CLOT
or
BLEED

A Painless Guide for People Who Hate Coag

About this guide
Hemostasis is one of those parts of pathology that lots of students find overwhelming. There's so much going on in the coagulation cascade alone - so many Roman numerals, so many arrows to different molecules, so many similar-sounding abbreviations...it's enough to make a person throw the book at the wall and go take a nap. As one student put it: "I don't know anyone who doesn't hate coag."

This guide is written for those of us who have had a difficult time understanding coag. It breaks hemostasis down into reasonably-sized sections, with easy-to-understand explanations, silly but helpful mnemonics, and answers to commonly-asked questions. By the end of this guide, you'll understand how clots are formed and what keeps us from clotting too much. You'll also know how to use coag and platelet laboratory tests in practice, and you'll have a good understanding of most bleeding and thrombotic disorders.

Extra help
If you are stuck, or frustrated, or if something just doesn't make sense, feel free to send me an email at pathology@pathologystudent.com. I'll do my best to get you unstuck and back on track.

Acknowledgements
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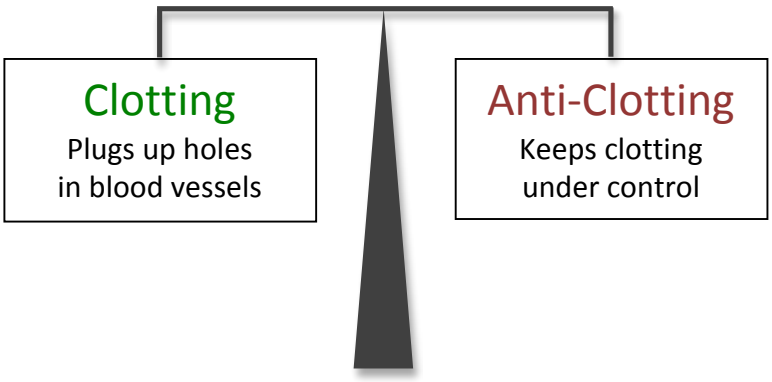
Chapter 1: How To Make a Clot

Before we begin

Hemostasis is a delicate balancing act. Lectures and textbooks tend to focus on the mechanisms of clotting (and we'll do so in this study guide too). But there's an equally important and robust anti-clotting system that keeps clotting under control. There has to be, obviously, or as soon as we got a small tear in a blood vessel (which happens all the time in capillaries), we'd become one big clot.



Both systems have to have all their parts intact and be able to function at the proper level. If your pro-clotting system is overactive, or if your anti-clotting mechanisms are less than stellar, the balance will tip towards the pro-clotting side, and you'll have an increased risk of thrombosis. Likewise, if your pro-clotting mechanisms are sluggish, or if your anti-clotting system is hyperactive, you'll be more likely to bleed. Causes of such imbalances can be either disease-related (e.g.., the patient has a coagulation factor deficiency) or drug-related (e.g., the patient is on warfarin, which inhibits vitamin K dependent coagulation factors).

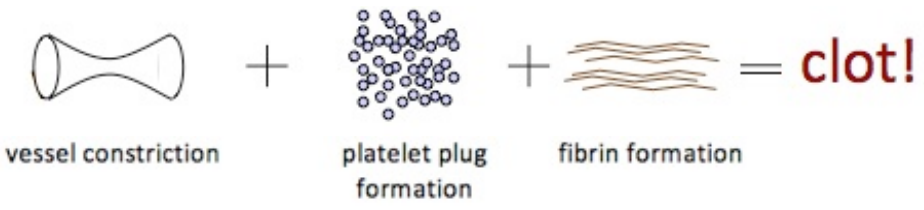
Keeping that balance in mind, let's talk about how to make a clot.

The three steps

The whole point of clotting is to plug holes in vessels so blood can't escape. There are three steps in this process:

1. constrict the blood vessel in the region of the hole
2. form a platelet plug
3. seal that plug with fibrin.

If you like drawings better than words (who doesn't?), you could summarize it like this:



Let's take a quick look at what's going on in each step (we'll get into more detail in a minute).

1. Blood vessels constrict

- This is a good way to quickly cut down on blood loss; vessel constriction leads to decreased blood flow, reducing the amount of blood lost.
- It also helps platelets and coagulation factors come into contact with each other and stick together when they bump into each other (which is necessary for the whole process to proceed). Normally, the speed and shear stress of blood flow "breaks" this binding affinity.
- Nobody talks much about this part of clotting (and neither will we) - probably because it works pretty well most of the time, and there aren't many diseases involving this step of clotting.

2. Platelets form a plug

- In this step, the platelets clump together and form a little mushy plug that covers the hole in the vessel. It's great - but without the final step (fibrin formation), it would fall apart and be useless.
- This step is often called "primary hemostasis" (in spite of the fact that it's not really the first thing that happens in clotting).

3. Fibrin seals the plug

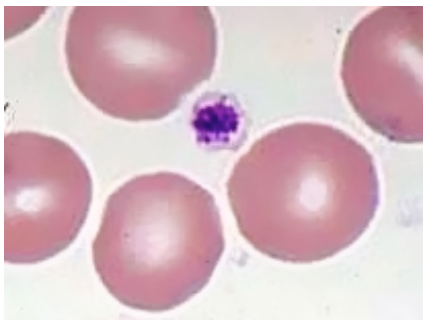
- In this final step, fibrinogen turns into fibrin, a long molecule that binds to and solidifies the platelet plug.
- There are a bunch of molecules involved in the process, each activating the next in a cascade fashion (hence the name "coagulation cascade").
- This step is often called "secondary hemostasis" (even though technically it's the third step in the process).
- There are several steps to fibrin formation:
  1. The initiator of the whole process is a molecule called tissue factor (TF).
  2. The coagulation cascade proceeds, one molecule activating the next, all the way down to the last step (which is the conversion of fibrinogen to fibrin).
  3. Fibrin superglues the platelets to each other, sealing and anchoring the plug.

Primary hemostasis

Primary hemostasis is that part of clotting in which you form a platelet plug. Let's take a look at platelets, and then we'll look at how they interact to form the plug.

Platelet morphology

Platelets are not really cells (they don't have a nucleus). They're fragments of cytoplasm shed by gigantic precursor cells called megakaryocytes that live in the bone marrow. Platelets look like this:



Normal platelet in blood smear

They are cute little irregularly-shaped fragments of cytoplasm with a central region of purplish granules (the "granulomere") and a peripheral region that is granule-free (the "hyalomere"). Most of the platelets live in the blood (and bone marrow); about a third are sequestered in the spleen.

Their simple appearance belies an amazingly complex structure. Both the membrane and the granules have a ton of important features.

Platelet membrane

The platelet membrane is critical to the whole process of clot formation:

- It provides a special phospholipid surface (historically referred to as platelet factor 3, or PF3) for coagulation factors to bind to. Coagulation factors need this unique phospholipid surface in order to function properly, and what better place than the surface of the platelets themselves?
- It displays a bunch of receptors that bind important molecules. Two important ones are glycoprotein (GP) Ib, which binds von Willebrand factor (vWF), and GP IIb-IIIa, which binds fibrinogen.

Platelet contents

There are lots of things inside platelets that help with the process of clotting:

- α granules ("specific granules") contain fibrinogen, von Willebrand factor (vWF), platelet-derived growth factor and P-selectin.
- δ granules ("dense granules") contain serotonin, ATP, ADP, and calcium (which is necessary for the vitamin K dependent coagulation factors).
- Contractile proteins (like actin) that help the platelet change shape during adhesion (a step that is essential for the release of granules).

How do platelets form a plug?

1. Endothelial damage exposes subendothelial proteins (like collagen), which attract platelets.
2. Platelets stick to the subendothelium (von Willebrand factor is the glue), a process called adhesion.
3. They change shape and release their granule contents, which attract more platelets.
4. They stick to each other and form a temporary plug (fibrinogen is the glue), a process called aggregation.
5. After a while, the platelets contract, which helps retract the clot and seal the vessel wall.

Secondary hemostasis

Secondary hemostasis is that part of clotting in which you make fibrin, which seals up the platelet plug. Let's take a look at how fibrin is made, which involves a process called the coagulation cascade (or, as one student put it, "the Roman numeral cascade of memorization hell").

The coagulation cascade is a series of enzymes ("factors") which activate each other in a cascade fashion (hence the brilliantly descriptive name). The whole point of the cascade is to turn fibrinogen (a big long precursor molecule which is used as a glue in early platelet plug formation, but which is useless as far as permanent sealing of a platelet plug goes) into fibrin (the incredible superglue that turns a mushy platelet plug into a rock solid blood loss barrier).

Here's something that you should tattoo on your thigh (okay, maybe just write it on your hand): The Whole Point of the Coagulation Cascade is to Turn Fibrinogen into Fibrin.

Why would you write that on your body?

Because it's way too easy to get so caught up in memorizing the names of these enzymes (plus all their Roman numeral designations, plus which one activates which) that you forget the seemingly obvious goal of the whole process, which is just the formation of fibrin. It's the old can't-see-the-forest-for-the-trees thing. It's also easy to forget that fibrin formation is only one part of clot formation! It wouldn't mean anything if you didn't already have a waiting platelet plug. Try to keep this in mind as we talk about the details of the coagulation cascade.

Coagulation factors

All the coagulation factors except fibrinogen are either enzymes or cofactors.

Enzymes

- These guys activate other coagulation factors.
- They circulate as inactive enzyme precursors. When the cascade is initiated, they are hydrolyzed by the preceding coagulation factor (though not always in numerical order), forming active enzymes. A little "a" after the Roman numeral indicates the active form of the enzyme.
- Most of these enzymes are serine proteases, which means they hydrolyze peptide bonds using a serine residue at their active center. The exception is XIII, a transglutaminase, which crosslinks and stabilizes fibrin polymers.

- Enzyme precursors include: II, VII, IX, X, XI, XII, and XIII. Each of these has a regular old name too (you can look these up in the reference section if you're into that kind of thing), but by convention, we use Roman numerals for all of them except II (which is usually designated by its regular old name, thrombin).

Cofactors

- These guys amplify the coagulation cascade.
- Some of them (V and VIII) are inactive until cleaved.
- Cofactors include: TF (tissue factor), V and VIII.

Intrinsic, extrinsic and final common pathways

- As you'll see in a scary diagram in a minute, the coagulation cascade is divided somewhat artificially into intrinsic and extrinsic pathways (followed by the final common pathway).
- In vitro, the intrinsic and extrinsic pathways operate separately.
- In vivo, the two pathways are big-time co-dependent. We need both intrinsic and extrinsic pathways for clotting. We know this because people with deficiencies of factors from either pathway have bleeding problems!

Things to make you look smart

Q. How come the intrinsic and extrinsic pathways are named like that?
A. The nomenclature arose out of laboratory observations of clot formation. To generate a clot via the intrinsic pathway in a test tube, you only need things that are *intrinsic* to whole blood. To generate a clot via the extrinsic pathway in a test tube, you need to add something *extrinsic* to whole blood (that is, tissue factor or some tissue-factor-like substance).

The extrinsic pathway

- Tissue factor is the activator of the extrinsic pathway.
- Tissue factor is a membrane protein that is found:
  1. on cells that are normally not in contact with blood (like smooth muscle cells in the subendothelium, and fibroblasts surrounding blood vessels). Vascular injury exposes this TF to the blood and initiates the coagulation cascade.
  2. in "microparticles" – little fragments of cell membrane found in normal blood. These microparticles have receptors for P-selectin. P-selectin is a cell adhesion molecule that lives in platelets and endothelial cells. When it's activated, it flips to the outside of the cell membrane, and the microparticles stick to it, and tissue factor accumulates in the region of the forming clot, and that's all she wrote.
  3. on endothelial cells, and on monocytes, when there's inflammation (but not under normal resting conditions – otherwise we'd be coagulating all over the place).
- TF binds to VII, activates it, and then this TF-VIIa complex kicks off the coagulation cascade via the extrinsic pathway.
- After a little Xa is formed, TFPI (tissue factor pathway inhibitor) turns off TF, and the extrinsic pathway is shut down, preventing over-clotting and thrombosis.

The intrinsic pathway

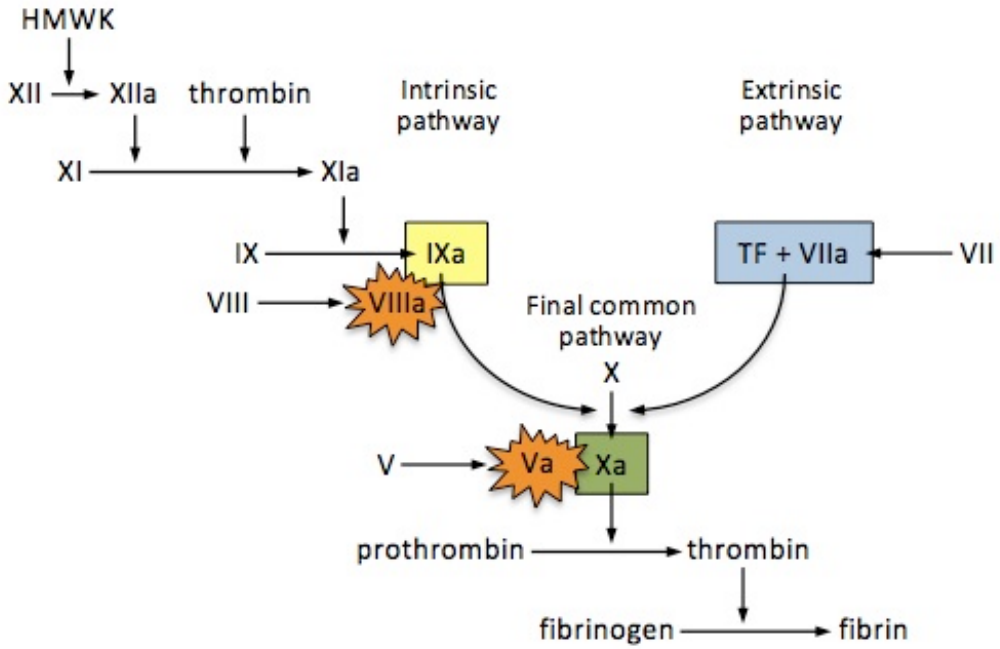
- Thrombin is the main activator of the intrinsic pathway *in vivo*. (Wait a minute, the diagram below shows it at the end of the cascade! Don't worry, we'll get to that later.)
- You'll notice that the "contact factors" (HMWK, prekallikrein, XII) are pretty much absent from much of our discussion. That's because although technically they are able to activate the intrinsic pathway, they don't play much of a role *in vivo*. XII, in particular, is only important in forming clots in test tubes. People with contact factor deficiencies don't have bleeding disorders! These factors are not crucial for activation of the intrinsic pathway *in vivo*.

The final common pathway

- This is the last leg of the cascade.
- It can be activated by either the intrinsic or extrinsic pathway.
- It consists of factors X, V, II (thrombin) and I (fibrinogen).

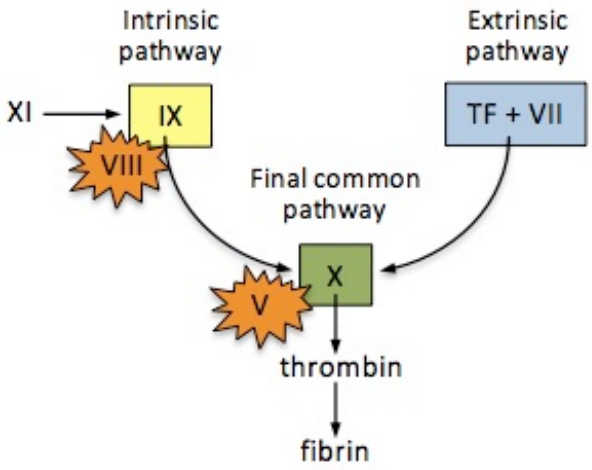
Okay, okay, just show me the diagram.

Fine! Here it is:



Scary, isn't it?

Actually, you can make it quite a bit simpler. If you take out some stuff at the top left (the so-called "contact factors" that you'll hear about from the biochemists, but which have basically no real importance *in vivo*) and remove the arrows showing the activation of each factor (you know that each one gets activated from proenzyme to enzyme - so you don't need to draw that out each time), it looks a little better:



You'll need to come up with some way to make this diagram memorizable. I have a little mnemonic that I use for my students that tends to work. It's silly, but hey, aren't most mnemonics? This one requires a short digression into the topic of women's shoes.

Shoes?

Yes, shoes. For the purposes of this discussion, we'll say that there are two kinds of shoes in the world: good ones and bad ones. And we're just talking high heels here - forget flats and boots and those disgusting mid-height heels.

A good shoe is elegant. It is simple, timeless, and clean in design. It draws attention not to itself but to the gorgeous leg it adorns. Like this:

A bad shoe, on the other hand, is tacky. It is complicated (think bows, zippers, fishing lures, or - if you're Lady Gaga - flank steak), frivolous, and busy. It is so distracting that it makes it impossible to discern the beauty of the woman; one is too caught up in trying to visually deconstruct the obnoxious shoe. Like this:

So it is with the coagulation cascade. There is the intrinsic arm of the cascade, which is unapologetically cluttered with so many Roman numerals and arrows that one cannot figure out (let alone remember) what is going on. And there is the extrinsic arm, which is simple, unfettered, and easy to understand.

Intrinsic: Roman numeral hell
Extrinsic: Two factors (TF and VII)
Common: Activated by both to produce fibrin

The big thing to remember about the cascade is which factors constitute the intrinsic arm and which ones constitute the extrinsic arm. If you can do that, everything else follows. It's easy to remember that one side has just tissue factor and VII, and the other side has every other factor in it...but you need to know which is which. You might not think this is important now, but when your attending asks you at three in the morning which pathway the prothrombin time measures, you'll thank me.

Back to the shoes. The intrinsic pathway is epitomized by the bad shoe. Both are complicated, cluttered, and (to exaggerate a bit) sinful. Behold: the SINtrinsic pathway! The extrinsic pathway is symbolized by the good shoe. Both are simple, elegant, and (with a little stretch of the imagination) sexy. Henceforth, then, this pathway shall be known as the SEXtrinsic pathway. (As an aside, when we get to coag tests, we'll see that the test names fall right into this simple/complicated analogy.)

Things to make you look smart

Attending: "Which pathway contains factor VII?"
Student (thinking quietly: let's see...that's the simple, elegant pathway with just tissue factor and VII, the one with the good shoe...): "The sextrinsic pathway."
Attending (eyes opened wide): "What?"
Student (recovering quickly): "Extrinsic. The extrinsic pathway."
Attending (considering a hearing aid): "Exactly."

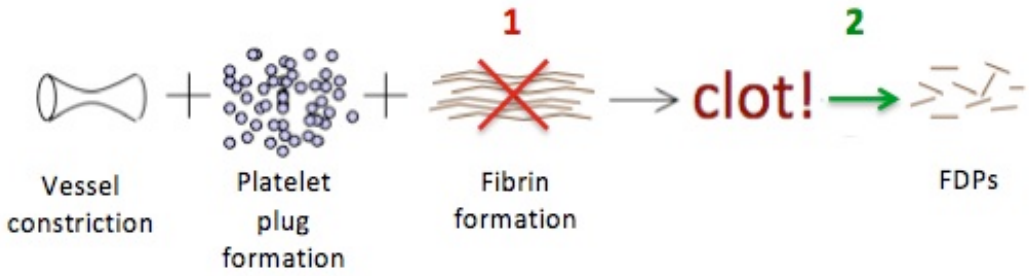
The interaction of the pathways in real life

So there are two pathways, both of which can be independently activated. Here's how the pathways fit together *in vivo*:

- Vascular injury exposes tissue factor to the bloodstream.
- Coagulation is started along the extrinsic pathway (making a little Xa and thrombin along the way).
- Xa quickly turns off the extrinsic pathway (through TFPI, which we'll talk about in the next chapter).
- Thrombin activates the intrinsic pathway.
- Thrombin also activates V and VIII, amplifying the intrinsic pathway (and final common pathway).
- Within a few hours, the initial mushy platelet plug is transformed into a solid mass by fibrin.

Chapter 2: How to Stop Clotting

There are two main ways of keeping clotting under control:
1. You can inhibit the coagulation cascade (number 1 in the fancy diagram below)
2. You can lyse portions of the clot to keep it down to a reasonable size (number 2 in the fancy diagram), a process which forms little fibrin fragments called fibrin degradation products, or FDPs.



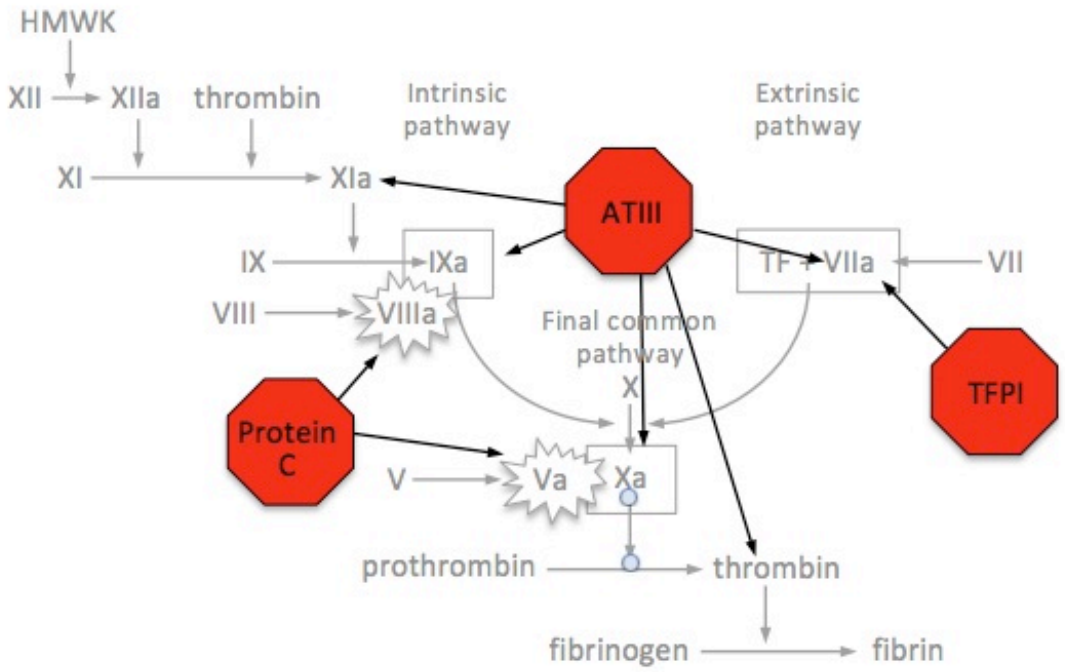
Let's take a look at each of these processes in a little depth.

Coagulation cascade inhibition

Several proteins keep the coagulation cascade – and thus, fibrin formation – under control. These natural anticoagulants include TFPI (tissue factor pathway inhibitor), ATIII (antithrombin III), protein C (Batman), and protein S (Robin). Let's take a look at where these proteins act.

Coagulation cascade inhibitors

- TFPI (tissue factor pathway inhibitor) does what its name says: it shuts off the tissue factor (extrinsic) pathway. It does this by inhibiting VIIa.
- Antithrombin III inhibits the serine proteases (IIa, VIIa, IXa, Xa, XIa, XIIa), shutting off all three pathways (intrinsic, extrinsic, and common). Who names these things? It's not just anti-thrombin, it's anti-a-whole-bunch-of-things. Heparin potentiates its action.
- Protein C is a serine protease which destroys Va and VIIIa (and hence the common and intrinsic pathways, respectively), effectively shutting down coagulation. Protein S is a cofactor that helps protein C (if protein C is Batman, protein S is Robin).



ATIII = antithrombin III
HMWK = high molecular weight kininogen
TFPI = tissue factor pathway inhibitor

Things to make you look smart

Q. So what turns on protein C?

A. Protein C is turned on by thrombin. Actually, thrombin binds to thrombomodulin (a receptor on endothelial cells that alters the thrombin molecule), and the resulting thrombin-thrombomodulin complex activates Protein C. That's pretty cool: thrombin regulates itself by turning on the thing that keeps it in check.

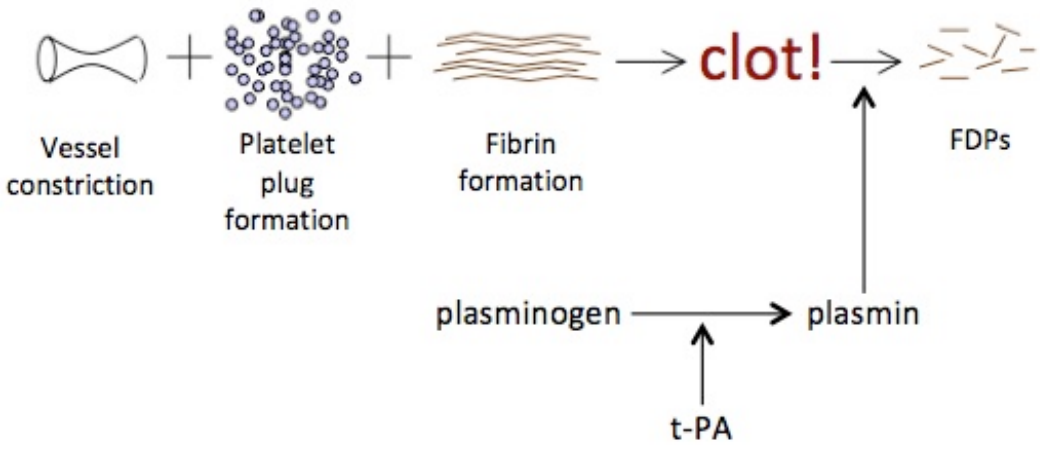
By the way, protein C does a couple other important things. It promotes fibrinolysis by helping out tissue plasminogen activator (t-PA) (see below). It also helps keep inflammation in check by inhibiting cytokine production. Because of its effects on clotting and inflammation, giving actual protein C can be useful in cases of really really bad DIC (disseminated intravascular coagulation).

Clot lysis

When the clot has done its job and bleeding has stopped, there are several proteins that help "remodel" the clot by breaking down fibrin into little chunks called fibrin degradation products (FDPs). These clot-busting proteins include t-PA (tissue plasminogen activator) and plasmin. t-PA is actually used as a drug for treatment of very recent ischemic strokes (but not hemorrhagic ones, obviously!).

Fibrinolytic agents

- t-PA binds to fibrin (a good idea, because that keeps its action localized to the clot!) and converts plasminogen to plasmin.
- Plasminogen is converted to plasmin, which breaks down fibrin into fibrin degradation products (FDPs), also called split products, which themselves inhibit thrombin and fibrin formation (how handy).
- Blood contains inhibitors of t-PA (plasminogen activator inhibitor, or PAI) and plasmin (α2-antiplasmin) so fibrinolysis doesn't get out of hand.



Chapter 3: Laboratory Tests

There are lots of different tests that evaluate a patient's clotting status. They can be broadly divided into those that evaluate platelets and those that evaluate the coagulation cascade. We'll go over the ones that you will order very frequently, as well as a few that you may encounter at some point (on the boards, at least).

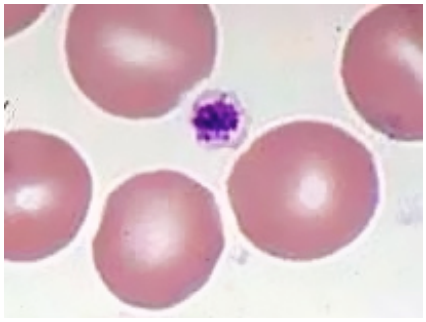
Platelet tests

Platelet count (Plt)

- Platelet counts are usually done by a big electronic particle counter.
- You can also estimate the count on a blood smear; you should see between 7 and 21 platelets per high power field (x1,000 oil immersion).
- The reference range is 150 – 450 x 10^9/L.
- This test does not evaluate platelet function; it just tells you the number of platelets present.

Platelet morphology

You can look at a blood smear under a microscope and see if the platelets look normal.



Normal platelet in blood smear

They almost always look like this (even when they don't work properly) - but on occasion, they will be bigger than usual, or lack granules, or both. We'll look at some platelet disorders that have funny morphology later.

Bleeding Time

What does the bleeding time measure?

- How platelets respond *in vivo* to vascular injury.
- Unfortunately, the bleeding time is affected by other factors, too (hemoglobin, skin quality, what kind of a mood the tech is in that day...).

How do you do a bleeding time?

- Put a blood pressure cuff on the patient's arm and inflate it to 40 mm Hg.
- Make a little incision (using a disposable spring-loaded lancet) on the inner surface of the patient's lower arm, noting the time.
- Blot drops of blood as they appear at the incision site.
- Wait (tedious!). When the incision stops bleeding, note the time.
- The total time elapsed between incision and bleeding cessation is called the bleeding time.
- The reference range is 2 to 9 minutes in adults (longer in children).

What kinds of things cause a prolonged bleeding time?

- Platelet disorders, such as von Willebrand disease.
- Severe thrombocytopenia.

When should you order a bleeding time?

- Some experts order it to screen symptomatic (bleeding) patients for inherited platelet disorders.
- Other experts consider it so unreliable that they don't use it at all, and go straight to the fancy, specific tests for von Willebrand disease and other platelet disorders.
- You can always call your friendly hospital pathologist and ask her what she would recommend.

Platelet aggregation studies

What do platelet aggregation studies measure?

- How well the platelets are able to aggregate (stick to each other).

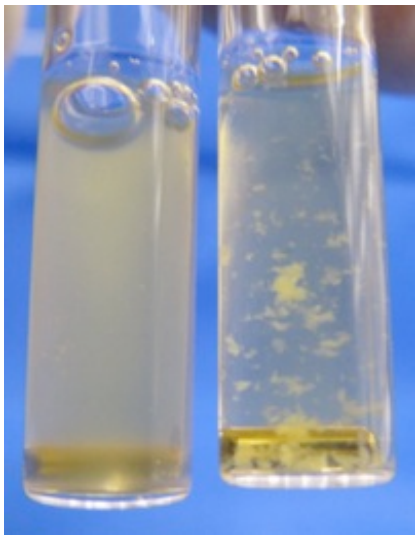
How do you do a platelet aggregation study?

- Add various aggregating agents (like ADP, collagen, ristocetin, arachidonic acid, epinephrine) to little tubes of the patient's platelets.
- Measure the decrease in turbidity of the platelet solution.
- The *pattern* of response to each agent helps narrow down your diagnosis.
    - Normal platelets will respond to every aggregating agent.
    - In platelet disorders, however, the platelets do not respond normally to every aggregating agent.
- One disorder may show normal aggregation with every agent except ristocetin. Another may show normal aggregation with ristocetin and arachidonic acid, but no response to any other aggregating agent. A third disorder may show no aggregation with any of the aggregating agents.

Wait, what?

- Okay, this is jumping ahead, but I think you can handle it.
- As an example: there's a platelet disorder called Bernard-Soulier which we'll talk about later. In this disorder, patients make abnormal GP Ib (the platelet receptor that binds von Willebrand factor).
- When you do a platelet aggregation study on these patients, their platelets respond normally to every aggregating agent except one: ristocetin.
- That's because ristocetin works by making platelets express their GP Ib receptors.

- So you can give all the ristocetin you want to platelets from patients with Bernard-Soulier, and the platelets just won't aggregate properly (because they can't express normal GP Ib).
- Von Willebrand disease, incidentally, has the same pattern, just for a different reason. In vWD, the patient has no vWF (or it's very abnormal). So you can mix these platelets with ristocetin, and they will go ahead and express their little GP Ib receptors, but there's no normal vWF around...so the platelets won't aggregate.
- Whew, that's enough. You can come back to his a little later, and it will make more sense then. Now back to our regularly-scheduled programming.



Platelet-rich human plasma before (L) and after (R) addition of ADP
(Dietzel65 from Wikimedia)

The left vial is murky because the platelets are suspended in the plasma. The right vial contains little clumps of platelets (they look like little flakes) which formed in response to the addition of ADP. Note that the right vial is less turbid; this decrease in turbidity is what is measured in the platelet aggregation assay. Both vials have yellow magnetic stirrers at the bottom.

Coagulation factor tests

These tests measure how long it takes to make fibrin. There are several tests, each of which start at a different point in the coagulation cascade (so you can tell where the problem is). You'll use these tests a lot when you're on the wards (and in clinic). You'll use the PTT and INR so frequently that you'll talk about them in your sleep.

Most coagulation tests are done like this:

- Draw the patient's blood into a special tube (containing citrate, which binds the calcium in the blood sample to prevent the blood from clotting).
- Back in the lab, spin the tube in a centrifuge, and decant the plasma (which is just serum plus coagulation factors).
- Put the patient's plasma in a test tube and add reagent.
- Measure how long it takes the patient's blood to form fibrin.

Prothrombin time (or protime) (PT)

What are the ingredients?

- Patient plasma
- Thromboplastin (a tissue-factor-like reagent)
- Calcium

What does the PT measure?

- This test initiates coagulation along the extrinsic pathway (through the addition of a tissue-factor-like substance to the test tube).
- So, it measures the extrinsic pathway (basically, factor VII). It also measures the common pathway (factors X, V, II and fibrinogen).
- The PT doesn't measure the patient's tissue factor! That's why it's called the "extrinsic" pathway - you have to add an extrinsic thing (tissue-factor-like substance) to the test tube to get the test to go.

What's the deal with factor VII?

- It's made by the liver
- It needs vitamin K for activation
- Its synthesis is inhibited by coumadin (warfarin)
- It has a *short* half-life (it doesn't take long to deplete your factor VII stores!)

What makes the PT go up (or put another way, what "prolongs" the PT)?

- Deficiency or inhibition of any of the factors in the extrinsic pathway (VII) or the common pathway (X, V, II, and/or fibrinogen). Some causes: liver disease, vitamin K deficiency, and "inhibitors" or anti-phospholipid antibodies, which we'll talk about in a minute.
- Coumadin (which affects factors in all three pathways - II, VII, IX, X).
- Heparin, to some extent (which affects factors in all three pathways: IIa, VIIa, IXa, Xa, and XIa).
- DIC (which screws everything up).

When should you order a PT?

- Never (trick question)! It's obsolete! There was so much variability between different reagents and instruments that the reference range was way too different from lab to lab - it's like there wasn't any way to be consistent and interpret the results.
- But…you still need to understand the PT, so you can understand (and order) the INR.
- All that being said, you'll still see this test ordered as a PT/INR...even though people only look at the INR part (see next).

International normalized ratio (INR)

Okay, so what's an INR?

- It's just a PT that's been mathematically corrected to account for differences between laboratories.
- The PT is a pretty annoying test, because a patient could get a PT in one hospital, and then go to another hospital down the street, and the PT would be totally different (because the stupid thromboplastin varies so much between manufacturers). So the results of the PT were really only useful within each particular hospital.
- To find out what the INR is, you do a PT (add thromboplastin and calcium to the patient's blood), and then you plug the result into a formula, which spits out the INR.
- That's cool, because now everyone is using the same scale of measurement. My INR at St. Mary's, for example, would be the same as my INR done at St. Luke's.
- Now we can use standards that apply to everyone, no matter where they got their test done! When someone has a thrombotic tendency, for example, the standard of care is to keep their INR (wherever it's performed) between 2 and 3.

When should you order an INR?

- To assess a patient's coagulation status (e.g., in unexplained bleeding, in DIC, or after lots of transfusions)
- To monitor coumadin therapy. The INR is the best test for monitoring coumadin (better than the PTT, which we'll talk about next). That's because the first factor to become depleted after coumadin administration is factor VII. So you want a test that measures factor VII, because that test will be the most sensitive. A test that only measured the other (longer-half-life) vitamin K dependent coagulation factors wouldn't be as useful - because by the time *that* test became abnormal, the patient would be at great risk of bleeding!
- When you are assessing liver function/damage (acute hepatitis, acetaminophen overdose)
- Maybe as an hospital admission or pre-operative screen (especially if patient is on coumadin or has a bleeding history)

Partial thromboplastin time (PTT)

What are the ingredients?

- Patient plasma
- Phospholipid extract
- Calcium

What's the *activated* partial thromboplastin time (APTT)?

- The APTT is just like the PTT, with the addition of an activating agent. This makes the reaction go faster in the laboratory instrument.
- The terms "APTT" and "PTT" are often used interchangeably. Don't worry about this.

Why is it called the partial thromboplastin time, when there's no thromboplastin in it? Are these people sadistic?

- Yes, they are sadistic.
- It's called the *partial* thromboplastin time because way back when they were creating this test, they figured out that if you just added a part of thromboplastin to a test tube, you could activate fibrin formation. It turns out that the part they were adding was just phospholipid, and that thromboplastin itself consists of both phospholipid and tissue factor.

What does the PTT measure?

- The PTT initiates coagulation along the intrinsic pathway (through the addition of just phospholipid and calcium).
- Coagulation won't occur along the extrinsic pathway in this test, because there's no tissue factor (or tissue-factor-like reagent) in the test tube.
- So, this test measures the intrinsic pathway (factors XI, IX, and VIII) and the common pathway (factors X, V, II, and fibrinogen)
- That's why the intrinsic pathway is named that way; to activate the pathway, you don't need to add anything "extrinsic" to the blood! (Yeah, you add phospholipid - but that's in normal blood already, in platelets, so you're just adding it back to the test tube.)

What makes the PTT go up (or, what "prolongs" the PTT)?

- Deficiency or inhibition of factors in either the intrinsic or the common pathway (so factors XI, IX, VIII, X, V, II, and/or fibrinogen). Some causes: hemophilia, von Willebrand disease (through increased degradation of factor VIII - see later), liver disease, vitamin K deficiency, and "inhibitors" or anti-phospholipid antibodies (see later).
- Heparin (which affects IIa, IXa, Xa, and XIa). The PTT is the best test for heparin monitoring, because although heparin affects coagulation factors on both sides of the pathway, it appears to affect those on the intrinsic side to a greater degree.
- Coumadin, to some extent (but the INR is a better test, for the reasons listed above. By the time the PTT goes up, the patient will be very anticoagulated, and possibly at risk of bleeding).
- DIC (which screws everything up).

When should you order a PTT?

- When you are assessing a patient's coagulation status (e.g., in unexplained bleeding, in DIC, or after lots of transfusions)
- When you are monitoring heparin therapy
- When you are assessing liver function/damage (acute hepatitis, acetaminophen overdose)
- Maybe as a pre-operative screen (to make sure your patient doesn't have hemophilia or von Willebrand disease before you do surgery)

You said these tests fall into the shoe analogy.

- They do (with a little stretch of imagination).
- The simple test (the PT) measures the simple, elegant, sextrinsic pathway.
- The complex test (the PTT, which is way more complex than the PT because it has three letters instead of two) measures the complex, tacky, sintrinsic pathway.

PTT mixing study

What are the ingredients?

- Patient plasma
- Pooled human plasma
- Phospholipid extract (PTT reagent)
- Calcium

What's the "mixing" part for?

- This test is basically just a regular old PTT test – with a bunch of pooled human plasma thrown in the tube too.
- So you're *mixing* the patient's (probably abnormal) plasma with some pooled (hopefully normal) plasma, and then running a PTT test, and seeing what the new PTT value is.

Why would you want to do that?

- To differentiate between the two main causes of a prolonged PTT (with a normal TT - see below for what the TT measures): factor deficiency or one of these "inhibitors" that we keep mentioning.
- If the new PTT value is within the normal range (if the PTT "corrects"), then you know the pooled human plasma must have supplied something to the patient's plasma to make it clot normally. (The "something" is usually a coagulation factor that the patient is missing.)
- If the new PTT value is still abnormal (if the PTT is still prolonged, and doesn't "correct"), then you know that even though you added a bunch of normal plasma to the mix, the patient's plasma still couldn't clot normally. There must be some other problem with the patient's plasma. (The "other problem" is usually an inhibitor.)

When should you order a PTT mixing study?

- when the PTT is prolonged but the TT is normal...
- …the PTT mixing study will help you narrow down your diagnosis to (1) a coagulation factor deficiency or (2) an inhibitor.

Thrombin time (TT)

What are the ingredients?

- Patient plasma
- Thrombin

What does the TT measure?

- The conversion of fibrinogen to fibrin. The last point in the common pathway. That's it.
- When you add thrombin to plasma, fibrinogen is converted to fibrin (bypassing the intrinsic and extrinsic pathways).

What makes the TT go up?

- Fibrinogen deficiency (quantitative or qualitative)
- The presence of FDPs (which inhibit the conversion of fibrinogen to fibrin)
- Heparin (which will inactivate the thrombin that you add to the patient's sample)

When should you order a TT?

- When a patient has a prolonged PTT, and you want to look for a fibrinogen problem
- When you are monitoring heparin therapy

Fibrin Degradation Products (FDP) Assay

What does an FDP assay measure?

- Fibrin degradation products (FDPs), which are the result of the breakdown of fibrin (in a clot) by plasmin.
- Unfortunately, the same end products (FDPs) are formed when plasmin circulates in the blood and breaks down fibrin*ogen* (this happens all the time at a very low rate, and does not mean that the patient has a clot).
- Fortunately, there is a special kind of FDP that is only formed by breaking down clots. It is called a D-dimer, and it is the result of the breakdown of *crosslinked* fibrin (which is only in clots!) by plasmin. If you see D-dimers floating around, you know that somewhere, there is a clot that is being broken down.

What causes FDPs to go up?

- Any kind of abnormal thrombotic state, like disseminated intravascular coagulation (DIC), pulmonary embolus (PE), or deep venous thrombosis (DVT).
- Unfortunately – or maybe fortunately – this test is very very sensitive. It can detect the most minute levels of fibrin degradation products (including D-dimers) in blood.

When should you order an FDP assay?

- Glad you asked. Since this test is sooooo sensitive, it's not a good test for proving that a patient DOES have an abnormal clot (like a pulmonary embolus or a deep venous thrombosis). If the test is positive, you can't be sure that the patient has a clot (because even normal, physiologic clotting can make your FDPs go up).
- But, since this test is sooooo sensitive, it's a great test for proving that a patient DOES NOT have an abnormal clot! If the test is negative, you can be pretty sure the patient does not have a clot. It's used quite a bit in ERs to rule out pulmonary embolism.

Fibrinogen Assay

What's fibrinogen again?

- Fibrinogen is the final coagulation factor way at the bottom of the cascade. It's the precursor to fibrin.
- It's made by the liver, and is rapidly depleted during clotting.

What causes the fibrinogen level to go down?

- In rip-roaring DIC (disseminated intravascular coagulation), the fibrinogen level often goes down (fibrinogen gets used up making all those clots).
- However, since fibrinogen is an acute phase reactant, a patient can be in florid DIC and still have a normal fibrinogen level.

When should you order a fibrinogen assay?

- When you're evaluating a patient for DIC (while realizing that a normal fibrinogen level does not rule out DIC).
- When you're massively transfusing a patient (because when someone comes in with a massive blood loss - say, from a bad car accident - you generally don't use whole blood; you use a combination of blood products, starting with red cells, and adding fresh frozen plasma as necessary.
- If you're not careful with your combination of replacement products, you might upset the coagulation/anti-coagulation balance, and the patient's fibrinogen could get diluted, putting him/her at risk for bleeding. So generally you'll order a fibrinogen level now and then when you're massively transfusing someone.

I'm exhausted. Just give me a quick summary.

- PTT = intrinsic pathway
- PT/INR = extrinsic pathway
- PTT mixing test = PTT with patient blood plus pooled plasma
- TT = fibrinogen to fibrin
- FDP/D-dimer = measures FDPs/D-dimers
- Fibrinogen = measures fibrinogen

I'm even more exhausted now. Remind me why I would order these tests.

- PTT: To measure a patient's general coagulation status; to check the intrinsic pathway in particular; to monitor liver function; to monitor heparin.
- PT/INR: To measure a patient's general coagulation status; to check the extrinsic pathway in particular; to monitor liver function; to monitor Coumadin.
- PTT mixing test: In a patient with a prolonged PTT (but a normal TT), to figure out whether the problem is a factor deficiency or an inhibitor.
- TT: To look for a fibrinogen problem; to monitor heparin.
- FDP/D-dimer: To rule out a thrombus.
- Fibrinogen: To monitor fibrinogen levels during massive transfusion.

Chapter 4: Bleeding disorders

General

Before we get into bleeding disorders

There are lots of things that can make you bleed: vascular disorders, thrombocytopenia, and problems with the clotting system. Our discussion will be limited to those bleeding disorders involving thrombocytopenia and the clotting system. Disorders resulting from thrombocytopenia include idiopathic thrombocytopenic purpura (ITP) and the thrombotic microangiopathies. Disorders involving the clotting system can result from coagulation abnormalities (e.g., hemophilia), platelet abnormalities (e.g., Bernard-Soulier), or both (e.g., von Willebrand disease and disseminated intravascular coagulation).

Thrombocytopenia

- ITP
- TTP/HUS

Problems with clotting

- Coag problems (e.g., hemophilia)
- Platelet problems (e.g., Bernard-Soulier)
- Both coag and platelet problems (vWD, DIC)

Clinical features

To a certain extent, the clinical picture (the location and manner of bleeding) depends on the underlying problem. Patients with thrombocytopenia tend to present with spontaneous skin and mucous membrane bleeding. Normally, the platelets lining the vessel walls provide a bit of physical protection against blood seepage between endothelial cells. If there are very few platelets around, there is less protection, and blood seeps from capillaries, forming little red dots (or petechiae).

Patients with coagulation disorders tend to bleed after a traumatic incident – but not right away. For example, they'll have their wisdom teeth pulled, and hours later they will have excessive bleeding from the surgical site. These patients can form a platelet plug just fine, so right after an injury, they are able to achieve hemostasis. But they are not able to make fibrin well, so the platelet plug isn't sealed up, and it just washes away after some time. Patients with severe factor deficiencies (like severe hemophilia) bleed into joints and soft tissue (they get large intramuscular bleeds).

Finally, patients with platelet disorders tend to bleed from mucous membranes and skin (they get nosebleeds, GI bleeds, and menorrhagia) with little or no apparent preceding trauma.

Those are generalizations, however - there's some crossover of symptoms, so it's not entirely black and white. The clinical picture just points you in a general direction (platelets vs. coagulation) so you know how to start the workup of your patient.

Bleeding disorders

We're going to divide the bleeding disorders into two big groups: those that are hereditary, and those that are acquired. Some of these are pretty rare (like the hemophilias) but you obviously still need to know a bit about them. Here's the outline we'll be following:

1. **Hereditary bleeding disorders**
    - von Willebrand disease (a disorder of both platelets and coagulation)
    - Hemophilia A (coagulation)
    - Hemophilia B (coagulation)
    - Other factor deficiencies (coagulation)
    - Hereditary platelet disorders (platelets)
2. **Acquired bleeding disorders**
    - Disseminated intravascular coagulation (DIC) (platelets and coagulation)
    - Idiopathic thromobocytopenic purpura (ITP) (thrombocytopenia)
    - Thrombotic microangiopathies: thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS) (thrombocytopenia)
    - Bleeding caused by deficiency of vitamin K dependent factors (coagulation)
    - Bleeding associated with liver failure (platelets and coagulation)

Hereditary bleeding disorders

Hereditary bleeding disorders include coagulation factor deficiencies and platelet abnormalities. In general, they are less common than acquired bleeding disorders (although as we'll see next, von Willebrand disease is pretty dang common).

Often a good history will elicit some comments from the patient with a hereditary bleeding disorder: "Yeah, now that you mention it, my sister gets the same nosebleeds I do" or "well, my mom says that my grandfather died when he was really young from some kind of blood problem." Sometimes, of course, there is no history that the patient knows about – or the patient is unconscious – so it's something you will want to consider when working up any patient with abnormal bleeding.

von Willebrand Disease (vWD)

General

- The most common hereditary bleeding disorder (1 in 100 people, by some estimates).
- Autosomal dominant inheritance, usually.
- You need to be aware of this disease because it is so common! You may never see someone with hemophilia (which happens in 1 in 10,000 people), but you'll for sure see someone with von Willebrand disease.
- Catching and diagnosing this disease early can reduce unnecessary procedures (like hysterectomies for heavy menstrual flow) and lessen risks (like excessive bleeding after tooth extractions).

The basic problem

- There's not enough von Willebrand Factor (vWF), or the vWF doesn't function properly.
- Type 1 vWD (70% of patients): decreased level of vWF
- Type 2 vWD (25% of patients): qualitatively abnormal vWF
- Type 3 vWD (5% of patients): complete lack of vWF

What's von Willebrand factor again?

- von Willebrand factor is a huge protein composed of multimers.
- It is synthesized by megakaryocytes and endothelial cells.
- It glues the platelets to the subendothelium.
- It is the carrier molecule for factor VIII (free factor VIII is destroyed).

Clinical findings

- The severity of bleeding is variable from patient to patient.
- Some patients (probably quite a few) don't have any symptoms at all.
- Typical vWD bleeding includes:
  - "platelet-type" bleeding - heavy menses (>24 pads/tampons a month), mucous membrane bleeding (e.g., nosebleeds), excessive blood loss from tiny cuts, easy bruising.
  - "factor-type" bleeding – late post-operative hemorrhage, joint bleeding (in severe cases).
- Patients with very severe (type 3) vWD may also show factor-deficiency-type bleeding, such as bleeding into large joints. This is because vWF is the carrier molecule for factor VIII - so if you don't have any vWF around, you're not going to have much viable factor VIII around.

Laboratory tests

- The bleeding time is prolonged (that's the whole point: you need vWF to bind platelets to endothelial cells).
- The INR is normal (because the extrinsic system is working fine)
- The PTT is usually prolonged (because of the whole factor VIII thing), and it "corrects" when you do a PTT mixing study (because the pooled plasma should have a normal amount of factor VIII in it).
- To confirm your diagnosis, you can order some fancy tests like:
  - a vWF level (low in type 1, absent in type 3, normal in type 2)
  - platelet aggregation studies (abnormal)

Treatment

- DDAVP (desmopressin) – stimulates vWF (and factor VIII) release from patient's endothelial cells. This is a common and easily-administered treatment (it's a nasal spray).
- Cryoprecipitate – this is a creamy white blood product that comes in a tiny pouch. It contains plasma proteins (including factor VIII and vWF) from a single human donor. If you need a lot, order a 6-pack (seriously).
- Factor VIII concentrate – this can be real factor VIII from pooled human plasma (not so good, because you worry about infectious diseases) or fake factor VIII from a drug company (a better idea; made using monoclonal antibodies and special DNA techniques).

Hemophilia A

General

- The most common coagulation factor deficiency.
- X-linked recessive inheritance, usually (about 30% of patients have a spontaneous mutation).
- Queen Victoria (British sovereign from 1837 until her death in 1901) had hemophilia and passed it on to three of her children: one affected son (Leopold) and two carrier daughters (Alice and Beatrice). Alice and Beatrice in turn passed the gene on to their children, and through these descendants, the disease proved disastrous for several of the royal houses of Europe. Check out the story of the "mad monk" Rasputin, who was consulted by Alix, wife of the Russian Czar Nicholas II, for treatment of their affected son, Alexis. Certain people didn't like the monk's influence on the Royal family, and this may have contributed to the eventual murder of the entire family in 1917.
- Anyway. One more interesting fact, and then we move on to the disease. Queen Victoria had no known family history of the disease – so she may have been one of the 30% of patients in which a spontaneous mutation is the cause of the disease.

The basic problem

- Factor VIII is decreased or totally absent.
- The factor VIII gene (at the end of the long arm of the X chromosome) is mutated.

What's factor VIII good for?

- It's one of the important coagulation factors in the intrinsic coagulation pathway (on the left-hand side of the little coagulation cascade imprinted on your brain).

Queen Victoria, Prince Albert and their children
Portrait by Franz Xaver Winterhalter in 1846.

Clinical findings

- Disease severity depends on how much factor VIII the patient can make.
- Typical bleeding in hemophilia includes recurrent, painful bleeding into joints following some sort of trauma, prolonged bleeding after dental extractions, and spontaneous mucosal hemorrhage.
- If disease is severe or untreated, joint deformity or disability can result.

Laboratory tests

- The PTT is prolonged, and it "corrects" when you do a PTT mixing study (Why? If you need to, go back and read about the PTT mixing study).
- The INR, TT, platelet count, and bleeding time are normal.
- Hey wait, the bleeding time is normal?
- Yes! The bleeding time is normal. That's because the bleeding time ONLY measures the platelet response to injury, not the coagulation cascade. Seems weird, but it's true. This is likely because patients with hemophilia can make a platelet plug just fine (so they stop bleeding normally after the bleeding time incision) - but they can't make fibrin to seal up that plug (so they will likely bleed at some point later on, after they've left the laboratory). Same principle as late bleeding following trauma or surgical procedures.
- To confirm your diagnosis, order quantitative and qualitative factor VIII assays.
- Diagnose the carrier state using DNA testing.

Treatment

- Patients with mild hemophilia (who can make a little factor VIII on their own) can use DDAVP (desmopressin), which stimulates the patient's endothelial cells to release factor VIII (along with vWF).
- Patients with severe hemophilia (who can't make any factor VIII at all) need factor VIII replacement. You want to give as little of this as you can, because some patients will start making antibodies to factor VIII (and then you have to give a ton of factor VIII to get any response).

Hemophilia B

General

- Inheritance pattern is the same as that of hemophilia A.
- Much less common than hemophilia A.

The basic problem

- Factor IX is decreased or totally absent.
- It's kind of nice that they named them this way: hemophilia A is factor VIII, and hemophilia B is factor IX.
- The factor IX gene (located next to the factor VIII gene) is mutated.

What's factor IX good for?

- It's one of the important coagulation factors in the intrinsic coagulation pathway (on the left-hand side of the little coagulation cascade imprinted on your brain).

Clinical findings

- Same as those of hemophilia A.

Laboratory tests

- Same INR, PTT, TT and bleeding time results as in hemophilia A.
- The only way to tell hemophilia A and B apart is by doing factor VIII and IX assays (and genetic tests).

Treatment

- Recombinant factor IX concentrate.

Other factor deficiencies

- Deficiencies of factors other than VIII and IX are very rare!
- **Factor XI** deficiency is seen mainly in Ashkenazi Jews, and usually causes excess bleeding only after trauma (such as surgery).
- **Factor XIII** deficiency causes a severe bleeding tendency (usually presenting with umbilical stump bleeding). It has a normal PTT! That's because factor XIII crosslinks fibrin molecules within the clot; it really has nothing to do with fibrin formation. So it's not measured by the PTT or the INR.

Hereditary platelet disorders

These are dang rare. So why are we covering them? Two reasons:

1. They have shown up on NBME part 1. I know, right? Ridiculous.
2. Several times after lecturing on this, someone has said, "I just had a patient with Bernard-Soulier!" or one of the others. So...whether something is rare or not, it happens (and to the patient who has it, it doesn't matter how rare it is).

Bernard-Soulier syndrome

- Autosomal recessive inheritance
- Patients have an abnormality of glycoprotein Ib (which binds von Willebrand factor), so their platelets can't bind to the subendothelium
- Giant platelets
- Severe bleeding

Glanzmann thrombasthenia

- Autosomal recessive inheritance
- Patients have an abnormality or absence of glycoprotein IIB-IIIa (which binds fibrinogen), so their platelets don't aggregate
- Severe bleeding

Gray platelet syndrome

- Platelets have few or no α granules (hence they look empty, or gray)
- Giant platelets
- Mild bleeding

δ granule deficiency

- Platelets have few or no δ granules (these aren't really visible by light microscopy anyway)
- Isolated, or part of a syndrome (like Chediak-Higashi)

Acquired bleeding disorders

Acquired bleeding disorders are more common than hereditary bleeding disorders. Unlike hereditary bleeding disorders, they involve multiple factor deficiencies.

Disseminated intravascular coagulation (DIC)

General

- DIC is a thrombo-hemorrhagic (clotting and bleeding) disorder that occurs as a complication of lots of different conditions.
- Something (see below) triggers the coagulation system, resulting in lots of little fibrin clots (which entrap platelets) throughout the body.
- These little clots (or microthrombi) do bad things: they snag and destroy red blood cells (causing a hemolytic anemia) and occlude small vessels (causing tissue hypoxia and infarction).
- All this rampant clotting uses up the platelets and coagulation factors, causing bleeding.
- To make matters worse, as the body tries to lyse the clots, fibrin is broken down into fibrin degradation products (which themselves inhibit clotting and aggravate the bleeding problem).

What kinds of things make a person go into DIC?

1. Things that dump procoagulant substances into the blood
    - obstetric complications (abruption, amniotic fluid embolism)
    - adenocarcinoma (especially really mucinous ones, like pancreatic adenocarcinoma)
    - venomous snake bites
    - acute promyelocytic leukemia
2. Things that damage endothelium or tissue
    - severe, gram-negative, toxin-producing bacterial infection
    - trauma or thermal burns
    - vasculitis (like systemic lupus erythematosus)

How am I supposed to remember all those things?

You'll do well if you remember the four things that cause most cases of DIC (Really! Listed this way, they spell “most!”):

- malignancy
- obstetric complications
- sepsis
- trauma

Things to make you look smart

Attending: “Tell me the four most common causes of DIC.”
Student (let’s see...M.O.S.T...M.O.S.T...): “Malignancy, OB complications, sepsis, and trauma.”
Attending: “I’m so impressed. Nice job. Every student should be as smart as you.”

Clinical findings

1. Onset may be fulminant (e.g., sepsis) or insidious (e.g., adenocarcinoma)
2. Symptoms are often in many organ systems:
    - respiratory system (dyspnea, cyanosis)
    - nervous system (seizures, coma)
    - renal system (oliguria, acute renal failure)
    - vascular system (circulatory failure, shock)
3. Signs of bleeding and thrombosis:
    - bleeding from venipuncture sites or surgical wounds
    - GI, lung, or obstetrical bleeding
    - petechiae
    - gangrene

Laboratory tests

1. Coagulation tests
    - The INR, PTT, TT and fibrinogen level are prolonged (because all factors are depleted)
    - Fibrin degradation products are increased (because of all the clotting going on)
2. Blood smear
    - The platelet count is low.
    - Fragmented red blood cells (schistocytes) are present; this is called a microangiopathic hemolytic anemia (MAHA) because the red cells are destroyed in small (micro) vessels.
    - The severity of DIC is reflected in the blood smear! Clinicians often order daily blood smears to see if the number of schistocytes is trending up or down.

Things to make you look smart

Attending: “If Mr. Jones really is in DIC, what should his blood smear look like?”
Student: “It will show a low platelet count and also some fragmented red cells, or schistocytes. We can order a smear every day and see if the number of schistocytes is going up or down.”
Attending: “Yes, that’s true. Man, where did you train? You’re brilliant! The rest of you, take note.”

Treatment

1. First of all, you need to diagnose and treat the underlying cause of the DIC as best as you can.
2. While you’re doing that, you can support the patient with:
    - fresh frozen plasma (FFP) – this contains coagulation factors
    - cryoprecipitate – this contains fibrinogen
    - platelets
    - red cells

Idiopathic thrombocytopenic purpura (ITP)

General

- Idiopathic thrombocytopenic purpura - also called immune thrombocytopenic purpura - is a disorder in which the patient makes antibodies against his/her platelets, which then get eaten up by macrophages.
- It is divided into chronic and acute forms, each of which has its own clinical features.

Clinical findings

1. Chronic ITP
    - Most common in adult women <40.
    - Primary (unrelated to any known risk factors) or secondary (occurring in the setting of other conditions, such as lupus, HIV, or chronic lymphocytic leukemia).
    - Onset often insidious. Symptoms include bleeding into skin and mucosal surfaces (petechiae), nosebleeds, easy bruising, melena, hematuria, and excessive menstrual flow.
    - Danger: subarachnoid hemorrhage and intracerebral hemorrhage (rare in treated patients though).
2. Acute ITP
    - Most common in children.
    - Abrupt onset; usually follows a viral illness.
    - Self-limiting in most cases.
    - In about 20% of children, though, it may persist and resemble chronic ITP.

Pathogenesis

- Auto-antibodies to platelet membrane glycoproteins IIb-IIIa, or Ib-IX.
- These anti-platelet antibodies bind to platelets, making the platelets yummy to phagocytes.
- Most of the eating happens in the spleen (if you take out the spleen, the symptoms usually go away).

Laboratory tests

1. Findings indicating platelet destruction include:
    - Low platelet count.
    - Normal/increased megakaryocytes in bone marrow.
    - Large platelets in blood (megakaryocytes are trying so hard to make platelets fast that they end up making big ones).
2. INR/PTT are normal! (because this is just a platelet problem)
3. There is no specific lab test to *prove* that the patient has ITP. It is a diagnosis of exclusion; only call it ITP after ruling out other causes of thrombocytopenia, such as:
    - Drug effect
    - Aplastic anemia
    - Bone marrow replacement (e.g., by tumor)
    - Big spleen
    - Non-immune-related platelet destruction (e.g., DIC, TTP, HUS)

Treatment

- Most patients respond to glucocorticoids (which inhibit phagocyte function).
- In patients who relapse, splenectomy often normalizes the platelet count. If that doesn't work, try immunomodulatory agents (like IV Ig or anti-CD20).

Thrombotic microangiopathies

General

- "Thrombotic microangiopathy" encompasses a bunch of clinical syndromes, the most common of which are thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS).

The definition for these diseases are shifting

1. Original definitions:

TTP	HUS
• MAHA • Thrombocytopenia • Fever • Transient neurologic deficits • Renal failure	• MAHA • Thrombocytopenia • More common in children

2. It's not so straightforward though. We now know:
   - TTP: patients may lack one or more of the five criteria.
   - HUS: some patients have fever, neurologic dysfunction, and/or renal failure.
3. It's sometimes hard to tell whether a patient has TTP or HUS!

Cause

- Thrombotic microangiopathy is caused by things (see "triggers," below) that activate platelets.
- The platelets clump together forming thrombi that get stuck in tiny vessels. This leads to MAHA and widespread organ dysfunction. To make matters worse, consumption of platelets leads to thrombocytopenia.
- Note: thrombotic microangiopathy has a different pathogenesis than DIC! While both have similar features (microthrombi and MAHA), in DIC the problem is activation of coagulation (so the INR and PTT are prolonged), but in thrombotic microangiopathies, the problem is platelet clumping (so the INR and PTT are normal!). Here's a little summary of the lab tests in TTP/HUS and DIC (let's throw in ITP, hemophilia and vWD just for the hell of it):

Things to make you look smart

Attending: "Can anyone tell me how you would use the INR and PTT to evaluate patients with a microangiopathic hemolytic anemia?"
Student: "Well, in DIC, the patient is consuming coagulation factors, so the INR and PTT are both prolonged. But there are also other causes of microangiopathic hemolytic anemia. In TTP and HUS, for example, the problem is not really a coagulation problem, but a platelet problem...so the INR and PTT are normal."
Attending: "Will you marry me?"
Student: "I'll think about it. Let's finish rounding first."

Triggers

1. TTP
    - Deficiency of a plasma enzyme called ADAMTS13 (also called vWF metalloprotease).
    - Hereditary (patients inherit an inactivating mutation in ADAMTS13) or acquired (patients develop an ADAMTS13-inhibiting autoantibody).
    - ADAMTS13 normally degrades very high-molecular-weight multimers of von Willebrand factor.
    - These are actually called Multimers of Ultra-large Size, or MOUS. Remember Princess Bride? Wesley is attacked by menacing rodents of unusual size, or ROUS, in the fire swamp. Kinda similar to the MOUS in TTP - big, honking, dangerous things to be avoided at all costs.
    - Without ADAMTS13, multimers accumulate and trigger platelet activation and aggregation.
    - You probably need some other, additional endothelial injury for TTP to occur (because people with hereditary ADAMTS13 deficiency don't always get TTP).

R.O.U.S from Princess Bride

A big, scary thing to be avoided at all costs.

2. HUS
    - Epidemic, "typical" HUS
        - Associated with gastroenteritis caused by E. coli O157:H7.
        - E. coli O157:H7 makes a nasty toxin that is absorbed from gut into blood.
        - Toxin injures endothelial cells and triggers platelet activation.
    - Non-epidemic, "atypical" HUS
        - Associated with defects in complement factor H, membrane cofactor protein (CD46) or factor I (proteins that normally keep the alternative complement pathway in check).
        - Inherited (various defects) or acquired (inhibitory autoantibodies).
        - Unclear how these defects trigger platelet activation.

Clinical findings

1. TTP
    - The classic pentad of findings is listed above. Here are the symptoms:
        1. blood in urine, jaundice (from the MAHA)
        2. bleeding, bruising (from severe thrombocytopenia)
        3. fever (Here's a little secret: you can always say "cytokine release" or "inflammation" when you don't know the exact cause of something)
        4. bizarre behavior, altered mental status, headache (from microthrombi in the CNS)
        5. decreased urine output (from renal failure)
    - Patients may also have other symptoms (besides those in the CNS and kidney) related to the deposition of microthrombi; any organ (heart, GI, others) can be affected.
    - Patients do NOT have to have the entire pentad to be diagnosed with TTP! In fact, most patients are missing one or more of the findings.
2. HUS
    - Epidemic HUS
        - Children and elderly are at highest risk.
        - Contaminated food (raw hamburger, spinach) is usually the culprit.
        - Classic symptoms: bloody diarrhea, followed a few days later by renal failure. Neurologic symptoms and fever are rare.
        - Patients can recover with supportive care; but irreversible renal damage and death occur in more severe cases.

Don't eat this!

Lebanese steak tartare by stu_spivack, from flickr

- Non-epidemic HUS
    - Symptoms are the same as in epidemic HUS, but without the diarrhea.
    - The disease course is relapsing-remitting.

Treatment

1. TTP
    - Plasmapheresis (to get rid of the antibodies) is useful in cases of acquired TTP.
    - Other immunosuppressive drugs (like steroids) can help too.
    - Patients with hereditary TTP are often given plasma every few weeks to replace their ADAMTS13.
2. HUS
    - Patients with HUS are treated supportively (dialysis, etc.).
    - If you're not sure whether it's HUS or TTP (often, it's hard to tell the difference!) you can do plasmapheresis for a while until you figure it out.

Other acquired bleeding disorders

Bleeding caused by deficiency or abnormality of vitamin K dependent factors

1. Vitamin K dependent factors: II, VII, IX, X, and proteins C and S (these guys all need to be γ-carboxylated by vitamin K in order to function).
2. Causes:
    - Coumadin (this causes a *functional* abnormality of vitamin K dependent factors. The *quantity* of II, VII, IX, X, and protein C and S is normal – but the quality is abnormal).
    - Poor diet (You get vitamin K from green leafy veggies and bacterial synthesis of vitamin K in the gut. Eat your veggies.).
    - Malabsorption (things affecting absorption: biliary obstruction, pancreatic disease, small bowel disease).
    - Prolonged treatment with antibiotics (much of our vitamin K is synthesized by our gut bacteria!)
    - Hemorrhagic disease of the newborn (newborns lack colonization of the bowel - so they get a shot of vitamin K at birth to tide them over. Without the shot, they can bleed during this period of time.)

Eat this!

Tuna multi greens salad by Mary Anne Enriquez, from flickr

Bleeding associated with liver failure

- There are *lots* of reasons that patients in liver failure bleed!
- Biliary obstruction causes impaired absorption of vitamin K, and therefore decreased synthesis of vitamin K-dependent coagulation factors.
- The liver makes most of the coagulation factors. If liver disease is really severe, the hepatocytes will make less – or dysfunctional – coagulation factors.
- Portal hypertension causes hypersplenism. With splenic sequestration of platelets, the platelet count decreases.
- Decreased thrombopoietin production by the liver contributes to the low platelet count.

Chapter 5: Thrombotic disorders

General

Before we get into thrombotic disorders

You'll be seeing lots of patients with thromboembolic events. Usually these take the form of deep venous thrombi (in the veins of the leg) or pulmonary emboli. The incidence of thrombosis goes up with age, and with the presence of certain risk factors (which we'll talk about). One of those risk factors is the presence of a thrombotic disorder (of which there are several types, as you'll see in a minute).

Just to give you some perspective: most patients who develop thrombi do *not* have a thrombotic disorder. Usually, the thrombus is due to a constellation of risk factors (for example, obesity plus smoking plus oral contraceptive use). So when a patient comes in with a thrombus, you wouldn't want to start working them up by looking for rare thrombotic disorders right away; you'd start by looking for common risk factors. If there aren't any, though, or if there are other weird findings (see below), that's when you should start looking for a thrombotic disorder.

Rudolf Virchow

If you haven't already read about Rudolf Virchow, you should stop reading this study guide immediately and go look him up in Wikipedia. I'm not kidding. Rudy (I like to call him Rudy) was one of those people in the 1800s that seemed to be able to do everything - and do it well. He was into anthropology, history, biology, politics, and on top of it all, he was a physician. He has a special place in the hearts of pathologists as he is considered "the father of pathology."

Rudolf Virchow
Drawing by Leslie Ward in Vanity Fair, 1893.

You'll hear his name a lot as you go through your training: Virchow's angle, Virchow's cell theory (which disproved spontaneous generation), Virchow's node, Virchow's autopsy method (which is a nice method, way better than that idiot Rokitansky's), Virchow-Robin spaces, the list goes on and on.

Virchow's Triad

One of his areas of discovery is particularly pertinent to our current discussion. Rudy coined the term "embolism" and then went on to define the causes thereof. He summarized them in a triad:

1. Endothelial damage

- Anything that rips up the endothelium, as we've discussed before, will increase the chances of clot formation.
- Atherosclerosis falls into this category, by the way, due to the damage that the plaques do to the endothelium

2. Abnormal blood flow

- Normally, blood should flow in a nice, laminar, smooth fashion. This keeps the clotting factors away from each other and from platelets.
- There are two types of abnormal blood flow that disrupt this process: stasis and turbulence.
- In stasis, the velocity of flow drops to a point where coagulation factors can attach to each other and platelets, initiating the coagulation cascade.
- In turbulence, flow is chaotic, and little eddies form. Blood is more static in these areas, allowing the factors and platelets to come into contact with each other as described above.
- Examples of stasis include varicose veins, atrial fibrillation (blood collects in the heart because it's not pumping uniformly), and prolonged immobilization (like in a plane ride, or after surgery). Turbulent blood flow can occur when there's a congenital heart defect (e.g., cardiac valve stenosis).

3. Hypercoagulability

- There are a bunch of seemingly unrelated risk factors that fall into this category, such as smoking, oral contraceptive use and adenocarcinoma (because the mucin in the tumor cells is a pro-coagulant).
- Thrombotic disorders (like we're talking about in this chapter) also fall into this part of Virchow's triad.

The triad is still a useful and correct concept today. if you look at all the known risk factors for thrombosis (see below), they all fall into one of these three categories. If you can't remember all those risk factors, you can probably remember Virchow's triad - and from there, you can list off a bunch of things that would increase your risk. Thanks, Rudy.

Risk factors for thrombosis

There are lots of risk factors for thrombosis. Most of these in and of themselves are not too dangerous...but if you start to get two or three or more, watch out: the risk goes way up.

- atherosclerosis
- trauma
- obesity
- prolonged immobilization
- varicose veins
- pregnancy
- oral contraceptive use
- myocardial infarction
- atrial fibrillation
- carcinoma (especially adenocarcinoma)
- smoking

When should you start worrying about a thrombotic disorder?

Most of the time, thrombosis is caused by the presence of too many risk factors. Sometimes, though, there is an underlying thrombotic disorder that is contributing to thrombosis. You should consider the possibility of a thrombotic disorder in your patient if:

- other family members have venous thrombosis
- the thrombosis is in a weird place ("normal" places = leg vein, lungs)
- your patient has had recurrent thromboses
- your patient is young (<50)
- your patient has had miscarriages (fetal losses)

Thrombotic disorders

We're going to divide the thrombotic disorders into two big groups, just like we did for bleeding disorders: those that are hereditary, and those that are acquired. Most of these are pretty rare, but for the reasons we talked about earlier, we still need to discuss them. The one exception is Factor V Leiden, which is surprisingly common. Here's the outline we'll be following:

1. Hereditary thrombotic disorders

- Factor V Leiden
- Antithrombin III deficiency
- Protein C (the anti-clotting Batman) deficiency
- Protein S (the anti-clotting Robin) deficiency
- Factor II (prothrombin) gene mutation
- Hyperhomocysteinemia

2. Acquired thrombotic disorders

- Antiphospholipid antibody syndrome

Hereditary thrombotic disorders

Sometimes, as mentioned before, patients have thromboses that seem to come out of the blue. In the past, patients like this would undergo tons of different tests - but a significant number would not be diagnosed with any thrombotic disorder at all. That's frustrating. Then a new disorder called Factor V Leiden was discovered, and it turned out to be responsible for a lot of these previously-unexplained thromboses. If you could only learn about one hereditary thrombotic disorder, Factor V Leiden would be the one.

Factor V Leiden

What's factor V Leiden?

- It's a slightly different factor V molecule, which is the product of a factor V gene with a single point mutation.
- Factor V Leiden is almost identical to normal factor V. The only difference is that there is a glutamine instead of an arginine at position 506 (virtually all patients with the disease have this mutation - a few other mutations have been described but they are rare).
- It's close enough in structure to normal factor V that it can participate in the coagulation cascade…
- …but different enough that *protein C can't cleave it* (and turn it off)!
- So, coagulation proceeds along its merry way with one less natural anti-coagulant to keep it in check.
- "Leiden" is for the city in the Netherlands where the mutation was first discovered.

The Oude Rijn ("Old Rhine") with drawbridge
Leiden, Netherlands

Things to make you look smart

Q. What's activated protein C resistance?
A. It's when protein C (after being activated) is unable to cleave factor V. There are lots of causes of activated protein C resistance - but Factor V Leiden is by far the most common.

How common is the factor V Leiden mutation?

- About half of the patients with "unexplained" thrombosis – that is, thrombosis occurring in the absence of known risk factors – turn out to have factor V Leiden.
- About 5% of Caucasians carry the mutation. No, that's not a typo: it's really 5%. People of Asian, Native American, or African descent are very unlikely to carry it.

What is the risk of getting a bad clot if you have the mutation?

- Heterozygotes have a thrombosis risk that is 7 times normal.
- Homozygotes have a thrombosis risk that is 80 times normal.

Things to make you look smart

The incidence of deep venous thrombosis in the general population is actually pretty small. If you adjust for age and sex, the annual incidence is somewhere around 120 people per 100,000. That number increases with age, and overall it's slightly higher in men than in women. Still: that puts things into perspective. If you have a risk of thrombosis that's double the normal risk, that sounds really scary - but in reality, your risk is still very low, since the normal risk is very very low.

How do you diagnose factor V Leiden?

- The INR and PTT are normal in these patients (Can you explain why? If not, drop us an email at pathology@PathologyStudent.com.), so these tests don't help you.
- You need to order genetic testing (sounds complicated, but it's not. It's a cheap PCR test that's becoming more and more common these days). This will tell you if your patient has the factor V Leiden mutation, and as a bonus, you'll find out whether your patient is homozygous or heterozygous.

How do you treat patients with the factor V Leiden mutation?

- In general, patients with factor V Leiden (even homozygotes!) should *not* be treated unless they have had a thrombosis.
- Coumadin is the only thing you can really give these patients. That, and tell them to avoid all the risk factors they can.

Antithrombin III deficiency

What is antithrombin III again?

It's a natural anticoagulant that inhibits the serine proteases (IIa, VIIa, IXa, Xa, XIa, XIIa), thus inhibiting all three pathways. Heparin potentiates its action.

What is wrong with the antithrombin gene in this disorder?

Lots of different mutations have been described, each with its own risk of thrombosis (compare this to factor V Leiden, in which virtually all patients have the exact same mutation).

How common is antithrombin III deficiency?

It's a *rara avis*. About 0.05% of the general population has antithrombin III deficiency.

What is the risk of getting a bad clot if you have it?

- Big! About half of heterozygous patients with this disorder will develop a thrombus – and many have recurrent thromboses. Homozygosity is incompatible with life.
- Heparin doesn't work in these patients (why?) - so antithrombin concentrates are used instead.

How do you diagnose antithrombin III deficiency?

- The best method is functional testing – which tells you if the patient's antithrombin III is working (and whether there's enough of it around).
- Antithrombin III functional testing is done by adding a known amount of thrombin to the patient's plasma, and then measuring the thrombin activity. In normal patients, the thrombin activity decreases. In patients with antithrombin III deficiency, the thrombin activity remains the same (because there's not enough antithrombin III to inhibit thrombin).
- It's best to do this test when the patient is not actively clotting or on anticoagulant therapy, because these things can temporarily change the level of antithrombin III. Which makes it kinda hard to find a good time to schedule this test.

Protein C deficiency

What is protein C again?

It's a natural anticoagulant that inactivates factors V and VIII, thereby shutting down clotting. It works with protein S to do this.

What's wrong with the protein C gene in this disorder?

Like antithrombin III deficiency, protein C deficiency can be caused by many different mutations in the protein C gene.

How common is protein C deficiency?

It's another *rara avis*. About 0.2% of the general population is heterozygous for the protein C mutation.

What is the risk of getting a bad clot if you have it?

- Heterozygotes have a thrombosis risk that is 7 times normal.
- Homozygous humans have not been reported. They likely abort spontaneously during gestation.
- In addition to the risk of thrombosis, patients also have a risk of skin manifestations: warfarin (coumadin)-induced skin necrosis and purpura fulminans.

Things to make you look smart

Q. What's warfarin-induced skin necrosis?

A. Warfarin-induced skin necrosis is a nasty problem that happens to people with previously-undiagnosed protein C deficiency. Here's how it happens:

- In addition to making factors II, VII, IX and X nonfunctional (by inhibiting their activation by epoxide reductase), warfarin (Coumadin) also inhibits proteins C and S activation.
- It turns out that protein C has a super short half-life (shorter than any of the other factors - even factor VII). So protein C becomes non-functional first, before any of the other factors.

Which means that right after Coumadin administration, patients are actually a bit hypercoagulable.
- Within a few days, the other factors are inhibited, tipping the balance in favor of anticoagulation.
- For most patients, the initial hypercoagulable state doesn't manifest clinically (giving a little heparin in the beginning is not a bad idea, though, because that should take care of any hypercoagulability during those first few days).
- For patients with protein C deficiency, however, that initial protein C inhibition is dangerous (since their protein C levels are already really low). It can manifest as widespread clotting that for some reason shows up most in the skin (with purpura and necrosis).

Q. What's purpura fulminans?

A. It's a similar situation - widespread coagulation with purpura and skin necrosis. It is most common in babies and small children, and it is usually associated with severe infection (often with a gram-negative organism). It is an acute and life-threatening condition that requires aggressive treatment.

How do you diagnose protein C deficiency?

- The best method is functional testing – which tells you if the patient's protein C is working (and whether there's enough of it around).
- It's best to do this test when the patient is not actively clotting or on anticoagulant therapy, because these things can temporarily change the level of protein C. So like antithrombin III testing, it can be a little tricky to get the patient in the right condition for the test.

Protein S deficiency

What is protein S again?

It's a cofactor for protein C. It helps protein C inactivate factors V and VIII, thereby shutting down clotting.

What's wrong with the protein S gene in this disorder?

There are many different mutations in the protein S gene that can lead to protein S deficiency.

How common is protein S deficiency?

Protein S deficiency is an *über rara avis* (how's that for mixing languages?). It's even more rare than protein C deficiency – but the exact incidence in the general population is not known.

What is the risk of getting a bad clot if you have it?

- Heterozygotes have a thrombosis risk that is 7 times normal.
- Homozygous infants are at risk for getting purpura fulminans.

How do you diagnose protein S deficiency?

- Same story as for antithrombin III and protein C: functional testing.
- Same caveats apply: you can't be sure of test results when the patient is actively clotting or on anticoagulant meds.

Factor II (prothrombin) gene mutation

What's wrong with the factor II gene in this disorder?
In this disorder, there is a gene mutation that causes the production of *excess* prothrombin! The prothrombin itself is normal in structure and function, though. It's just that there's too much of it.

How common is the factor II mutation?

- About 5% of Caucasians carry the mutation.
- People of Asian, Native American, or African descent are very unlikely to carry it.

What is the risk of getting a bad clot if you have the mutation?

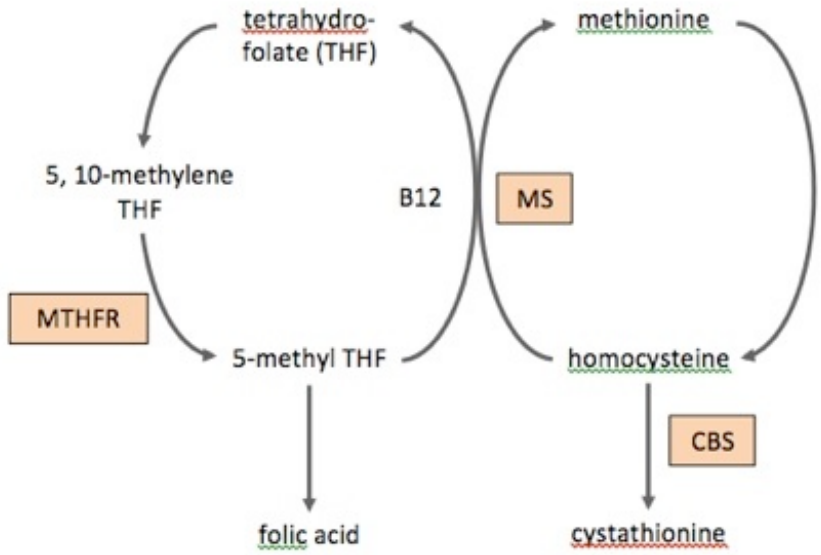
- Heterozygotes have a thrombosis risk that is 2 times normal.
- Homozygotes have a thrombosis risk that is 20 times normal.

How do you diagnose the factor II gene mutation?

- There is a PCR test, like in factor V Leiden.

Hyperhomocysteinemia

What's homocysteine again?
Homocysteine is made from dietary methionine. It's important in maintaining myelin, and it is essential for the conversion of dietary folate into the form of folate that participates in DNA synthesis. Check out the B12/folate/homocysteine/methionine diagram below. You've seen before in biochem (shudder) and will no doubt see again, in different iterations, in other courses. It shows how methionine (which comes from your diet) is turned into homocysteine, some of which gets metabolized into cystathionine and some of which gets recycled into methionine (with the help of B12 and folate).



CBS = cystathionine beta synthase
MTHFR = methylenetetrahydrofolate reductase
MS = methionine synthase

So how do you get hyperhomocysteinemia?

- Classic homocystein*uria* is a rare autosomal recessive disorder caused by a deficiency in the cystathionine beta synthase (so homocysteine doesn't get broken down into cystathionine). It turns out that these patients get vascular disease and thrombosis at a much earlier age, and to a greater extent, than patients without homocysteinuria. It's thought that the vascular disease/thrombosis is caused by the high homocysteine levels – but it hasn't been definitively proven.
- But you can get elevated levels of homocysteine by other means:
  - Some people have mutated methyl tetrahydrofolate reductase gene (the gene that produces the enzyme with the unfortunate abbreviation MTHFR). Europeans, Middle Easterners, and Japanese patients have the highest carrier rates. This mutation can obstruct the normal folate cycle, leading to mild homocysteinemia.
  - Dietary deficiencies of folate, B12, and B6 can also lead to mild homocysteinemia.

What's so bad about having hyperhomocysteinemia?

- Hyperhomocysteinemia appears to be associated with a greater risk of thrombosis and atherosclerosis (leading to arterial and venous thromboses, myocardial infarction, and stroke).
- Exact mechanisms are not clear, but it is known that too much homocysteine can have bad effects on hemostasis (by causing tissue factor expression) and vessel walls (by causing intimal thickening).

What is the risk for getting a bad clot if you have it?

- The risk for venous thrombosis is 2.5 times normal.
- The risk for myocardial infarction is 10 times normal.

How do you diagnose hyperhomocysteinemia?

- Measure the plasma level of homocysteine (homozygotes have very high levels; but heterozygotes or patients with dietary deficiencies may have only slightly elevated or even normal levels).
- There is a PCR test for the MTHFR gene mutation (expensive, but diagnostic).
- You might also want to look for folate deficiency (using serum and red cell folate levels) and B12 deficiency (using methylmalonic acid levels).

Acquired thrombotic disorders

The most important acquired thrombotic disorder is the antiphospholipid antibody syndrome.

Antiphospholipid antibody syndrome

What are antiphospholipid antibodies?

- Autoantibodies in the patient's plasma that are directed against various phospholipids.
- These autoantibodies also just happen to bind to the phospholipid part of the PTT reagent (and, sometimes, the PT reagent). Then there's not enough usable reagent in the test tube, and the patient's specimen doesn't clot. The reaction is therefore *falsely* prolonged, making one think the patient has a bleeding tendency (though this would not fit the clinical picture). Remember: treat the patient, not the laboratory values!
- By the way, these antibodies can also bind to the reagent in the syphilis test, causing a false positive result.
- They are sometimes called "inhibitors" because they appear to *inhibit* coagulation in the test tube.
- The weird thing is that *in vivo*, these antiphospholipid antibodies can actually cause thrombosis (through a bunch of different proposed mechanisms). That's a weird paradox: patients look like they should bleed more easily (if you look at their PTT), but actually, they may be at increased risk of thrombosis.
- There are a bunch of different types of antiphospholipid antibodies, including anticardiolipin antibodies, anti-β2-glycoprotein antibodies, and lupus anticoagulants (which were discovered in patients with lupus).

How do you get these antiphospholipid antibodies? Are they dangerous?

- Children sometimes develop them after an infection. In this setting, the risk of thrombosis is only slightly increased.
- Adults sometimes develop them as part of an autoimmune disorder, like lupus (in fact, antiphospholipid antibodies in whatever clinical setting are often called "lupus inhibitors" or "lupus anticoagulants" because of this association). In this setting, the risk of thrombosis is moderately increased.
- Elderly adults may develop them in association with drugs. In this setting, the risk of thrombosis is not increased.
- When a patient with an antiphospholipid antibody has severe consequences, like recurrent miscarriage and renal failure, it's called antiphospholipid antibody syndrome.

What do you do if you think your patient might have an antiphospholipid antibody?

- Order a PTT. If it's prolonged, it should *not* correct if you do a PTT mixing study (Why? If you need a refresher, go back and look at the PTT mixing study in chapter 3.)
- The tricky part is this: even if the PTT is normal, your patient still may have an antiphospholipid antibody! That's because the prolongation of the PTT depends on the particular PTT reagent your lab is using.
- There are a bunch of other, fancy tests you can do to detect antiphospholipid antibodies. Just call the laboratory and ask them to do whatever test they offer.

Reference

Blood products

Blood product	Contents	Use
Whole blood	Red cells, plasma, white cells, platelets	Not used much, except in massive blood loss (say, >40% of blood)
Red blood cells ("packed red cells")	Mostly red cells (with a little plasma, a few white cells and a few platelets)	Low hemoglobin
Platelets	Platelets	Bleeding due to low platelet count
Fresh frozen plasma (FFP)	Fibrinogen and all other coagulation factors	Coagulopathy due to multiple factor deficiencies (like in liver disease), protein C or S deficiency, or TTP/HUS
Cryoprecipitate	Fibrinogen, factors VIII and XIII, and von Willebrand factor	Fibrinogen deficiency, hemophilia A, factor XIII deficiency, and severe von Willebrand disease
Granulocytes	Mostly neutrophils (with some plasma, platelets and red cells)	Severe neutropenia

Coagulation factors: common names and functions

I	Fibrinogen	Forms fibrin part of the clot
II	Prothrombin	Serine protease
III	Tissue factor	Inhibitor/cofactor
V	Labile factor; accelerin	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart-Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman factor	Serine protease
XIII	Fibrin stabilizing factor	Transglutaminase
-	Prekallikrein (Fletcher factor)	Serine protease
-	High molecular weight kininogen (Fitzgerald factor)	Cofactor

Common abbreviations

ADAMTS13	A disintegrin and metalloproteinase with a thrombospondin type I motif, member 13
aPTT	Activated partial thromboplastin time
ATIII	Antithrombin III
DIC	Disseminated intravascular coagulation
DVT	Deep venous thrombosis
FDP	Fibrin degradation products
FFP	Fresh frozen plasma
GP	Glycoprotein (as in GP Ib, GP IIb-IIIa)
HMWK	High molecular weight kininogen
HUS	Hemolytic-uremic syndrome
INR	International normalized ratio
ITP	Idiopathic (or immune) thrombocytopenic purpura
MAHA	Microangiopathic hemolytic anemia
MTHFR	Methyl tetrahydrofolate reductase (no, not what you were thinking!)
PAI	Plasminogen activator inhibitor
PE	Pulmonary embolus
Plt	Platelet count
PT	Prothrombin time
PTT	Partial thromboplastin time
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
t-PA	Tissue plasminogen activator
TT	Thrombin time
TTP	Thrombotic thrombocytopenic purpura
vWD	Von Willebrand disease
vWF	Von Willebrand factor

Sources

Here are some good reference sources for further reading and explanations.

1. Hoffbrand A.V. *Essential Haematology*. Fifth edition 2006: Blackwell Science, England
2. Robbins pages 115 – 121 (normal hemostasis)
3. Robbins pages 121 – 129 (thrombosis and consequences)
4. Robbins page 666 (lab tests)
5. Robbins pages 667 -674 (bleeding disorders)

Fun activities

1. Draw out your own little map of the coagulation cascade, including the natural anticoagulants, in whatever way makes sense to you. Include shoes if you like. Promptly forget this after boards. Redraw it as an intern and put it in the little book you carry in your white coat.

2. Fill in the following tables:

Test	Measures what?	When would you order it?
PT		
INR		
PTT		
TT		
PTT mixing study		
FDP assay		
Fibrinogen assay		
Bleeding time		
Platelet aggregation studies		

Disorder	Basic problem	Bleeding risk	Diagnostic test(s)
Von Willebrand disease			
Hemophilia A			
Hemophilia B			
Bernard-Soulier			
Glanzmann thrombasthenia			
Gray platelet syndrome			
Delta granule deficiency			
DIC			
ITP			
TTP			
HUS			

Disorder	Hereditary?	Race	Thrombosis risk	Diagnostic test(s)
Factor V Leiden				
Factor II gene mutation				
Hyperhomocysteinemia				
Protein C deficiency				
Protein S deficiency				
ATIII deficiency				
Antiphospholipid antibody syndrome				
DIC				
ITP				
TTP				
HUS				

3. Write down as many risk factors for thrombosis as you can think of. Go back to Robbins p. 122, table 4-2, and fill in the ones you missed.

4. Eat mapo doufu, an ancient Chinese longevity dish that turns out to be an excellent source of anticoagulants (see Hammerschmidt D, Szechwan Purpura, NEJM 1980; 302(21):1191-3). Use as excuse to go unshaven for a week.

Mapo Doufu

¼ cup dried tree ear mushrooms plus 1 cup boiling water
3" piece of fresh ginger
8 or more cloves garlic (for a yield of 2 Tbsp. chopped garlic)
5 scallions
6 fresh water chestnuts (can substitute jicama)
½ lb. ground pork or beef
2 lbs. fresh bean curd, cut into ½" cubes
2 Tbsp. soy sauce
1 tsp. sesame oil
1 Tbsp Chinese rice wine or cooking sherry
2 tsp. cornstarch plus ¼ cup water, combined in a small bowl
6 Tbsp. peanut oil
1 ½ tsp. hot pepper flakes in oil
1 Tbsp. hot pepper paste
2 Tbsp. oyster sauce (optional)
1 tsp. sugar
another 3 Tbsp. soy sauce, plus 1/3 cup water
½ tsp. ground, roasted Szechwan peppercorns
another 1 tsp. sesame oil
1 tsp. salt, or to taste

Put the tree ears in a small bowl and pour the boiling water over them. Let them soak for about 15 minutes, until they become soft and gelatinous. Peel the ginger, then chop it into tiny pieces, about the size of a match head. Clean the scallions, then chop them, both the white part and about one-third of the green, into pieces slightly larger than the ginger, about 1/4" in diameter. If a food processor is not available, march-chop the ground meat for a finer texture. Add 1 Tbsp. of the chopped ginger and one scallion's worth of the chopped scallions to the ground meat, along with the soy sauce, sesame oil, and wine, and mix thoroughly. If a food processor is available, process the ginger, scallion, ground meat, soy sauce, sesame oil, and wine all together. Set aside the meat mixture for about 30 minutes.

Peel the garlic, then chop it coarsely. Combine it with the rest of the chopped ginger and mince them together until they reach the consistency of a thick paste (or use a food processor). Mrs. Chiang insists that the finer you chop the garlic and ginger the more interesting the finished dish will be.

Cut the dark skin off the outside of the water chestnuts, then chop them into pieces about the size of a match head. Drain the tree ears, then rinse them and pick them over carefully to remove the tiny impurities, like little pieces of wood, that might still be embedded in them. Then mince them into little pieces the size of a match head.

Just before you are ready to begin cooking, add the cornstarch to the meat mixture and blend thoroughly. Heat your wok or pan over a moderately high flame for about 15 seconds, then add

the peanut oil. It should be hot enough to cook with when the first small bubbles begin to form and a few small wisps of smoke appear.

When the oil is ready, quickly throw in the garlic and ginger and vigorously stir-fry them over a medium flame for about 30 seconds, using your cooking shovel or spoon to scoop the ingredients from the sides of the pan and then stir them around in the middle, so they won't burn or stick.

Continue to stir-fry while you add the hot pepper flakes in oil, hot pepper paste, water chestnuts, tree ears, and oyster sauce. Then stir-fry for another 30 seconds.

Add the meat mixture and keep stirring it as it cooks, taking special care to break up any large chunks of meat that stick together. After the meat has cooked for about 1 minute and has lost its pinkish color, throw in the bean curd and the remaining chopped scallions and stir-fry everything together. Then add the sugar and stir-fry for another 30 seconds.

Pour in the 3 Tbsp. soy sauce and the 1/3 cup water and wait for the liquid to boil, then let the contents of the pan cook over a moderate flame for 2 minutes more. Add the peppercorns and stir thoroughly. At this point, determine how much sauce there is in the pan. If the dish seems watery, you should get ready to add the cornstarch and water mixture that you have already prepared. But if there does not seem to be much liquid, you won't need the cornstarch.

Make sure that you stir up the cornstarch mixture before you pour it into the pan, then stir-fry everything over a medium flame for a few seconds until the sauce becomes clear and slightly thickened. Add the 1 tsp. sesame oil and stir it in thoroughly; then, just before serving, taste the dish for salt. It should taste sharp and clear, with just a hint of sweetness. Stir in the 1 tsp. salt, if you want, and serve.

Het Hu Nu (tree ear mushroom)
(clayirving on Flickr)