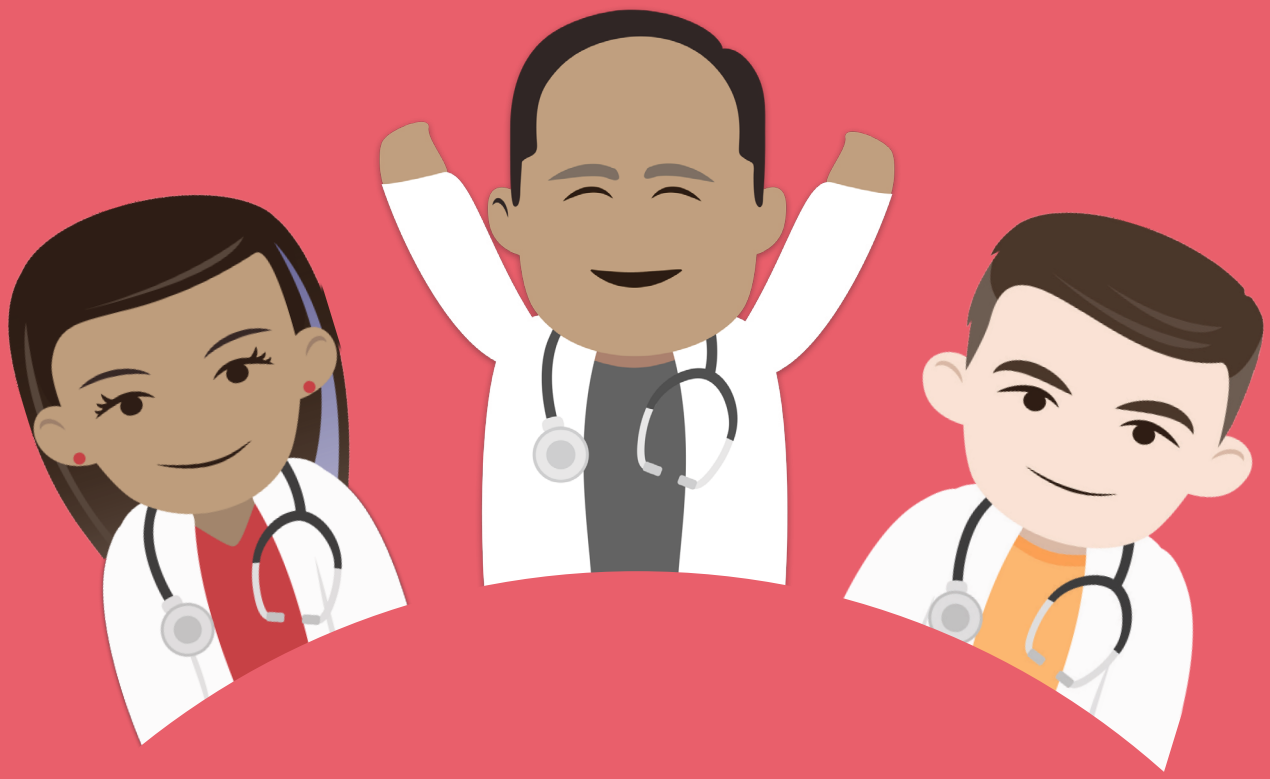
Hematology and Coagulation Essentials
Chapter 7

PINPOINTING THE CAUSE OF PROLONGED CLOTTING



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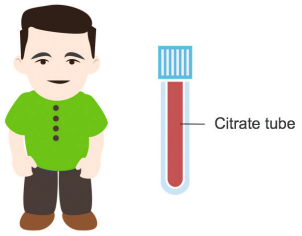
Pinpointing the cause of prolonged clotting

INTERPRETING THE PROTHROMBIN TIME (PT) TEST

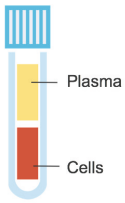
Prothrombin time (PT)

Prothrombin time (PT) is a common test of secondary hemostasis which involves measuring clotting time. PT measures the integrity of the extrinsic and common pathways.

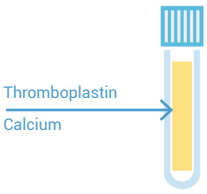
The following steps are involved



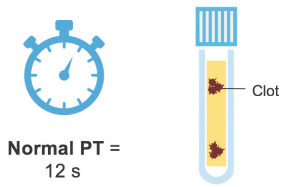
1. Collect patient's blood in citrate tube



2. Centrifuge to separate plasma



3. Add thromboplastin (factor III) and calcium



4. Measure time it takes blood to clot, in seconds

NOTE

Since the thromboplastin used may vary from lab to lab and country to country, results are best reported as an international normalized ratio (INR).

$$INR = \left[\frac{\text{Patient PT}}{\text{Mean normal PT}}\right]^{ISI}$$

Isolated prolonged PT

Since PT is a measure of the extrinsic and common pathways, if a patient has normal partial thromboplastin time (PTT) (which measures the intrinsic and common pathways) but prolonged PT, then the defect must be in the extrinsic pathway.

The extrinsic pathway involves factors III and VII. Since factor III (thromboplastin) is added during a PT test, if PT is prolonged (with normal PTT) then the abnormality must be in factor VII.

Such an abnormality may be due to

- Inherited factor VII deficiency
- Acquired factor VII deficiency (e.g., liver disease, vitamin K deficit, warfarin)
- Factor VII inhibitor

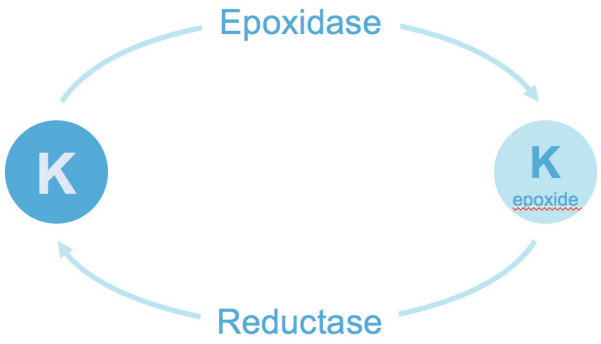
Vitamin K and clotting factors

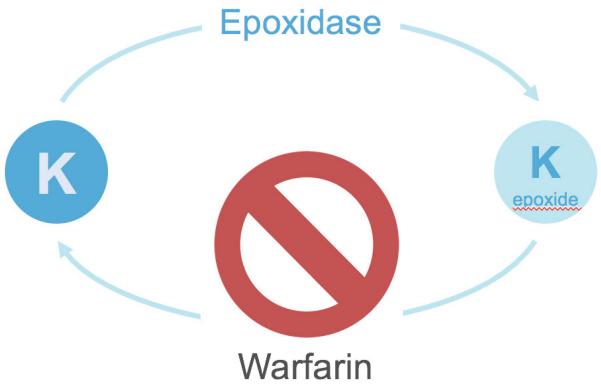
Vitamin K dependent proteins associated with clotting are II, VII, IX, X, and protein C and S.

The vitamin K dependent factors (II, VII, IX, X) have 9–12 glutamic acid residues near the amino terminal end, which needs to be carboxylated (vitamin K dependent).

Coumarin / warfarin blocks reductase, leading to the accumulation of non-functional vitamin K epoxide.

PT is used to monitor the efficacy of warfarin.





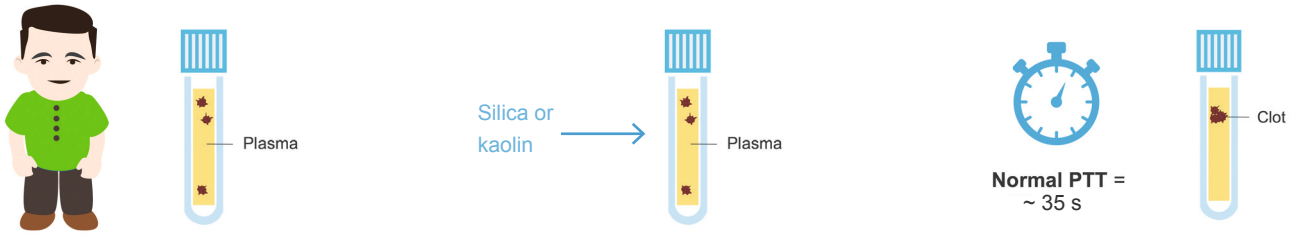
Pinpointing the cause of prolonged clotting

INTERPRETING THE PARTIAL THROMBOPLASTIN TIME (PTT) TEST

Partial thromboplastin time (PTT)

Partial thromboplastin time (PTT) is a common test of secondary hemostasis which involves measuring clotting time. PTT measures the integrity of the intrinsic and common pathways.

The following steps are involved



1. The patient's platelet-poor plasma is mixed with a surface activating agent (silica or kaolin) and platelet substitute (crude phospholipid).
2. Clotting time is determined.

Isolated prolonged PTT

Since PTT is a measure of the intrinsic and common pathways, if a patient has normal prothrombin time (PT) (a measure of the extrinsic and common pathways) but prolonged PTT, then the defect must be in the intrinsic pathway.

Such an abnormality may be due to

Deficiencies in HMWK, Prekallikrein or factor XII
- These deficiencies are not associated with bleeding.

Deficiencies in factors VIII, IX, and XI
- These represent inherited disorders—hemophilia A, B, and C, respectively.

Presence of an inhibitor of factors VIII, IX, or XI

Von Willebrand disease (VWD)
- These patients have low factor VIII.

Heparin

Presence of lupus anticoagulant (LA)

Pinpointing the cause of prolonged clotting

MAKING SENSE OF A MIXING STUDY

Let us consider a patient who is undergoing a screen for coagulation tests. Most often this would include a complete blood count (looking for thrombocytopenia), measurement of prothrombin time (PT), and partial thromboplastin time (PTT). If either the PT or PTT are prolonged then the logical question would be

Is the abnormal test result due to clotting factor deficiency or due to an inhibitor?

In order to answer this question, we should order a mixing study.

Deficiency Inhibitor

Mixing study

In a mixing study the patient's plasma is mixed with an equal volume of control (normal) blood. The basic principle is to measure PT and PTT before mixing and twice afterwards. The first time will be immediately after mixing and the second time will be from 30 minutes to 2 hours after mixing. The abnormal PT or PTT will either correct (i.e., normalize) or fail to correct (i.e., not normalize).

PT measures the integrity of the extrinsic and common pathways.

Therefore, if PT is prolonged, the causes include deficiency of

- Factor VII
- Factor X
- Factor V
- Factor II
- Factor I

Of these, deficiency of factor VII is most common.

Alternatively, rather than a deficiency, an inhibitor to any of these factors may be present. The presence of inhibitors is rare; however, when they are seen, factor V inhibitor is the most common.

With a PT mixing study, two outcomes are possible

1. PT normalizes immediately after mixing, and remains normal for the duration of the test.
   This suggests a factor deficiency.

2. PT does not normalize immediately after mixing, or at any point during the test.
   This suggests the presence of a factor inhibitor.

With a PTT mixing study, three outcomes are possible

1. PTT normalizes immediately after mixing and remains normal for the duration of the test.
   This suggests a factor deficiency.

2. PTT does not normalize immediately after mixing, or at any point during the test.
   This suggests the presence of a factor inhibitor.

3. PTT normalizes immediate after mixing but remains prolonged at later time points.
   This is typical of the presence of a factor VIII inhibitor.

If the patient is clinically bleeding, then the presence of a factor VIII or IX inhibitor is most likely.

If the patient is not bleeding, but rather has thrombus formation (e.g., stroke) then the presence of lupus anticoagulant is more likely.

Prolonged PT and PTT

When both PT and PTT are prolonged, this suggests a deficiency of one or more factors in the common pathway (e.g., X, V, prothrombin, fibrinogen). However, deficiencies of multiple factors in both the intrinsic and extrinsic pathways can also result in prolonged PT and PTT. This is commonly seen in liver disease, vitamin K deficiency, and disseminated intravascular coagulation (DIC).

Inhibitors to FV (exposure to bovine thrombin), FX, and exposure to drugs such as heparin, DTI, warfarin or superwarfarin (brodifacoum), can also cause prolonged PT and PTT.

Pinpointing the cause of prolonged clotting

DIAGNOSING INHIBITORS OF COAGULATION

When the conclusion of a mixing study is a factor deficiency we should order the appropriate factor assay.

For example, if an individual has prolonged partial thromboplastin time (PTT) and mixing study shows correction, then we should order assays for factor VIII and IX.

If the mixing study suggests the presence of an inhibitor, we should test for factor VIII or IX inhibitors, or lupus anticoagulant (LA), since these are the three most common inhibitors.

If the patient is clinically bleeding, then the presence of factor VIII or IX inhibitor is most likely.

If the patient is not bleeding, but rather has thrombus formation (e.g., stroke) then the presence of lupus anticoagulant is more likely.

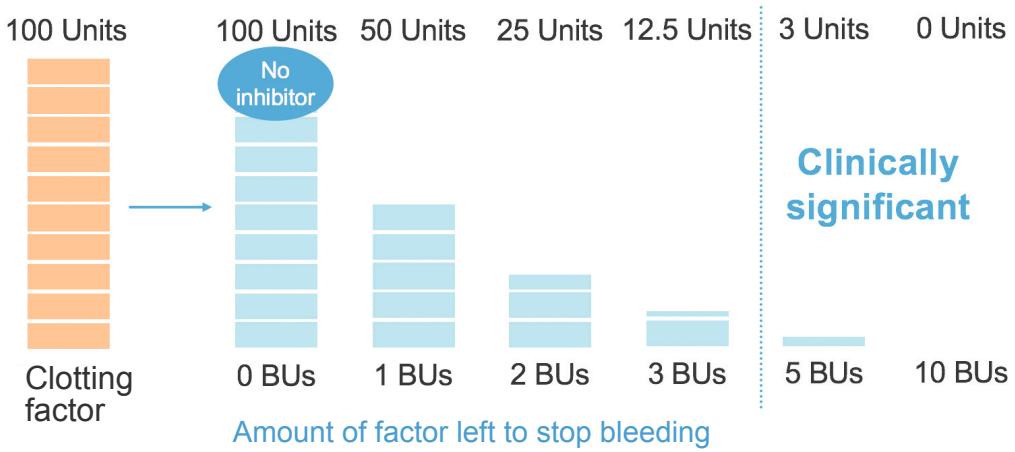
Factor VIII inhibitor may be present in hemophilia A patients receiving factor VIII. Sometimes individuals who are not hemophiliacs may develop spontaneous factor VIII inhibitor. These patients may develop bleeding, since inhibition of factor VIII disrupts proper clot formation.

Factor IX inhibitor may be seen in hemophilia B patients receiving factor IX. Sometimes individuals who are not hemophiliacs may develop spontaneous factor IX inhibitor. These patients may also develop bleeding, since inhibition of factor IX also disrupts proper clot formation.

When a factor VIII or IX inhibitor is detected, the next step is to quantify the amount of inhibitor present.

Testing for factor VIII and IX inhibitors

The Bethesda assay is used to quantify the levels of factor VIII or IX inhibitors in blood. The units are Bethesda units. If the quantity of an inhibitor is less than 0.5, then the result is considered to be negative. Levels below 5.0 are considered to be clinically insignificant.

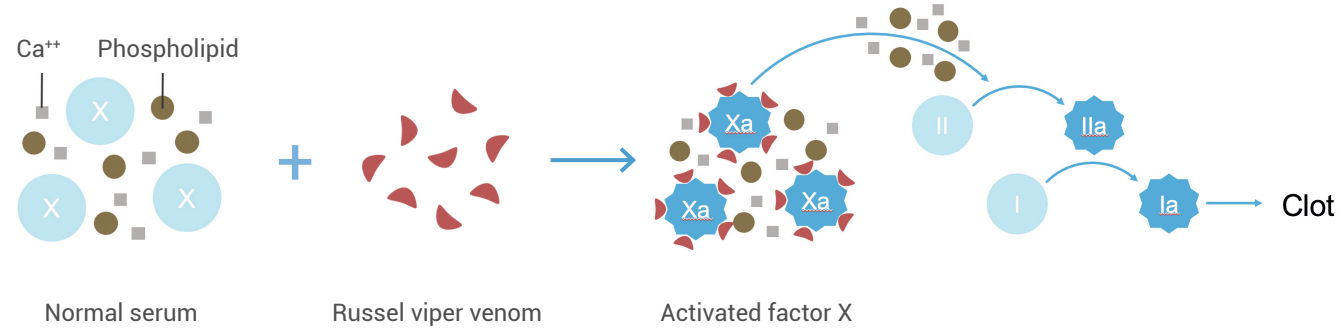


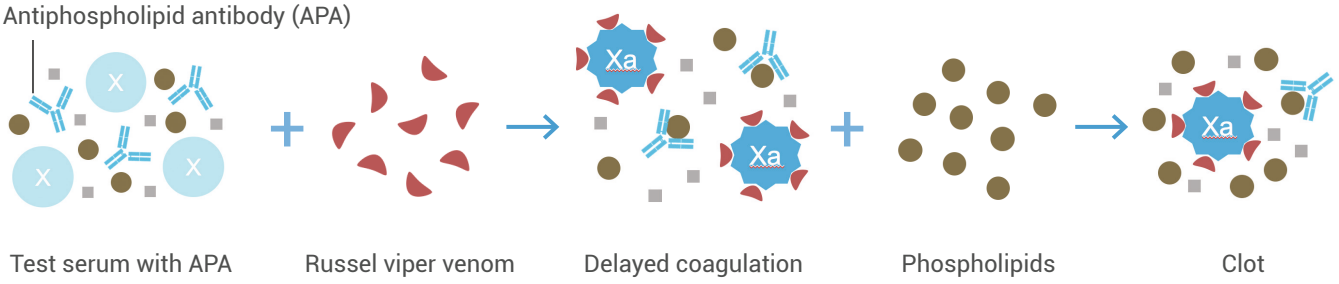
Testing for lupus anticoagulant

Lupus anticoagulant is an antibody that binds to phospholipids and proteins in the cell membrane, and may cause thrombosis in vivo. However, the lupus anticoagulant also acts against phospholipids used in coagulation testing and its presence leads to prolonged PTT. Despite its name, patients with lupus anticoagulant do not have bleeding, but instead actually have an increased risk of thrombosis.

The **Russell viper venom test** is used to detect lupus anticoagulant. Russell viper venom activates factor X and starts the clotting pathway. However, if there is lupus anticoagulant present, it will bind to phospholipids and delay the clotting time.

Thus, when the mixing study points towards an inhibitor we may order all three tests, or test for lupus anticoagulant if patient has a history of thrombosis, or test for factor VIII and factor IX inhibitors if there is a history of bleeding.





Pinpointing the cause of prolonged clotting

MANAGING A BLEEDING PATIENT WITH NORMAL PT AND PTT

Excessive bleeding in a patient may result from a wide variety of causes.

Family history of bleeding is important. Hemophilia A and B are inherited as X-linked recessive disorders—thus, males are more commonly affected. Medication history is also important, since administration of antiplatelets and anticoagulants can lead to bleeding.

When we workup a patient for bleeding, we should start by ordering

- Complete blood count (CBC)
- Prothrombin time (PT)
- Partial thromboplastin time (PTT)
- Thrombin time (TT)
- Peripheral smear exam

The two most common causes of prolonged TT are heparin administration and hypofibrinogenemia or dysfibrinogenemia. A peripheral smear may provide clues based on morphology—for example, grey platelet syndrome is where platelets appear grey in color.

These tests will pick up the common causes of bleeding, including thrombocytopenia, factor deficiency, and the presence of factor inhibitors.

If the PT and PTT are prolonged we should order a mixing study and based on the result, move onto factor assays, or test for inhibitors.

Sometimes the CBC, PT, and PTT are all normal. In such situations we need to consider mild VWD disease, thrombocytopathia, factor XIII deficiency, or fibrinolysis.

Von Willebrand disease (VWD)
We should order a VWD panel to rule it out.

Thrombocytopathia
PFA-100 may be used to screen for thrombocytopathia; however, a platelet aggregation study is the gold standard.

Factor XIII deficiency
To diagnose factor XIII deficiency we may order a factor XIII screen, which is based on clot solubility in a 5M urea solution. If the screen is positive, we should order a factor XIII assay (Bethesda assay).

Fibrinolysis
There are several tests to pick up fibrinolysis.

- Thromboelastograph (TEG)
  - The Ly30 values are high in patients with fibrinolysis when tested by TEG.
- Diesseminated intravasal coagulation (DIC) screen
  - DIC screen panel would show low fibrinogen and increased values of fibrin degradation products (FDP) values in fibrinolysis
- Euglobin clot lysis time

If an abnormality is detected, which points toward abnormal fibrinolysis then more specific tests may be ordered.

- Plasminogen assay
- Tissue plasminogen activator
- Alpha-2-antiplasmin levels
- Plasminogen activator inhibitor

These more specific tests are rarely ordered, and are generally only available for testing in a reference laboratory.