Review Article

Corresponding Author: Glenlyd Helmut A. Romanillos, Brian M. Denney, Emmalyn B. Cutamora, Graduate School, Cebu Doctors' University, Mandaue City, Cebu, Philippines

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Laboratory Assessment and Advancements in Hemostasis Testing During the Pandemic: Its Role in the Diagnosis of COVID-19-related Coagulopathies

Glenlyd Helmut A. Romanillos, Brian M. Denney, Emmalyn B. Cutamora

Graduate School
Cebu Doctors' University

Abstract

Hemostasis is the physiological process by which blood flow is halted at the injury site to prevent excessive bleeding. Laboratory assessment of hemostasis refers to a series of tests that are performed to evaluate various components of this process, including platelet function, coagulation factors, and fibrinolysis. Overall, laboratory assessment of hemostasis is important for diagnosing and managing bleeding and clotting disorders. These tests can help identify the disorder's underlying cause and guide appropriate treatment. The COVID-19 pandemic has prompted some innovations and updates to the laboratory assessment of hemostasis, particularly concerning managing patients with COVID-19 who may be at increased risk of thrombosis.

Keywords: COVID-19-related coagulopathies, fibrinolysis testing, hemostasis testing, advancements in hemostasis

Introduction

Hemostasis, which translates to stoppage of bleeding, involves the formation of a blood clot composed of platelets and a protein called fibrin (Encyclopedia Britannica, 2021). Hemostasis has three main stages: primary hemostasis, secondary hemostasis, and fibrinolysis (Rodak et al., 2015). In primary hemostasis, platelets are activated and aggregate at the injury site to form a temporary plug. In secondary hemostasis, a cascade of coagulation factors leads to fibrin

formation, stabilizing the platelet plug and forming a permanent clot. Fibrinolysis is when the clot dissolves once the injury has healed.

Hemostasis abnormalities are conditions characterized by disturbances in the normal blood clotting and coagulation processes, leading to excessive bleeding or clotting (Rodak et al., 2015). These abnormalities can arise from inherited or acquired factors and result in clinical manifestations. Diagnosing hemostasis abnormalities involves a comprehensive evaluation of the patient's medical history, physical examination, and laboratory testing. Laboratory assessments may include tests such as complete blood count, coagulation profile, platelet function assays, and specific factor assays (Hoffman et al., 2019). Laboratory evaluation of hemostasis is an important tool for diagnosing and managing bleeding and clotting disorders. Hemostasis testing includes screening and confirmatory tests to evaluate various stages of hemostasis. Screening tests include platelet count, bleeding time, prothrombin time (PT), and activated partial thromboplastin time (aPTT) (Adcock & Brophy, 2013). Confirmatory tests confirm abnormal screening test results and identify specific coagulation factor deficiencies. These tests include factor assays, mixing studies, and inhibitor assays. In addition to traditional hemostasis testing, newer assays have been developed to evaluate specific aspects of hemostasis. These include thrombin generation assay and rotational thromboelastometry (ROTEM) assay (Rodak et al., 2015).

The COVID-19 pandemic has highlighted the importance of understanding the hemostatic abnormalities associated with the disease and the role of laboratory assessments in managing patients. Hemostasis laboratory assessment is crucial in diagnosing and monitoring COVID-19-related coagulopathy, characterized by a prothrombotic state and increased risk of thrombotic events. Laboratory evaluation of hemostasis in COVID-19 patients includes standard tests such as Complete Blood Count (CBC), Activated Partial Thromboplastin Time (aPTT), Prothrombin Time (PT), D-dimer, and Fibrinogen levels (Pavoni et al., 2020). These tests help assess the overall coagulation profile and identify abnormalities, such as thrombocytopenia, prolonged clotting times, and elevated D-dimer levels. In addition to the standard tests, there have been several updates and measures during the COVID-19 pandemic. These include the measurement of markers associated with hypercoagulabilities and endothelial dysfunction, such as the von Willebrand factor antigen, factor VIII, and soluble P-selectin (Pavoni et al., 2020). These markers provide insights into the underlying pathophysiology of COVID-19-associated coagulopathies. Thromboelastography (TEG) and ROTEM are viscoelastic assays that comprehensively assess clot formation, strength, and stability (Rodak et al., 2015). These tests have been utilized in COVID-19 patients to identify hypercoagulability, guide anticoagulation therapy, and assess bleeding risk. Furthermore, point-of-care testing (POCT) has been emphasized during the pandemic, enabling the rapid and decentralized testing of coagulation parameters. POCT devices, such as handheld coagulometers and portable viscoelastic analyzers, have facilitated timely decision-making in critical care settings (Pavoni et al., 2020).

Review and Discussion

The Hemostasis Process

Primary hemostasis is the initial stage in response to a blood vessel injury.[2] It involves the formation of a platelet plug to prevent bleeding (Hoffman et al., 2019). This process has three main steps: platelet adhesion, activation, and aggregation. Platelet adhesion is the first step in achieving primary hemostasis. When a blood vessel is damaged, von Willebrand factor (vWF) binds to exposed collagen fibers in the damaged vessel wall. Platelets adhere to vWF using their glycoprotein receptors, particularly the glycoprotein Ib/IX/V complex. This adhesion helps anchor platelets to the site of injury. Platelet activation is the next step in achieving primary hemostasis. Once adhered, various agonists activate platelets, including thrombin, adenosine diphosphate (ADP), and thromboxane A2 (TXA2) (Rodak et al., 2015). Activation leads to a cascade of intracellular events, including shape changes, granule release, and exposure of glycoprotein IIb/IIIa receptors found on the platelet surface. Platelet aggregation is the final step in achieving primary hemostasis. Activated platelets bind to each other by interacting with their exposed glycoprotein IIb/IIIa receptors and fibrinogen molecules (Hoffman et al., 2019). This crosslinking of platelets forms a platelet plug, reinforcing the initial seal at the injury site.

Secondary hemostasis, the coagulation cascade, is the second stage that follows primary hemostasis (Rodak et al., 2015). It involves a complex series of interactions between proteins known as clotting factors that form a fibrin clot, further reinforcing the platelet plug and stabilizing the clot (Furie & Furie, 2020). The coagulation cascade follows two pathways, intrinsic and extrinsic, which converge on a common pathway leading to fibrin formation (Furie & Furie, 2020). The intrinsic pathway activates when factors XII (Hageman factor) and XI come in contact with the subendothelial components exposed during vessel injury (Rodak et al., 2015). These events lead to the activation of factor IX, which forms a complex with factor VIII and the calcium ions on the phospholipid surface. Activated factor IX (IXa) activates factor X. The extrinsic pathway activates when tissue factor (TF) is released into the blood during tissue damage (Rodak et al., 2015; Furie & Furie, 2020). The TF forms a complex with factor VII, and this TF-VIIa complex activates factor X. Both pathways converge at Factor X activation. Activated factor X (Xa) combines with factor V, calcium ions, and phospholipids to form a prothrombinase complex. This complex converts prothrombin (Factor II) to thrombin (Factor IIa). Thrombin plays a central role in secondary hemostasis. It converts fibrinogen (factor I) into fibrin, polymerizing and forming a mesh-like network stabilizing the platelet plug and forming a clot.[4] Thrombin also amplifies the coagulation cascade by activating factors V, VIII, and XI. Anticoagulant mechanisms, including the protein C pathway and tissue factor pathway inhibitor (TFPI), tightly regulate the clotting process to prevent excessive clot formation and maintain homeostatic balance (Furie & Furie, 2020).

Fibrinolysis is a physiological process after clot formation to dissolve fibrin clots and restore blood flow. It is a crucial mechanism for maintaining hemostatic balance and preventing the formation of excessive or unwanted blood clots (Rodak et al., 2015). The main enzyme involved in fibrinolysis is plasmin, derived from its inactive precursor, plasminogen (Machlus & Colleoni, 2018). Plasminogen is converted into plasmin through the action of tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA), which are serine proteases. Endothelial and other cells produce and release these activators in response to various stimuli. Once plasmin is generated, it degrades fibrin, the major component of blood clots, by cleaving it into smaller fragments called fibrin degradation products (FDPs) or fibrin split products (FSPs). Plasmin degrades other clot components, such as fibrinogen, factors V and VIII, and complement proteins (Rodak et al., 2015; Machlus & Colleoni, 2018). The fibrinolytic process is regulated by several inhibitors, including plasminogen activator inhibitor-1 (PAI-1) and alpha-2-antiplasmin. PAI-1 inhibits tPA and uPA, whereas alpha-2-antiplasmin directly inhibits plasmin activity (Machlus & Colleoni, 2018). These inhibitors prevent excessive fibrinolysis and help maintain the integrity of the clot until it is no longer required.

Hemostasis Abnormalities

Inherited hemostasis abnormalities include bleeding disorders, such as hemophilia, von Willebrand disease, and platelet function disorders (Hoffman et al., 2019). Deficiencies in clotting factors cause Hemophilia, primarily factor VIII (hemophilia A) and factor IX (hemophilia B). Von Willebrand disease is characterized by a deficiency or dysfunction of the von Willebrand factor, an essential protein involved in platelet adhesion and factor VIII stabilization. Platelet function disorders can result from platelet adhesion, activation, or aggregation defects, leading to impaired primary hemostasis. Acquired hemostasis abnormalities often arise due to medical conditions, medications, or medical interventions (Rodak et al., 2015; Hoffman et al., 2019). These include liver disease, vitamin K deficiency, disseminated intravascular coagulation (DIC), and anticoagulant therapy. Liver disease can disrupt the production of clotting factors, while vitamin K deficiency impairs the synthesis of factors II, VII, IX, and X. DIC is a complex disorder characterized by widespread coagulation activation, leading to clotting and bleeding. Anticoagulant therapy, such as warfarin or direct oral anticoagulants, can increase the risk of bleeding (Hoffman et al., 2019).

Diagnostic Tools To Evaluate Hemostasis

Screening Tests for Hemostasis Assessment

Screening tests evaluate the overall function of the hemostatic system. The most commonly used screening tests include the platelet count, bleeding time, and PT and aPTT (Rodak et al., 2015). The platelet count quantifies the number of platelets in the blood circulation. Thrombocytopenia, characterized by a low platelet count, can result in bleeding due to decreased platelets available to form a clot. A high platelet count, or thrombocytosis, can result in clotting due to increased platelet function. The bleeding time measures the time it takes bleeding to stop

after a small incision is made on the skin (Rodak et al., 2015). It evaluates platelet adhesion function, as platelets form the initial plug at the injury site. A prolonged bleeding time may indicate platelet dysfunction. The PT and aPTT evaluate the extrinsic and intrinsic coagulation pathways, respectively (Rodak et al., 2015). The PT measures the time it takes for clot formation to occur after adding a tissue factor. In contrast, the aPTT measures the time it takes for clot formation to occur after adding activators of the intrinsic pathway. Abnormal PT or aPTT results may indicate a deficiency in coagulation factors or the presence of an inhibitor.

Confirmatory Tests for Hemostasis Assessment

Confirmatory tests confirm out-of-normal range screening test results and identify specific coagulation factor deficiencies (Adcock & Brophy, 2013). These tests include factor assays, mixing studies, and inhibitor assays.

Factor assays measure the activity level of specific coagulation factors. Abnormal factor assay results may indicate a deficiency of a specific factor. Factor assays use plasma samples collected in citrate anticoagulant tubes to prevent clotting. The plasma is then separated by centrifugation and stored frozen until the assay can be done. The most common factor assays include factor VIII, factor IX, factor XI, and factor XII. Each assay is specific for a particular factor that uses a different reagent or activator (Adcock & Brophy, 2013).

Factor VIII assay: Factor VIII is an essential cofactor for activating factor X by factor IXa (Adcock & Brophy, 2013). Factor VIII deficiency leads to hemophilia A, a bleeding disorder characterized by spontaneous bleeding into joints and muscles (Rodak et al., 2015). The factor VIII assay measures the activity of factor VIII in plasma samples (Verma, 2017). The assay uses a reagent containing a phospholipid surface and a source of factor IXa. The time required for clot formation is measured, and the result is reported as a percentage of normal.

Factor IX assay: Factor IX is a serine protease that activates factor X in the intrinsic pathway of the coagulation cascade (Adcock & Brophy, 2013). Factor IX deficiency leads to hemophilia B, a bleeding disorder similar to hemophilia A (Rodak et al., 2015). The factor IX assay typically involves two main steps: factor IX activation and measurement of its activity level (Peyyandi et al., 2016). The assay is performed by mixing the patient's plasma with a reagent that triggers the intrinsic pathway of coagulation. The time required for clot formation is measured, and the result is reported as a percentage of normal.

Factor XI assay: Factor XI is a serine protease that activates factor IX in the intrinsic pathway of the coagulation cascade (Adcock & Brophy, 2013). Factor XI deficiency leads to Hemophilia C (Rodak et al., 2015). The factor XI assay involves mixing the patient's plasma with reagents that trigger the intrinsic pathway of coagulation (Martinuzzo et al., 2014). The time it

takes for clot formation to occur is measured, and the result is compared to a standard curve or reference range to determine the factor XI activity level in the patient's plasma.

Factor XII assay: Factor XII is a serine protease that activates factor XI in the intrinsic pathway of the coagulation cascade (Adcock & Brophy, 2013). Factor XII deficiency is rare and is not associated with bleeding disorders (Rodak et al., 2015). The factor XII assay measures the activity of factor XII in plasma samples. The assay uses a reagent containing a negatively charged surface, such as kaolin and calcium ions (Adcock & Brophy, 2013). The time required for clot formation is measured, and the result is reported as a percentage of normal.

Mixing studies are performed in the laboratory assessment of hemostasis to differentiate between factor deficiencies and inhibitors (Verma et al., 2017). These studies involve mixing patient plasma with an equal volume of normal plasma containing normal levels of coagulation factors and inhibitors (Rodak et al., 2015; Verma et al., 2017). If the clotting time of the mixed plasma is corrected to normal, it suggests a factor deficiency, while if the clotting time remains prolonged, it suggests the presence of an inhibitor. Mixing studies are performed using various activators, such as kaolin or tissue factor, and the clotting time is measured using a coagulation analyzer. The test is performed twice, once with the patient's plasma alone and once with the mixture of a patient and normal plasma, and the clotting times are then compared (Verma et al., 2017).

Inhibitor assays detect and quantify the presence of inhibitors to coagulation factors in a patient's blood (Rodak et al., 2015). Inhibitors are antibodies that interfere with the normal function of coagulation factors, and their presence can cause bleeding disorders (Verma et al., 2017). Inhibitors may be acquired, such as in cases of autoimmune diseases, or inherited, such as those seen in hemophilia A and B. Several methods for inhibitor assays include the Bethesda assay, the Nijmegen assay, and the modified Nijmegen-Bethesda assay (Verma et al., 2017). These assays measure the inhibitory activity of antibodies in patient plasma by comparing the clotting time of a plasma sample to that of a control plasma sample with known factor activity. The results are reported in Bethesda units (BU) or Nijmegen-modified Bethesda units (NBU), which indicate the degree of inhibition of the factor (Verma et al., 2017).

The Bethesda assay detects and quantifies factor VIII and factor IX inhibitors in hemophilia A and B, respectively. The Nijmegen assay assesses inhibitors to von Willebrand factor (VWF) and factor VIII in patients with von Willebrand disease, whereas the modified Nijmegen-Bethesda assay detects and quantifies inhibitors to factor VIII in patients with hemophilia A treated with factor VIII replacement therapy (Verbruggen et al., 2018).

Hemostasis Laboratory Assessment during the COVID-19 Pandemic

One important development is the recognition of COVID-19-associated coagulopathy (CAC), which refers to a prothrombotic state in some patients with severe COVID-19 (Dorgalaleh et al., 2020). CAC is an interplay of several factors, including inflammation, endothelial dysfunction, and coagulation system activation (Dorgalaleh et al., 2020). Laboratory assessment of hemostasis can play a critical role in diagnosing and managing CAC.

In addition to standard tests for platelet count and coagulation factors, some additional tests that may be used in the context of COVID-19 include:

1. **D-dimer**: This fibrin degradation product is released when a clot lyses. Elevated levels of D-dimer can indicate the presence of a blood clot (Rodak et al., 2015). D-dimer levels are frequently elevated in patients with severe COVID-19 and may be used to monitor for thrombotic events (Dorgalaleh et al., 2020).
2. **Anticoagulant Monitoring**: Patients with severe COVID-19 receiving anticoagulant therapy may require closer monitoring of their coagulation status. More frequent testing of clotting factors and adjusting the dosage of anticoagulant medication can be done as needed (Dorgalaleh et al., 2020).
3. **Thromboelastography (TEG)**: This test measures the viscoelastic properties involved during blood clot formation and lysis (Rodak et al., 2015). TEG can provide information about clot strength, stability, and fibrinolysis, which may be particularly useful in CAC.
4. **Point-of-Care Testing (POCT)**: POCT devices provide rapid and accurate results for hemostasis testing in patients with COVID-19 (Lippi et al., 2020). POCT devices are particularly useful in managing critically ill patients, where rapid decision-making is essential.

Thromboelastography for Hemostasis Assessment of COVID-19 patients

TEG is a viscoelastic assay that measures the mechanical properties of a clot as it forms and matures (Rodak et al., 2015). TEG has been increasingly used for the laboratory assessment of hemostasis in patients with COVID-19, as it provides a rapid assessment of coagulation status and the identification of hypercoagulability (Lippi et al., 2020).

In COVID-19, abnormal coagulation parameters such as elevated D-dimer levels and prolonged prothrombin time have been associated with increased mortality (Dorgalaleh et al., 2020). TEG can provide additional information on clot formation and lysis that may not be reflected in traditional coagulation assays (Lippi et al., 2020). A recent study found that COVID-19 patients with severe disease had significantly shorter R and K values and higher maximum amplitude (MA) values on TEG, indicating a hypercoagulable state (Spyropoulos et al., 2020).

TEG can also guide the use of anticoagulation therapy in COVID-19 patients. A study of critically ill COVID-19 patients found that TEG-guided anticoagulation therapy led to lower

mortality rates and improved clinical outcomes compared to standard prophylactic anticoagulation (Zampieri et al., 2021).

Conclusion

Laboratory assessment of hemostasis plays a crucial role in diagnosing and managing bleeding and clotting disorders. The COVID-19 pandemic has brought challenges to laboratory testing, including disruptions in the supply chain due to closed borders and increased demand for certain tests. However, advancements in technology and testing methods have allowed for the development of new assays and the adaptation of existing assays to serve the needs of patients during the pandemic time.

One important advancement in hemostasis laboratory testing during the pandemic has been viscoelastic testing, which allows for rapid assessment of coagulation status and identification of hypercoagulability in COVID-19 patients. Additionally, point-of-care testing has increased in importance, as it allows for rapid testing and treatment decisions at the bedside.

Overall, the laboratory assessment of hemostasis during the COVID-19 pandemic has required flexibility and adaptation on the part of laboratory professionals. While challenges remain, advancements in testing technology and methodology have allowed for the continued provision of high-quality care to patients with bleeding and clotting disorders, including those affected by COVID-19.

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