Screening Coagulation Assays, Factor XIII and D-dimer

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Physiologic Hemostasis

Hemostasis is the physiologic means by which the body both maintains circulatory flow and stops the loss of blood [1]. It is an intricate and complex process that relies on the interaction of multiple cellular components and protein pathways that serves to both maintain blood in a fluid state yet functions to develop a physical barrier to prevent excessive bleeding from injured blood vessels when needed. The system is highly regulated through a number of different mechanisms, in part being composed of proenzymes, procoagulant proteins, and anticoagulant proteins. Clot formation initiates the fibrinolytic system that functions to limit clot size and in some circumstances results in clot dissolution. The fibrinolytic system is also highly regulated containing proenzymes as well as profibrinolytic and antifibrinolytic proteins. Disorders in either clot formation or fibrinolysis can enhance bleeding potential and in some instances result in spontaneous hemorrhage.

Hemostasis progresses through three steps, beginning with (1) vasospasm which occurs nearly simultaneously with (2) platelet plug formation (referred to as primary hemostasis), followed by (3) the development of a fibrin clot (secondary hemostasis) [1–3]. Each step is triggered by injury to the blood vessel wall with resultant exposure to subendothelial matrix constituents. As vascular spasm or vasoconstriction helps limit blood loss from the vessel, primary and secondary hemostasis function together to form a fibrin clot. Primary hemostasis is described in more detail in Chapter 1.

Secondary hemostasis, also referred to as blood coagulation, is initiated by exposure of the plasma to tissue factor (TF) [1]. TF is found in the subendothelial matrix and can also be expressed by select cells in response to certain stimuli. A trace amount of procoagulant factor VII circulates in the activated rather than proenzyme form (activated factor VII [FVIIa]), and this serves to keep the hemostatic system "primed." FVIIa binds exposed TF resulting in the activation of factor X (activated FX [FXa]). This ultimately initiates the sequential activation of multiple coagulation proenzymes into functional serine proteases or functional cofactors, the activated forms of the procoagulant factors. Activated procoagulant factors bind the activated platelet and red blood cell surface, and this enables interaction with their respective cofactors and required cations, allowing the generation of highly efficient coagulation factor complexes that result in bursts of thrombin formation. Thrombin cleaves soluble fibrinogen creating fibrin polymers that polymerize electrostatically. An insoluble fibrin gel is formed when activated factor XIII covalently cross-links the fibrin polymers.

Most proteins involved in hemostasis are produced in the hepatic parenchyma with the exception of factor VIII, which is believed to be synthesized by hepatic sinusoidal endothelial cells, and a subunit of factor XIII [4]. Due to hepatic immaturity at birth, the normal reference interval for most clotting factors varies with age, a concept coined "developmental hemostasis" [5].

Laboratory Evaluation of Blood Coagulation

In the laboratory, secondary hemostasis is measured by determining the time required for a fibrin clot to form in platelet-poor plasma, when exposed to a coagulation activator and calcium. This system does not evaluate the platelet component of hemostasis, nor does it reflect the activity of the naturally occurring anticoagulants. Furthermore, lysis of the fibrin clot is evaluated using a distinct set of assays. Global screening assays that evaluate both the hemostatic and fibrinolytic systems require whole blood testing and are discussed in Chapter 5, "Hyperfibrinolysis." In addition to performing screening laboratory assays, the evaluation of a

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bleeding patient should include a complete history, physical examination, and review of medications, including prescribed and over-the-counter preparations, as well as any naturopathic remedies.

Secondary hemostasis can be evaluated using a simple battery of three assays which should be performed in all bleeding patients [1, 2]. These assays each measure the time to fibrin clot formation following the addition of different coagulation activators. The tests that evaluate secondary hemostasis include the prothrombin time (PT), activated partial thromboplastin time (aPTT), and functional fibrinogen level or a thrombin time (TT) (a surrogate fibrinogen assay when heparin is not in the specimen). In vitro, fibrin clot formation can be initiated through the intrinsic system by adding a contact activator (e.g., ellagic acid, silica, kaolin), through the extrinsic system by adding tissue factor, or it can be initiated by the addition of thrombin, each of which is added to separate aliquots of the anticoagulated plasma sample [1]. Calcium is required to reverse the anticoagulant effect of sodium citrate. Evaluation of functional fibrinogen or a thrombin time is needed in the evaluation of a bleeding patient, as the PT and aPTT are insufficiently sensitive to clinically significant decreases in fibrinogen levels. Important limitations of the PT and aPTT include the following: (1) both assays can be prolonged factitiously due to a number of different pre-analytical variables, (2) neither assay detects abnormalities of certain critical factors such as factor XIII and proteins in the fibrinolytic system nor platelet function defects, and (3) either assay may be prolonged due to conditions that do not increase bleeding risk [6]. A significant limitation of these screening assays is that in vitro fibrin clot formation does not adequately mimic the physiologic clotting process and does not evaluate the interaction of plasma factors with cellular components and the vasculature. Although the PT, aPTT, and functional fibrinogen assay are inadequate measures of in vivo hemostasis, these assays are a convenient and readily available means to provide, albeit limited, evaluation of secondary hemostasis.

Screening Coagulation Assays

Proper sample acquisition and handling is critical to obtaining accurate coagulation results [7–9]. Samples should be collected in 3.2% sodium citrate using the proper 9:1 blood to anticoagulant ratio. Incomplete filling of a sodium citrate tube, hematocrit >55%, or combining the contents of two incompletely filled sodium citrate tubes may lead to spuriously prolonged clotting times. While samples with hematocrits >55% require adjustment by reducing the amount of liquid anticoagulant in the collection tube, no adjustment is required for samples with reduced hematocrits. Samples should be collected from a peripheral vein, whenever possible, to avoid contamination from a port or intravenous infusion. Samples should be adequately mixed immediately following collection and processed in a rapid fashion. Hemolysis should be avoided as this may interfere with clot-based results.

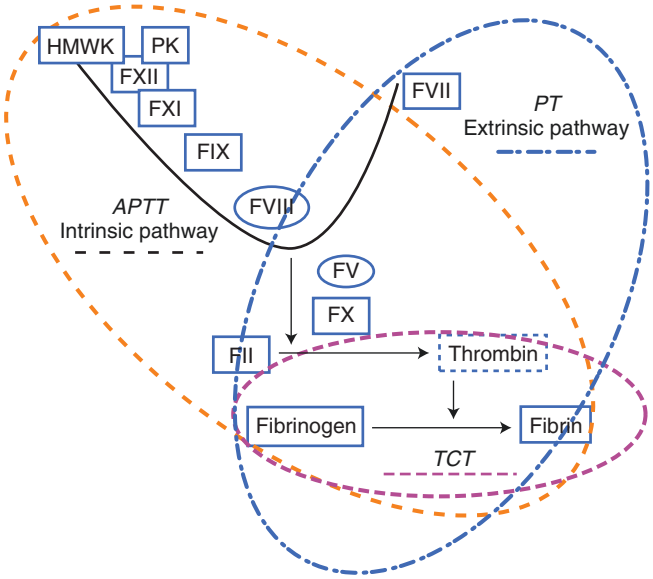

Fig. 2.1 In vitro model of secondary hemostasis

The PT evaluates factors of the extrinsic pathway (factor VII), common pathway (factors X, V, and II), and fibrinogen (Fig. 2.1) [2, 3, 10]. In the PT assay, coagulation is initiated by adding tissue factor and phospholipids (this combination is called thromboplastin) to recalcified plasma, and the time to form a fibrin clot is measured in seconds. Most PT reagents contain a heparin neutralizer, and therefore the PT does not typically elevate in the presence of heparin up to 1–2 units/mL depending on the PT reagent. The aPTT evaluates factors of the contact pathway (high molecular weight kininogen [HMWK], prekallikrein [PK], factor XII), intrinsic pathway (factors VIII, IX, and XI), and common pathway (factors X, V, and II) as well as fibrinogen [2, 3, 10]. In the aPTT, fibrin clot formation is initiated by addition of a contact activating agent, calcium, and phospholipid.

Prolongation of the aPTT or PT typically occurs when a single factor in the appropriate pathway falls below approximately 50% (a normal reference interval for factor activity is often in the range of 50–150%). An important caveat to this is that the aPTT is relatively insensitive to the clinically important intrinsic factors (factors VIII, IX, and XI), and the aPTT typically does not prolong until one of these factors falls below about 30% [3, 10]. Reagent responsiveness to factor deficiency varies between reagent manufacturers and, therefore, between laboratories. The aPTT may also elevate when multiple factors are in the lower end of the normal range, especially in a pediatric patient [11]. Neither the aPTT nor PT prolongation demonstrates a linear relationship with

decreased factor levels, and these assays prolong in an exponential fashion as functional factor concentrations decrease [12]. The thrombin time measures the time to fibrin clot formation following the addition of thrombin. The thrombin time is an indirect measure of functional fibrinogen, and in the evaluation of a bleeding patient, either a thrombin time or functional fibrinogen should be measured [13]. Functional fibrinogen is measured using a citrated plasma sample exposed to thrombin in the presence of phospholipids and calcium, which is called Clauss method. The time in seconds for fibrin clot to form is read in relationship to a standard curve of known fibrinogen concentration [1].

Once it is determined that the aPTT, PT, and/or TT are abnormal, plasma mixing studies can be performed to help determine if the prolongation of the clotting time reflects a factor deficiency or the presence of an inhibitor [2]. In the presence of a factor deficiency(ies), addition of an equal volume of normal plasma to the patient plasma typically corrects the clotting time into the normal reference interval. In the presence of a specific or non-specific factor inhibitor, addition of normal plasma typically does not lead to correction of the patient clotting time into the reference interval, although this is dependent on the reagent and type of inhibitor. Some factor inhibitors, such as specific factor VIII and factor V inhibitors, often require incubation at 37 °C for 1 to 2 hours in order to demonstrate their inhibitory effect [14]. Specific factor inhibitors are antibodies that neutralize the activity of a single coagulation factor and often lead to a bleeding diathesis. These antibodies are detected and their strength measured in the laboratory using specific coagulation factor inhibitor assays commonly referred to as a Bethesda assay [14]. Non-specific inhibitors interfere with multiple factors or can interfere with a reagent component such as phospholipid. Lupus anticoagulants and certain anticoagulant drugs including heparin, direct Xa, and direct thrombin inhibitor agents act as non-specific inhibitors in the coagulation laboratory and may lead to incomplete correction in normal plasma mixing studies [14, 15]. In order to determine the basis of aPTT, PT, and/or TT prolongation, further testing such as specific factor assays or evaluation for a lupus anticoagulant may need to be performed.

Abnormalities of the aPTT and PT are common in the critically ill as well as the trauma patient. In trauma patients, a PT and/or aPTT ratio >1.5 (compared to the control) predicts excessive bleeding [16]. However, in trauma patients, these parameters are not entirely useful within the first 1–2 hours of the trauma or until the patient stabilizes [17]. In hypothermic patients, the values of the PT and aPTT may underestimate the coagulopathy because the PT and aPTT reactions are performed on samples warmed to 37 °C [17].

A number of hemorrhagic diatheses may occur despite a normal aPTT, PT, and functional fibrinogen level (see Table 2.1). A normal aPTT does not rule out mild deficiencies of factor VIII, IX, and/or XI as these factor activities must fall to 20 to 40% or less, depending on the reagent used in the assay and how the reference range is established, before the aPTT prolongs [10]. FXIII activity, platelet dysfunction, and fibrinolytic factors are not assessed by these screening assays. The most important contributors to bleeding that are missed by the PT and aPTT are platelet dysfunction and hyperfibrinolysis [16].

Table 2.1 Bleeding with a normal APTT and PT and TT/fibrinogen

Cause	Comment
Mild factor VIII, factor IX, or factor XI deficiency	Single factors generally have to fall below approximately 30% before the aPTT is prolonged
Hereditary or acquired FXIII deficiency	Can lead to significant bleeding potential with spontaneous bleeding
Alpha 2-antiplasmin deficiency	
Abnormal platelet number or function	
Vascular or collagen abnormality	Such as Ehlers-Danlos syndrome, vasculitis
Anatomical cause	

Table 2.2 Isolated prolonged PT (normal aPTT and normal fibrinogen/TT)

Cause	Comment
Deficiency or inhibitor of factor VII	Severe deficiency may be associated with spontaneous bleeding. Factor VII is the first factor to decrease with vitamin K deficiency or antagonism and liver disease. Inhibitor is rare
Mild deficiency of factor X, factor V, or factor II	Single factor deficiencies in the 30–60% range may lead to isolated prolongation of the PT. Moderate to severe deficiency causes prolongation of aPTT as well
Anticoagulant therapy: direct Xa inhibitor anticoagulant (e.g., rivaroxaban, apixaban, edoxaban)	May cause prolongation of the aPTT depending on drug concentration. Will not affect the TT

Prolonged PT, Normal aPTT, and Normal Functional Fibrinogen

When the PT is prolonged but the aPTT and functional fibrinogen (or TT) are normal, a deficiency of factor VII should be considered (see Table 2.2) [1, 2, 18]. Mild deficiencies of factors X, V, and II may also cause isolated prolongation of the PT, as PT reagents are more sensitive to deficiencies of these common pathway factors than are aPTT reagents [19]. Certain drug therapies, such as direct Xa inhibitor anticoagulants and warfarin, can cause isolated prolongation of the PT but can also prolong the aPTT with stable warfarin therapy [15, 20].

In the presence of factor deficiency, a PT mixing study generally demonstrates correction unless the prolongation is due to vitamin K deficiency, vitamin K antagonism, or sometimes liver disease, where the PT mixing study tends to demonstrate near correction into the normal reference interval, but not completely within the normal reference interval. For example, if the normal interval of the PT is 11–14.1 seconds and a patient has a PT result of 35 seconds, a mixing study that demonstrates complete correction would have a 1:1 mix result that falls into reference interval (11–14.1 seconds), and one that demonstrates near correction may correct to a PT of 14.2–15 seconds. A PT mix that demonstrates incomplete correction would have a 1:1 mix result usually above about 18 seconds (with a normal reference interval of 11–14.1 seconds), and this occurs in the presence of a factor inhibitor such as a FVII inhibitor. This may also occur with a direct thrombin or Xa anticoagulant or can occur with a specific factor V, X, or II inhibitor, although each of these drugs and inhibitors typically also elevates the aPTT [20–22]. Select factor II (prothrombin) inhibitors, specifically those that occur in association with lupus anticoagulants, commonly function as clearing rather than neutralizing antibodies [23, 24]. See Chapter 23. These antibodies cause prolongation of the PT due to rapid clearance of the factor II-antibody complex, resulting in low factor II levels and hence factor II deficiency. A PT normal plasma mixing study demonstrates correction as these clearing coagulation factor inhibitors will not be detected with a factor inhibitor (Bethesda) assay.

Factor VII has the shortest half-life of all coagulation factors and therefore is the first factor to decrease with liver disease as well as vitamin K deficiency or vitamin K antagonists. Hereditary deficiency of factor VII is rare as is the development of acquired inhibitors to FVII [1, 25, 26]. Deficiency of factor VII prolongs the PT without affecting the aPTT or TT. A hereditary deficiency of any of the common pathway factors (factors X, V, and II) is rare [1, 25]. Heterozygous (mild) deficiencies of any of these factors may or may not be associated with bleeding. Bleeding is more typical of heterozygous factor II deficiency than heterozygous factor V, VII, or X deficiency [1, 25]. Spontaneous bleeding associated with these rare heterozygous factor deficiencies typically involves skin and mucous membranes. Heterozygous deficiency of factor V, X, or II may present with an isolated elevated PT [19]. Homozygous deficiency of any one of the common pathway factors would lead to significant factor deficiency and cause prolongation of both the PT and aPTT and may lead to a significant bleeding tendency, both spontaneous and with provocation [25]. Acquired deficiency of factor X can occur with amyloidosis and, depending on the level of deficiency, may lead to an isolated, elevated PT and increased bleeding risk [19].

Prolonged aPTT, Normal PT, and Normal Functional Fibrinogen (or Normal TT)

Prolongation of the aPTT with a normal PT and functional fibrinogen (normal TT) may reflect a deficiency or inhibitor of a contact pathway factor (factor XII, HMWK, PK), deficiency or inhibitor of an intrinsic pathway factor (factor VIII, IX, XI), or the presence of a lupus anticoagulant (see Table 2.3) [1–3, 27]. Heparin therapy or contamination, as well as direct thrombin inhibitor (DTI) anticoagulants, elevates both the aPTT and thrombin time [15]. Certain PEGylated drugs (e.g., PEG interferon) may cause prolongation of the aPTT, yet this PEG interference poses no increased bleeding risk [28]. Select lipoglycopeptide antibiotics including daptomycin and telavancin may elevate the aPTT, and in some circumstances the PT, without increasing bleeding risk [29, 30]. The degree of prolongation depends on the concentration of the antibiotic in the plasma and the reagent sensitivity to the drug. It has been reported that elevated levels of C-reactive protein may cause spurious elevation of the aPTT with commonly used aPTT reagents [3]. Severe contact factor deficiencies typically greatly prolong the aPTT, yet clinically are not associated with an increased bleeding potential. Mild deficiencies of a contact factor pathway generally will not affect the aPTT.

Table 2.3 Isolated prolonged aPTT (normal PT and TT/fibrinogen)

Cause	Comment
Intrinsic factor (factor XI, factor IX, factor VIII) deficiency or inhibitor	Severe deficiency may be associated with spontaneous bleeding; aPTT may be normal with mild single factor deficiency
Contact factor (factor XII, prekallikrein, high molecular weight kininogen) deficiency or inhibitor	Severe deficiency may lead to marked prolongation of the aPTT, but no increased bleeding risk. Heterozygous deficiency of a single contact factor will not likely affect the aPTT
Acquired factor VIII, factor IX, or factor XI inhibitors	Factor VIII inhibitor by far the most common and may lead to severe spontaneous bleeding, can occur as alloantibody (in hemophilia A) or autoantibody; inhibitors against factor IX or XI are rare
Lupus anticoagulant (LA)	Increases risk for thrombosis and obstetric complications. Leads to bleeding only when LA is associated with thrombocytopenia or deficiency of prothrombin (factor II). Factor II deficiency would cause a prolonged PT
Select PEGylated drugs	Prolongation of APTT is not associated with increased bleeding risk
Lipoglycopeptide antibiotics, select	Select agents effective against MRSA[a] such as daptomycin and telavancin. Prolongation of aPTT is not associated with increased bleeding risk. May also prolong the PT depending on plasma drug concentration and reagent used in laboratory

[a]Methicillin-resistant *Staphylococcus aureus*

Table 2.4 Causes of factor VIII deficiency

Cause	Comment
Hemophilia A	X-linked; therefore typically males affected; female carriers may bleed with provocation
von Willebrand disease (VWD)	In type 2N VWD, VWF activity and antigen may be normal and FVIII activity reduced
Acquired hemophilia A	May be associated with severe spontaneous bleeding
Acquired von Willebrand syndrome	May be associated with severe spontaneous bleeding
Spurious decrease	Can occur with some lupus anticoagulants or incorrect sample type (i.e., serum or EDTA plasma)

In a previously healthy male or female patient with a prolonged aPTT and normal PT and fibrinogen, who presents with acute, possibly catastrophic, spontaneous hemorrhage into soft tissues and muscle, acquired hemophilia (an acquired factor VIII inhibitor) or acquired von Willebrand syndrome (AVWS) should be considered (see Table 2.4) [31–34]. Factor VIII inhibitors (acquired hemophilia A) can develop in an older population for no apparent reason, or they may present in patients with underlying autoimmune disorders, underlying solid tumors, or lymphoproliferative malignancies and may also occur in association with pregnancy [31, 32]. Acquired hemophilia A should be considered early in the evaluation of abnormal bleeding in the postpartum setting [35]. FVIII inhibitors may develop 1 to 4 months and rarely as late as 1 year postpartum. Acquired factor VIII inhibitors are rare in children but have been reported [36]. In the presence of a FVIII inhibitor, the aPTT is elevated, while the PT is normal, and aPTT mixing studies may or may not demonstrate correction upon immediate mix, but typically demonstrate prolongation with incubation over time at 37 °C. Factor VIII activity and FVIII inhibitor (Bethesda) assays should be performed and also possibly von Willebrand factor activity and antigen assays (see next paragraph). Acquired inhibitors to factor VIII are the most frequent acquired factor inhibitors reported. Acquired inhibitors to factor IX or XI are rare [37, 38].

Acquired factor VIII deficiency can also occur as a feature of AVWS [34, 39, 40]. In a bleeding patient without a history of hemophilia A, who has a low factor VIII activity (<10%), von Willebrand factor antigen and activity should be measured to rule out AVWS, especially if there is no evidence of a specific FVIII inhibitor. AVWS may occur in both pediatric and adult patients [39]. There are many different underlying conditions that are associated with AVWS such as underlying lymphoproliferative disorders, certain cardiac valve disorders, ventricular septal defects, essential thrombocythemia, Wilms tumor, and hypothyroidism, to name a few. The laboratory diagnosis of AVWS is essentially the same as hereditary von Willebrand disease (VWD), and both type 1 (quantitative) and type 2 (qualitative) deficiencies may occur.

Congenital factor VIII (hemophilia A) and IX deficiencies (hemophilia B) are X-linked disorders and therefore typically present in males and rarely in females (except in specific situations, e.g., skewed lyonization, homozygosity for the hemophilia gene resulting from consanguinity, and deletions such as Turner syndrome), with an isolated prolonged aPTT and isolated deficiency of either factor VIII or factor IX [41–43]. Severe hemophilia A or B (≤1% factor VIII or IX activity, respectively) presents with spontaneous hemorrhage, while moderate (2–5% factor level) to mild (6–40% factor level) hemophilia may go undiagnosed until a patient is challenged. Hemophiliacs may develop specific factor inhibitors in response to factor replacement therapy, and when present this significantly complicates replacement therapy. Female carriers of hemophilia A and B have an increased bleeding tendency, even when factor levels are in the 40–60% range, especially when their hemophiliac relatives have a severe form of the disease [42]. Factor levels are not always a good predictor of bleeding in hemophilia carriers [44]. Recent studies have highlighted the increased incidence of postpartum hemorrhage, in the range of 20–40%, in this population [45].

Factor XI deficiency (hemophilia C) is autosomal in inheritance and affects both males and females [46, 47]. The incidence of factor XI deficiency in most populations is 1 in 1,000,000, although it is significantly greater in an Ashkenazi Jewish population occurring at a frequency of 1 in 450. Severe deficiency is defined by factor levels less than 15%. The bleeding tendency is variable and does not always correlate to factor XI activity levels, but is more likely to occur with severe deficiency and when an injury involves an area with high fibrinolytic potential, such as the oral cavity or urogenital tract. Spontaneous bleeding with hemophilia C is rare. Bleeding typically occurs only with provocation. Development of inhibitors in response to replacement therapy is unusual but reported. Acquired factor XI inhibitors are rare and may occur in those with underlying autoimmune disorders [37, 38].

VWD is the most common inherited bleeding disorder. It has an autosomal mode of inheritance and therefore affects both males and females. VWD is due to a deficiency or defect of von Willebrand factor (VWF) [1, 40, 48, 49]. VWF serves as the carrier protein for procoagulant factor VIII and also serves to bind platelets to the site of vascular injury, and therefore, VWF serves an important role in primary hemostasis. When VWF is decreased, factor VIII activity is decreased concordantly. The aPTT is not an adequate screen for VWD as the aPTT will not prolong until factor VIII levels fall below 20–30%, depending on the reagent [10]. To screen for VWD, VWF antigen and activity, as well as factor VIII activity, should be measured in plasma. Deficiencies of VWF alone do not affect the aPTT, PT, or thrombin time.

Lupus anticoagulants (LA) are a common cause of an isolated prolonged aPTT. Although historically termed an "anticoagulant," lupus anticoagulants are more commonly associated with an increased thrombotic risk and risk of certain obstetric complications, rather than increased bleeding risk [27, 50]. LA are non-specific inhibitors, and aPTT mixing studies generally demonstrate incomplete correction, although this is reagent dependent. Diagnosis of the presence of LA is made by comparing the results of phospholipid dependent assays performed in the presence of low and high phospholipid concentrations. Shortening of the clotting time in the presence of increased phospholipids is characteristic of LA [27, 50]. As PT reagents contain a greater phospholipid concentration compared to aPTT reagents, most PTs are not prolonged in the presence of LA, although certain PT reagents may demonstrate sensitivity to lupus anticoagulants. Lupus anticoagulants may interfere with the phospholipids required in factor VIII, IX, and/or XI activity assays, making the activities appear factitiously low. Assay interference should always be considered in a patient without bleeding, but with decreased factor VIII, IX, and XI activity results, especially when the values of all three factors are low. If LA interference is suspected, a chromogenic factor VIII or factor IX activity assay should be used as these assays are more accurate in the presence of LA [51]. Another option is to measure the intrinsic factors using an aPTT reagent that is not LA sensitive, and this may require sending the sample to a reference laboratory. In contrast to spurious LA interference in intrinsic factor assays where factors VIII, IX, and XI may appear decreased, severe liver disease is associated with decreased factor IX and XI activities but normal to elevated factor VIII activity. In vitamin K deficiency/antagonism, factor IX activity is low and factor VIII and XI activities are normal.

Prolonged TT, Normal PT, and Normal aPTT

In practice, this pattern of results occasionally occurs due to the presence of low molecular weight heparin or unfractionated heparin (either from low-dose therapy or contamination) or direct thrombin inhibitor (DTI) therapy (see Table 2.5) [20]. As drug concentration increases, the aPTT will elevate with heparin, and both the aPTT and PT prolong in the presence of DTI [15]. In fact, many laboratories perform a thrombin time as a quality control measure in an effort to rule out heparin therapy or contamination of a sample. In general, conditions or drugs that prolong the thrombin time that would lead to spontaneous or an enhanced bleeding diathesis would also elevate the PT and aPTT. The thrombin time is sensitive to both the amount and functionality of fibrinogen. Thus, both hypofibrinogenemia and dysfibrinogenemia may elevate the thrombin time [1, 52, 53]. Typically, the aPTT and PT will elevate when fibrinogen levels fall below around 80 to 100 mg/dL, but this depends on the reagent used in the laboratory.

Table 2.5 Isolated prolonged TT (normal aPTT and normal PT)

Cause	Comment
Deficiency of fibrinogen	Fibrinogen levels less than approximately 100 mg/dL result in prolongation of the PT and aPTT. The PT is more sensitive to low fibrinogen than the PTT
Abnormal fibrinogen (dysfibrinogen)	Tends to cause prolongation of the PT and aPTT, although the TT is the most sensitive assay. May be associated with major hemorrhage
DOAC of anti-Xa action	The PT and aPTT may be prolonged or normal
Fibrin split products at high concentration	Interferes with fibrin polymerization and may lead to prolongation of the PT and/or aPTT, although TT is most sensitive. Does not increase bleeding risk by itself
Monoclonal antibodies, select	Interferes with fibrin polymerization and may lead to prolongation of the PT and/or aPTT, although TT is most sensitive. Does not increase bleeding risk by itself

Substances that can interfere with fibrin polymerization such as fibrin(ogen) degradation products and paraproteins may elevate the TT, but are not consistently associated with an enhanced bleeding potential [54]. Interference with fibrin polymerization may also cause prolongation of the aPTT and PT, although the TT is the most sensitive of the three assays. Inhibitors of thrombin activity, such as antibodies to thrombin formed after exposure to thrombin glue, may elevate the TT but typically elevate the aPTT and PT as well [55]. Increased level of D-dimer is another cause of the prolongation of the TT.

Prolonged PT and Prolonged aPTT, Normal Functional Fibrinogen (or Normal TT)

Prolongation of the PT and aPTT with a normal functional fibrinogen (or normal TT) may reflect multiple factor deficiencies, a deficiency or inhibitor of a common pathway factor (factors X, V, and II), vitamin K deficiency, vitamin K antagonist (warfarin), superwarfarin (rat poison), or an anti-Xa inhibitor anticoagulant (see Table 2.6) [1–3, 15, 20, 56]. Some lipoglycopeptide antibiotics, such as daptomycin or telavancin, may elevate the aPTT and PT, presumably due to interference with the phospholipids required in the assay, but are not associated with an increased bleeding risk [29, 30]. aPTT and PT mixing studies demonstrate correction with factor deficiency(ies). An exception to this is factor II inhibitors that develop in association with a lupus anticoagulant as these antibodies are clearing and not neutralizing [23, 24]. Incomplete correction of both aPTT and PT mixing studies

Table 2.6 Prolonged aPTT and prolonged PT (normal fibrinogen/TT)

Cause	Comment
Vitamin K deficiency or vitamin K antagonists (warfarin or rat poison)	Can lead to significant bleeding potential with spontaneous bleeding
Factor X, factor V, or factor II deficiency	Severe deficiency may lead to spontaneous bleeding. Mild deficiency does not typically prolong the aPTT
Multiple factor deficiencies	Due to factor deficiencies involving both the intrinsic and extrinsic pathways or a single deficiency in the common pathway factors X, V, and II
Liver disease	If severe, may prolong the thrombin time due to hypofibrinogenemia or dysfibrinogenemia
Anticoagulant therapy: direct Xa inhibitor anticoagulant and warfarin	The PT is more sensitive to drug effect than is the aPTT
Dilutional coagulopathy	Associated with massive transfusion or massive fluid resuscitation
Lupus anticoagulant with hypoprothrombinemia (see Chapter xx)	May result in significant bleeding potential; the inhibitor causing decreased factor II is a clearing and not a neutralizing antibody, and therefore the PT mix typically corrects, and a factor II inhibitor titer is negative
Spurious, i.e., Hct >55% and volume of sodium citrate in collection tube not corrected, short draw, incorrect sample type (i.e., serum or EDTA plasma)	Not associated with an enhanced bleeding potential

occurs in the presence of a factor V inhibitor, factor X inhibitor, or Xa inhibitor anticoagulant therapy. While a direct thrombin inhibitor (DTI) anticoagulant can also prolong the aPTT and PT, the thrombin time would also be prolonged [20]. A lupus anticoagulant may rarely elevate both the aPTT and PT with certain PT reagents, although the PT is typically only mildly prolonged in these instances.

In a previously healthy male or female patient with a prolonged aPTT and PT and normal thrombin time or functional fibrinogen, who presents with acute, spontaneous hemorrhage, acquired vitamin K deficiency, or antagonism, should be strongly considered [15]. Vitamin K is crucial to the synthesis of functional factors II, VII, IX, and X. Infants are born naturally deficient in vitamin K, and it should be administered shortly after birth [57]. Infants who have not received vitamin K may suffer life-threatening intracranial and retroperitoneal hemorrhage occurring between days one to seven of life. Although a fat-soluble vitamin, a daily requirement exists because vitamin K is not effectively stored in the body [58]. Vitamin K is obtained through diet (e.g., leafy green vegetables) and intestinal flora. Deficiency of vitamin K should be considered particularly in patients who have experienced prolonged antibiotic use and malnourishment and in patients with biliary obstruction. Individuals with fat malabsorption disorders, including inflammatory bowel disease and cystic fibrosis, as well as individuals administered certain medication including cephalosporin, cholestyramines, anticonvulsants, and certain sulfa drugs, may be at increased risk of vitamin K deficiency [59]. Typical presenting symptoms include easy bruising and bleeding that may manifest as nosebleeds, bleeding gums, blood in the urine, blood in the stool, and tarry black stools. Bleeding may be severe and manifest as life-threatening intracranial and retroperitoneal hemorrhage. With vitamin K deficiency or warfarin, the aPTT and PT are elevated, and both may be so prolonged they yield "no clot detected." The thrombin time is normal as is fibrinogen, unless there is significant bleeding leading to consumption. Normal plasma mixing studies demonstrate correction of the aPTT into the normal range and typically near correction of the PT. Factor assays reveal decreased factor II, VII, IX, and X activities (the vitamin K-dependent factors) and normal factor XI, VIII, and V activities. In contrast, in liver disease, all factors (including functional fibrinogen) are decreased except for factor VIII, and in severe liver disease, the thrombin time tends to be elevated. In disseminated intravascular coagulation (DIC), all factors may be decreased due to consumption, including factor VIII, and fibrinogen may also be significantly decreased. A relatively efficient screen to distinguish vitamin K deficiency/warfarin from liver disease in a patient with a prolonged aPTT and PT, but normal TT, is to perform factor IX and V activities. With vitamin K deficiency/warfarin, factor IX is decreased, but factor V normal. In liver disease, both factor IX and V activities are decreased (see Table 2.7).

Anticoagulant rodenticides (rat poisons) are long-acting anticoagulants similar to warfarin [56, 60–62]. They act as vitamin K antagonists but are significantly more potent and longer-acting than warfarin. Also referred to as superwarfarins, anticoagulant rodenticides include bromadiolone, chlorophacinone, difethialone, diphacinone, and brodifacoum [61]. Brodifacoum is the agent most commonly used as a rat poison in the United States. Anticoagulant rodenticides are toxic when eaten or inhaled and when they come in contact with the skin. Clinical manifestation depends on the severity of the exposure and may result in fatal hemorrhage. Simple ingestion of a single dose, as may occur in a pediatric population, is usually asymptomatic [62]. With severe poisoning, the aPTT and PT may result in "no clot detected" while the thrombin time remains normal. Confirmation of rodenticide anticoagulant exposure requires serum determination of their presence. These agents can be measured using high-pressure liquid chromatography or mass spectrometry [63]. Serum warfarin shows no cross-reactivity with superwarfarins, and

Table 2.7 Impact of common coagulopathies on the aPTT, PT, and TT

Disorder	PT	APTT	Thrombin time	Comment
Vitamin K deficiency/ antagonism including rat poison	↑	↑	→	With rat poison, the aPTT and PT improve with administration of vitamin K but prolong with time as more rodenticide is released from the adipose tissue
FVIII deficiency	→	↑	→	FVIII deficiency can be hereditary or acquired
Liver disease	↑	↑	→↑	PT prolongs initially followed by aPTT and then thrombin time when severity increases
Disseminated intravascular coagulation	↑	↑	↑	

a serum warfarin assay will not detect their presence. Because these agents are lipophilic, their effect can be long-lasting. A classic feature of anticoagulant rodenticide poisoning is that the PT and aPTT are both greatly prolonged and correct with the administration of large amounts of vitamin K (ranging from 50 to 800 mg), only to prolong again with time as the rodenticide is released from the adipose tissue. Vitamin K must often be administered for prolonged periods of time on a daily or twice daily basis, sometimes up to 1 year's duration [56, 62].

Prolonged PT and Prolonged aPTT and Decreased Fibrinogen (or Prolonged TT)

This pattern may occur with a significantly decreased or abnormal fibrinogen (where the functional fibrinogen value is less than 80 to 100 mg/dL), severe liver disease, or multiple factor deficiencies as may occur with DIC. This pattern of results may also occur with DTI therapy, depending on the plasma concentration of DTI and reagent sensitivity to drug (see Table 2.8).

With liver disease, patients tend to present with bruising, epistaxis, bleeding from venipuncture sites, oral mucosa, gastrointestinal mucosa, and esophageal varices [64]. The etiology of the coagulopathy in liver disease is complex [65]. Liver disease will lead to impaired synthesis of all factors produced by the liver, as well as impaired clearance of activated factors, and increased fibrinolysis. It is important to note that both pro- and anticoagulant factors will be reduced in liver disease and thus the overall balance may not necessarily lead to anticoagulation, and in fact, thrombotic events, particularly of the mesenteric and portal veins, may occur in patients with cirrhosis [66, 67]. Further, while most coagulation factors are produced by the liver, VWF and factor VIII are not. Thus, in liver disease, all coagulation factors tend to decrease with increasing disease severity (although typically 90% of more of hepatic functionality must be lost before factor levels tend to decrease), with the exception of factor VIII, which may elevate as an acute phase protein, and this may somewhat compensate physiologically for decrease in liver-produced procoagulants. Diseases of the liver and biliary tract tend to more significantly impair production of vitamin K-dependent coagulation factors, and thus the PT will tend to be more dramatically prolonged than the aPTT (as compared to other processes such as DIC). Fibrinogen also decreases with severe liver disease and often becomes dysfunctional. Fibrinogen function may decrease over time as the fibrinogen becomes dysfunctional (even if the fibrinogen antigen levels do not) due to increased sialic acid residues, and this may, in turn, elevate the thrombin time (or decrease functional fibrinogen levels) [68]. To effectively distinguish

Table 2.8 Prolonged aPTT, prolonged PT, and prolonged TT

Cause	Comment
Severe liver disease	Can lead to significant bleeding potential with spontaneous bleeding
Disseminated intravascular coagulation	Can lead to significant bleeding potential with spontaneous bleeding; elevated D-dimer levels
Fibrinogen deficiency or dysfibrinogenemia	May result in significant bleeding potential
Anticoagulant therapy: direct thrombin inhibitor anticoagulant	May increase bleeding depending on plasma drug level
Thrombolytic therapy	May lead to significant bleeding potential with spontaneous bleeding; plasminogen is also typically decreased

liver disease from vitamin K deficiency or antagonism, factors V, VIII, and VII (or one of the other vitamin K-dependent factors) should be measured. With vitamin K deficiency, factors V and VIII will be normal and factor VII decreased, while with liver disease factors V and VII are decreased, while factor VIII is normal to elevated.

DIC may be difficult to distinguish from severe liver disease, particularly since these entities may occur together [69]. In contrast to liver disease, in DIC, fibrin degradation products and D-dimer levels tend to be more greatly elevated, both PT and aPTT are often both markedly elevated due to consumption of all factors, the platelet count is decreased to a greater degree, and signs of microangiopathic anemia may be seen on blood smear (e.g., schistocytes). Also, DIC is a more dynamic and unstable process, and thus coagulation screening tests, platelet count, and fibrin degradation products vary more over time in DIC as compared to liver disease. Fairly rapidly changing coagulation parameters over time is an important distinguishing laboratory feature.

Abnormalities of fibrinogen, either deficiency of or a dysfunctional fibrinogen protein, may lead to elevation of the aPTT, PT, and thrombin time. In patients with dysfibrinogenemia, all three screening assays are typically prolonged, while the thrombin time is the most sensitive of the three [52, 53, 69, 70]. A reptilase time, an assay similar to the thrombin time that measures the conversion of fibrinogen to fibrin, is also typically prolonged with dysfibrinogenemia [52]. A reptilase time may be used to differentiate prolongation of the thrombin time due to heparin from a fibrinogen abnormality, as the reptilase time is normal in the presence of heparin but elevated with a hypo- or dysfibrinogenemia. In dysfibrinogenemia there is a discrepancy between the concentration of fibrinogen measured by immunologic methods and its functional activity based on a clotting assay [52]. Typically with dysfibrinogenemia, fibrinogen antigen levels are normal, while fibrinogen functional activity is low, although multiple variations have been reported [69]. Congenital dysfibrinogenemia may be asymptomatic or may be associated with either a bleeding or thrombotic tendency. In those with a bleeding tendency, 11% report major bleeding and often present with bleeding following surgery or trauma. Postpartum hemorrhage is also a common presentation as is menorrhagia [71]. Patients may report easy bruising and prolonged bleeding with minor injuries. Spontaneous life-threatening bleeding is rare [71]. Acquired dysfibrinogenemia may also occur in association with cirrhosis of the liver and hepatocellular carcinoma [70].

Hypofibrinogenemia and afibrinogenemia can be hereditary or acquired and may lead to elevation of the aPTT, PT, and thrombin time. Afibrinogenemia is an autosomal recessive disorder with an incidence of 1–2 cases per million [1, 43]. Bleeding manifestations range from mild to severe, and bleeding associated with surgery or trauma is common. Most cases manifest in the neonatal period but can present at a later age. The major cause of death is intracranial hemorrhage. Another characteristic feature of afibrinogenemia is spontaneous splenic rupture [43]. In distinction, with hereditary hypofibrinogenemia, patients tend to bleed with provocation rather than suffer spontaneous bleeding, and fibrinogen levels are generally in the range of 100 mg/dL. Afibrinogenemia may also occur as an acquired condition in association with exposure to certain snake venoms, such as the Western diamondback rattlesnake [72, 73]. These patients present with greatly elevated aPTT and PT (possibly even "no clot detected") with immeasurable fibrinogen levels. Clinical bleeding in these cases is variable.

Normal PT, Prolonged aPTT, and Prolonged TT with Normal or Low Fibrinogen

This pattern of results suggests the presence of an anticoagulant including heparin or a DTI (see Table 2.9). Depending on the plasma concentration of DTI and reagent responsiveness, the PT may also elevate. As most PT reagents contain a heparin neutralizer, the PT tends not to elevate in the presence of heparin or heparin-like anticoagulants unless the anticoagulant present is so great in concentration that it overwhelms the heparin neutralizer in the PT reagent. Heparin contamination of blood samples is not uncommon when samples are collected through a port or indwelling catheter. In general, laboratory neutralization of heparin will correct the aPTT and thrombin time to normal range (in an otherwise normal specimen); however, larger concentrations of heparin may not be entirely neutralized by laboratory protocols, and thus the thrombin time or, less often, the aPTT may show residual elevation even after neutralization. Residual elevations of the aPTT and thrombin time following heparin

Table 2.9 Prolonged aPTT, normal PT, and prolonged TT with normal or low fibrinogen

Cause	Comment
Anticoagulant therapy: direct thrombin inhibitor anticoagulant (dabigatran)	The TT is exquisitely sensitive such that a normal TT can rule out significant dabigatran effect. May cause prolongation of the aPTT depending on drug concentration
Anticoagulant therapy: heparin or heparin contamination	Typically does not prolong the PT as PT reagents contain heparin neutralizers
Acquired heparin-like inhibitor (see Chapter xx)	A very rare acquired cause of bleeding, may occur with anaphylaxis or certain malignancies. Tends to prolong the aPTT as well, but not the PT as PT reagents contain heparin neutralizers. Anaphylaxis may also be associated with hyperfibrinogenolysis leading to reduced fibrinogen activity levels

neutralization may require investigation (preferably when the patient is off heparin therapy).

Heparin-like anticoagulants may develop rarely in association with certain malignancies and have been described following anaphylaxis induced by a wasp sting. See Chapter xx. This has been shown to elevate the thrombin time and greatly elevate the aPTT and may lead to measurable heparin levels in a chromogenic anti-Xa assay. Anaphylaxis is associated with mast cell activation and secretion of mediators, including heparin [74]. Secretion of tryptase from mast cells in anaphylaxis may lead to hyperfibrinogenolysis and decreased fibrinogen activity.

As mentioned in the previous section, clinically significant defects or deficiencies of fibrinogen will generally elevate the thrombin time, aPTT, and PT.

Factor XIII

Factor XIII (FXIII), also known as fibrin stabilizing factor, is necessary for the formation of a firm hemostatic plug. FXIII functions to stabilize the clot by cross-linking fibrin molecules and renders the clot resistant to fibrinolysis [75]. A deficiency of factor XIII can be hereditary or acquired. Hereditary deficiency, an autosomal condition, can be classified as severe (less than 2–5% FXIII), moderate (5–30%), and mild (30–60%). Severe factor FXIII deficiency has an estimated incidence of one in four million. Patients with severe deficiency may present with spontaneous major hemorrhage including hemarthrosis, subcutaneous hemorrhage, and intracranial bleeding, which is the leading cause of death. In women during their reproductive years, intraperitoneal bleeding may occur with ovulation. Bleeding may be delayed following surgery or trauma due to premature lysis of the hemostatic plug. Moderate deficiency may present with mild spontaneous bleeding or bleeding with provocation. Heterozygous factor XIII deficiency has an estimated frequency of 1 in 1000. Patients have plasma levels in the range of 30–60% and do not bleed spontaneously, but rather with provocation.

Acquired FXIII deficiency may reflect decreased synthesis, increased consumption, or inhibitor development [76, 77]. Consumption may occur with sepsis, trauma, or DIC and generally leads to levels in the range of 30–60%. Whether this enhances bleeding potential in these conditions must be proven. Inhibitor development is rare and can develop in deficient patients following replacement therapy but is more likely to develop de novo in association with another disease such as systemic lupus erythematosus or lymphoproliferative disorders or in response to certain medications (e.g., penicillin, ciprofloxacin, isoniazid, phenytoin). Bleeding with a FXIII inhibitor may be life-threatening and is difficult to treat. Morbidity associated with factor XIII inhibitors is high, even when treated. The presence of an inhibitor can be investigated by identifying low to undetectable FXIII activity levels and then performing FXIII activity mixing studies which demonstrate lack of correction with addition of normal plasma.

Deficiency of factor XIII does not affect the aPTT, PT, or thrombin time, which are each normal. A qualitative urea solubility test is often used to screen for FXIII deficiency, although this will only detect severe deficiency (<1–2%) and this test is no longer recommended. A quantitative FXIII activity assay is the recommended assay to evaluate functional factor XIII levels and to make a diagnosis of hereditary or acquired FXIII deficiency, though this assay may be of limited availability.

Quantitative D-Dimer

D-dimer is a terminal degradation product from the breakdown of fibrin. Unlike other fibrin degradation products, D-dimer is formed only after fibrin has been cross-linked by activated factor XIII and lysed by plasmin [78]. Quantitative D-dimer is most often used in the evaluation of venous thrombosis and DIC. There are many varied conditions, however, associated with an elevated D-dimer level [78, 79]. D-dimer will increase post-operatively and in normal pregnancy, as well as in a variety of pathologic states including venous thrombosis, DIC, consumptive coagulopathy associated with certain snakebites, visceral malignancies, and atherosclerotic vascular disease to name a few. Also, because fibrin degradation products are metabolized by the liver and secreted by the kidneys, both liver and kidney disease can affect D-dimer clearance and, hence, plasma levels. Thus, elevation of D-dimer is non-specific and must be viewed in context with other laboratory results and clinical history.

Because D-dimer represents the breakdown products of cross-linked fibrin clot, clinical conditions that cause breakdown of early fibrin formation or lysis of fibrinogen result in hypofibrinogenemia and elevated fibrin degradation products, but not elevated D-dimer levels. Examples include treatment with thrombolytic therapy and primary hyperfibrinogenolysis, as may occur with some prostate cancers [80].

The D-dimer assay is often performed in the bleeding patient, along with the clot-based coagulation screening assays, to determine the presence of in vivo clot formation and breakdown, particularly in a patient with suspected DIC. In DIC, the aPTT and PT are often prolonged, and the fibrinogen decreased, due to activation of coagulation and ongoing consumption. The platelet count is typically decreased and/or shows a decreasing trend over time. Both fibrin degradation products and D-dimer levels are elevated in the majority of cases due to fibrinolysis. Importantly, as DIC is a dynamic and unstable process, serial monitoring of

these parameters is often necessary to make the diagnosis, or to follow progression of response to treatment over time. Increasing PT, aPTT, and D-dimer levels with a decreasing platelet count are highly suggestive of DIC. In addition, the trends in PT, aPTT, and platelet count may help guide transfusion therapy in a bleeding patient or a patient undergoing an invasive procedure. DIC is discussed in more detail in Chapter 16, "Bleeding Associated with Disseminated Intravascular Coagulation."

Snakebite coagulopathies may closely mimic DIC. Snake venoms from *Agkistrodon* snakes, such as copperheads, contain thrombin-like enzymes and FX activators and lead to a venom-induced consumption coagulopathy associated with prolongation of the aPTT, PT, and thrombin time, decreased fibrinogen, and elevated D-dimer [81, 82].

Most typically, laboratories employ a latex agglutination method to quantitate D-dimer, as this type of assay provides relatively rapid and reliable results using automated instruments. The units and magnitude for D-dimer reporting are often an issue of confusion [78]. D-dimer can be reported in either D-dimer units (DU) or fibrinogen equivalent units (FEU), and there are approximately 2 FEU to 1 DU. Additionally, the magnitude for reporting may vary, with some using nanograms vs micrograms and milliliters vs liters [83]. It is advisable to pay particular attention to D-dimer units when evaluating published algorithms, or comparing results between laboratories.

Screening APTT, PT, and TT Result Combinations

(Note that reference intervals for these assays are typically validated such that approximately 2.5% of a normal healthy population will have results that fall just above reference interval.)

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