicine practitioners use the international normalized ratio (INR) to describe PT results. This is a direct result of the use of multiple testing methodologies and reagents across laboratories. This variability led to disparities in laboratory results for PT, and difficulty when comparing results between laboratories. To alleviate the effect of this variation, each thromboplastin reagent is assigned an international sensitivity index (ISI) value. A lower ISI translated into a more sensitive test, and generally to longer times for PT results. The INR is calculated by the following formula:

$$\mathrm{INR} = (\mathrm{PT}_{\mathrm{patient}}/\mathrm{MNPT})^{\mathrm{ISI}}$$

where the patient's PT value is divided by the mean of no fewer than 20 normal patient PT readings (MNPT), and this product is raised to the power of the ISI. The INR provides a metric to allow accurate dose adjustment of vitamin K antagonist medications.[9] Most hand-held PT analyzers designed for at-home monitoring of vitamin K antagonist therapy report an INR value. Some laboratories in veterinary medicine would report an INR value, but this is not standard practice, with most veterinary reference laboratories reporting individual coagulation reference intervals from healthy patients.

The aPTT assesses the integrity of the intrinsic and common pathways. The intrinsic pathway is also termed the contact pathway, as it is activated in vitro by contact with artificial, negatively charged surfaces. The "partial" in aPTT refers to the addition of phospholipid (but not TF), and the "activated" refers to the contact activators used to accelerate fibrin formation. Common contact activators include ellagic acid, micronized silica, celite, or kaolin. Benchtop instruments add phospholipid, calcium and the contact activator to citrated plasma at 37°C, and monitor for time to clot formation.[7] POC devices use citrated or fresh whole blood (FWB) (cartridges specific for either), to which a proprietary contact activating substance, calcium, and phospholipid are added, and clot formation is detected by the machine. As with the PT test, reference ranges are machine and reagent specific and are not interchangeable.

Several POC devices have been evaluated in human and veterinary medicine for the testing of PT and aPTT. The Coag DX (Idexx, Westbrook, ME) evolved from the SCA2000 (Synbiotics) and is a hand-held POC device for testing PT and aPTT on citrated or FWB from feline, canine, and equine patients. The Coag Dx has an internal electronic QC. This device has specific individual cartridges for PT and aPTT testing and also specific separate cartridges for citrated blood or FWB. For PT testing, the device uses a proprietary thromboplastin reagent and calcium. The aPTT cartridges use a proprietary contact activating substance, calcium, and phospholipid (but not thromboplastin). The analyzer aspirates the required amount of blood into the test channel where it is mixed with the reagents. The sample is mechanically moved back and forth and monitored for fibrin clot formation by a series of optical detectors. As clot formation begins, the blood flow through the cartridge slows, and the end of the test occurs when the movement decreases below a predetermined rate.[1]

This technology, as the SCA2000, was evaluated in a population of healthy and ill dogs to evaluate PT, aPTT, and activated coagulation time (ACT).[8] The methodology and reagent differences led to differences in the reference ranges of both the PT and aPTT compared with common reference laboratory methods (the aPTT reference ranges being 5-6 times longer than the PT). Despite the difference in absolute clotting times, the POC device was able to

[1] IDEXX Coag Dx Operators Guide, IDEXX Laboratories, Westbrook, Maine.