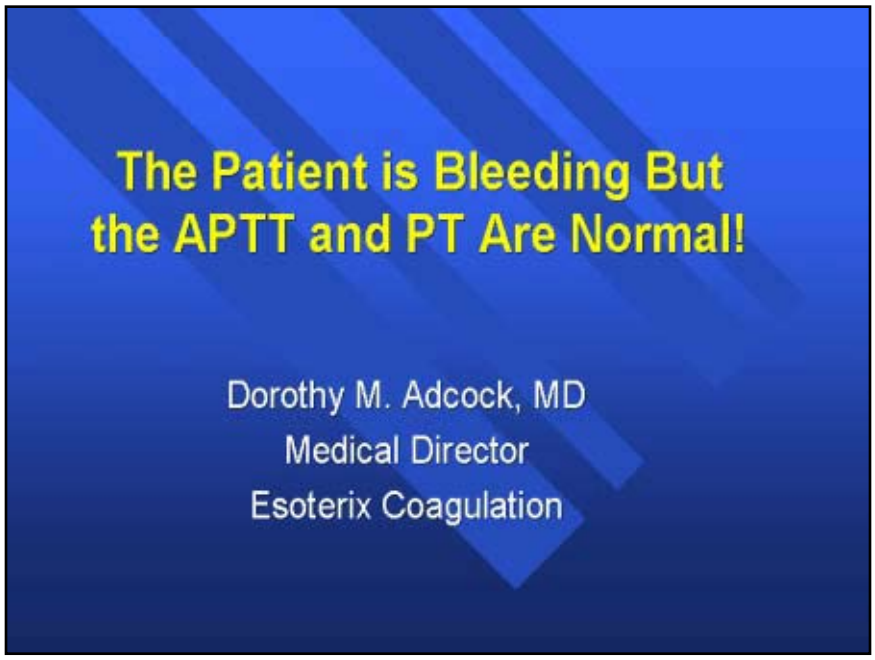
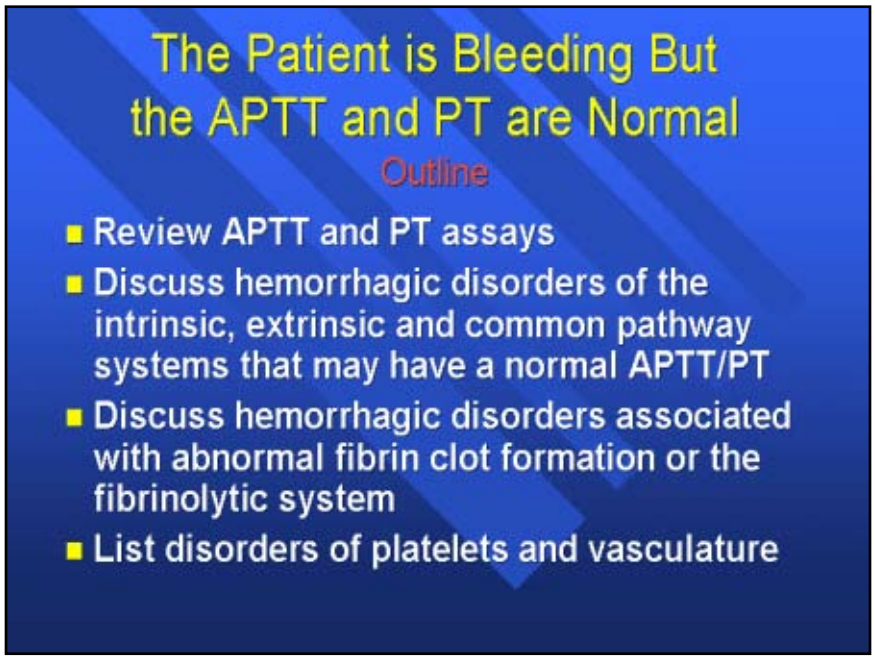
The Patient is Bleeding & the PT/aPTT are Normal
Dorothy Adcock, MD

Title: The patient is bleeding but the APTT and PT are normal



As Dr. Worfolk mentioned, the topic of the discussion this morning, the patient is bleeding, but the aPTT and PT are normal.

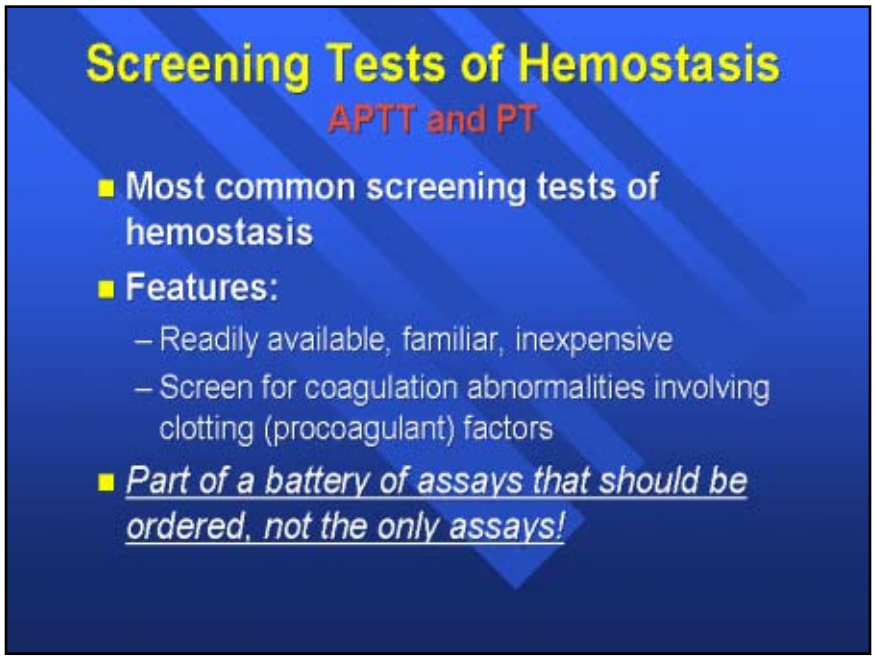
Title: The patient is bleeding but the APTT and PT are normal - Outline



What we will discuss this morning is, first of all, we will review the aPTT and PT assays, and I will be referring to the aPTT as just the PTT in the future. We will discuss hemorrhagic disorders of the intrinsic, extrinsic, and common pathway systems that may have a normal PTT and PT.

We will then discuss hemorrhagic disorders associated with abnormal fibrin clot formation of the fibrinolytic system. And then finally, we will list disorders of platelets and vasculature that may be associating with bleeding diathesis as well.

Title: Screening tests of hemostasis - APTT and PT



Now I am certain that all of you are aware that the PTT and PT are the most common screening tests of the hemostatic system. These tests are of course readily available to us in our laboratories; they are familiar to us and

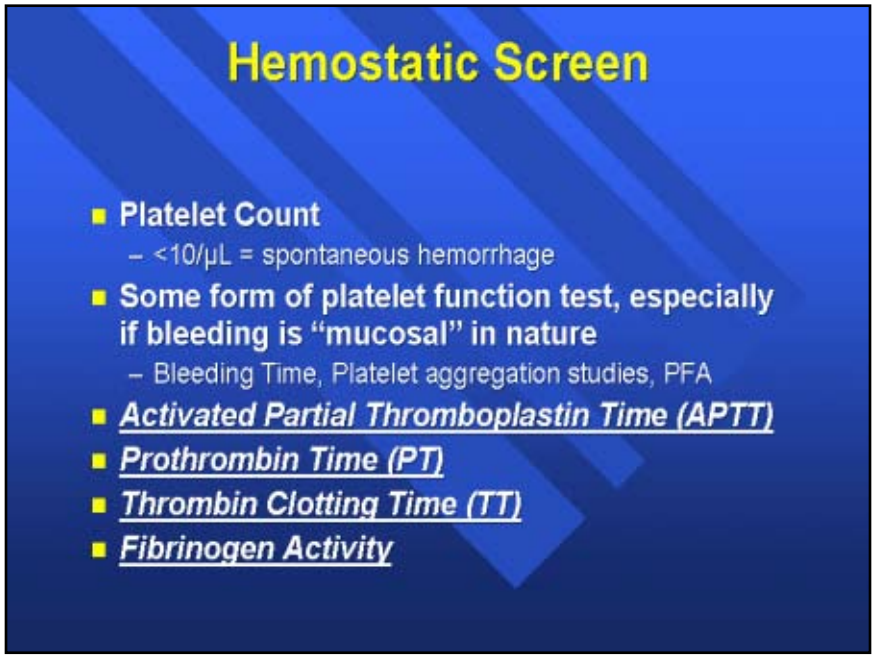
inexpensive.

And really what I would like to do this morning is bring some more material to us regarding these assays. In the sense that, since these assays have been in the laboratory for such a long period of time, we tend to take these assays for granted, and perhaps expect too much out of these assays.

So what I would like to review with you this morning are some of the limitations of these assays. So that we can use them to their best advantage in order to help the patients that we're treating. Now, the PTT and the PT of course are assays that are used to screen for coagulation abnormalities involving the procoagulant or clotting factors.

Keep in mind that if you are evaluating a patient for an abnormality of the hemostatic system, that the PTT and PT are really only part of a battery of assays that should be ordered. These are not the only assays that we should consider evaluating in a patient with a bleeding diathesis.

Title: Hemostatic screen



A hemostatic screen, a thorough hemostatic screen, should include the following assays. You should of course perform a platelet

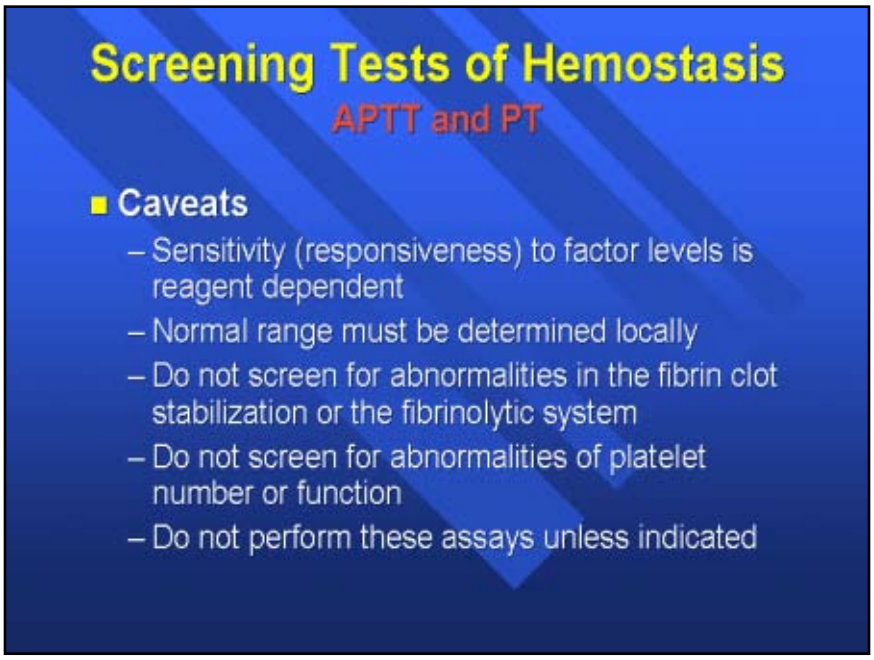
count. Keep in mind that if a patient has less than 10,000 platelets, they are at a risk for spontaneous hemorrhage.

And the typical number of platelets that is required for a surgical procedure is about 50,000 platelets. So certainly a low platelet count is a cause of abnormal bleeding, as is platelet dysfunction. If your patient has a bleeding diathesis, some sort of platelet function test is in order.

This is particularly true if the patient suffers what is called mucosal bleeding. And we'll talk about what that means in a few minutes. Now any sort of a platelet function test is probably adequate whether it's a bleeding time, platelet aggregation studies or some sort of an automated platelet function test such as a platelet function analyzer evaluation.

The hemostatic screen should also include of course the PTT; the PT; and if your laboratory can do it, a thrombin time; as well as fibrinogen activity. So keep in mind that this is the, that hemostatic screen should include all of these assays, but we're going to focus today on the PTT and the PT to begin with.

Title: Screening tests of hemostasis - APTT and PT - Caveats



Now there are a few caveats that we'll begin with when we talk about the PTT and the PT. The sensitivity or the responsiveness of these assays to factor levels is very reagent dependent, and we will talk about how to look at responsiveness of the various reagents.

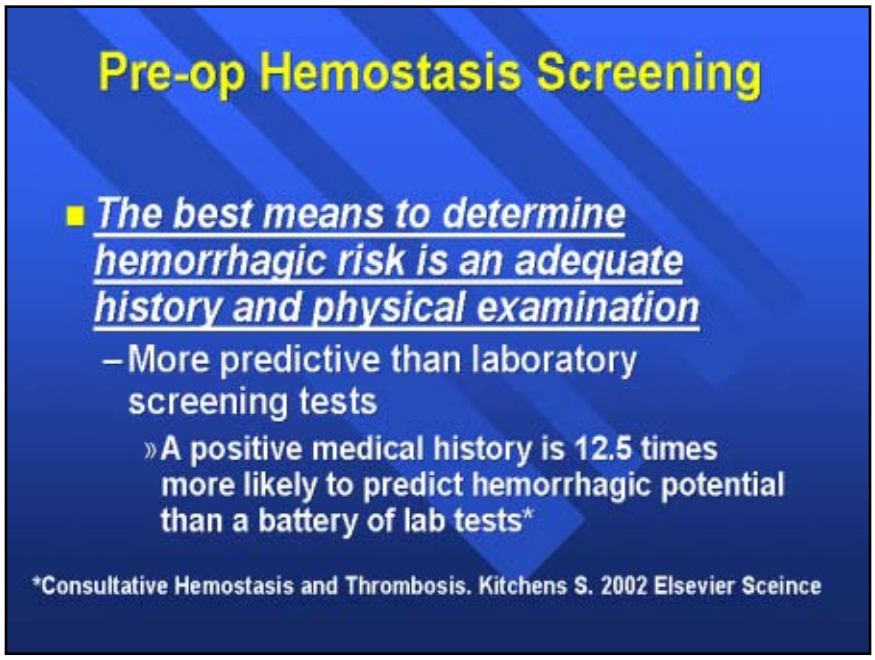
Your normal range of course must be determined locally, and this range as you all know must be determined every time a lot change is brought in to your laboratory.

The normal range is really critical, because it is only by defining a normal range that you determine what abnormal is. If your normal range is too broad, if you perhaps use a 3 SD normal range rather than a 2 SD normal range, then you will be missing clinically significant abnormalities.

So how you define your normal range is really critical, because that of course determines what abnormal is. Keep in mind also that the PTT and the PT do not screen for abnormalities in fibrin clot stabilization, nor in the fibrinolytic system.

And we will take this opportunity this morning to review some of those abnormalities. And of course they don't screen for abnormalities of platelet number and function. Now it's also important to keep in mind that we should only perform these assays when it's clinically indicated. These assays should not be performed unless they will be useful.

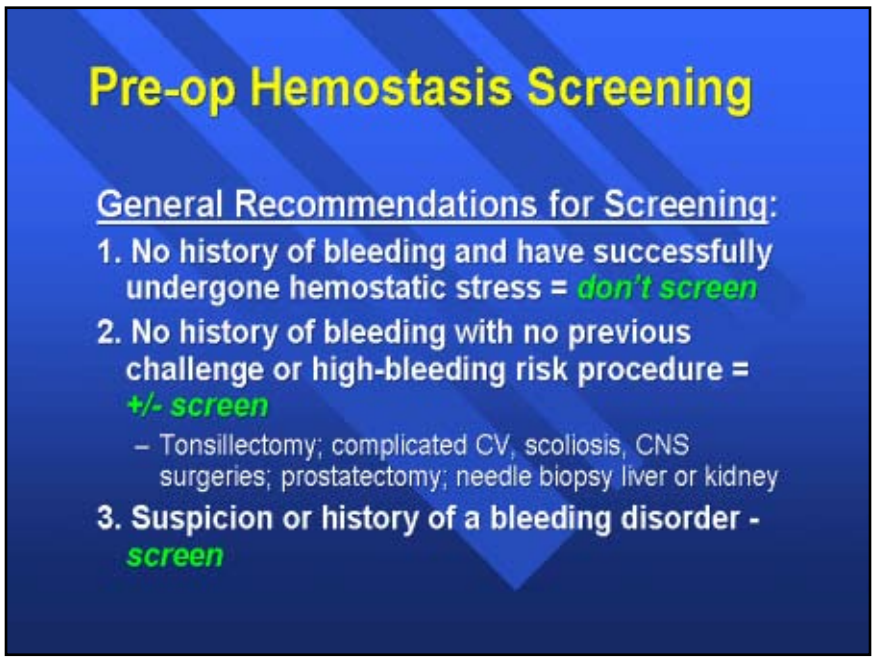
Title: Pre-op hemostasis screening - The best means to determine...



If you're evaluating the patient preoperatively, the very best means to determine hemorrhagic risk is an adequate history and physical examination. These in fact are more predictive than any laboratory screening assays.

In a study reported in a book by Dr. Kitchens, he has demonstrated that a positive medical history is 12-1/2 times more likely to predict a hemorrhagic potential than a battery of laboratory tests. So that's always an important consideration. When should you be performing these tests?

Title: Pre-op hemostasis screening - General recommendations for screening



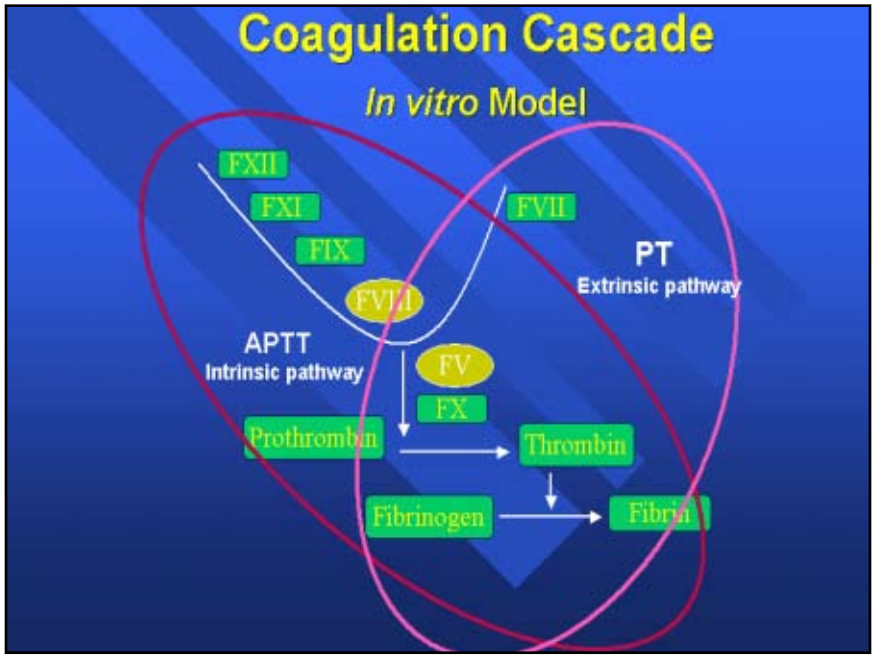
All right, so here are the general recommendations for screening that I think are really very useful. If your patient has no history of bleeding, and they have successfully undergone some sort of a hemostatic stress in the past, that is some sort of a surgical procedure, dental procedure, then it really isn't necessary to screen these patients preoperatively with the PT or a PTT.

If the patient has no history of bleeding, but no previous challenge, and they will be undergoing a high-risk procedure, you may consider screening.

And I've listed for you some of the surgical procedures that may be associated with a bleeding diathesis where you really may want to screen your patient with the PT and the PTT, then of course if your patient has a suspicion or a history of bleeding, a bleeding disorder, then you should screen these patients.

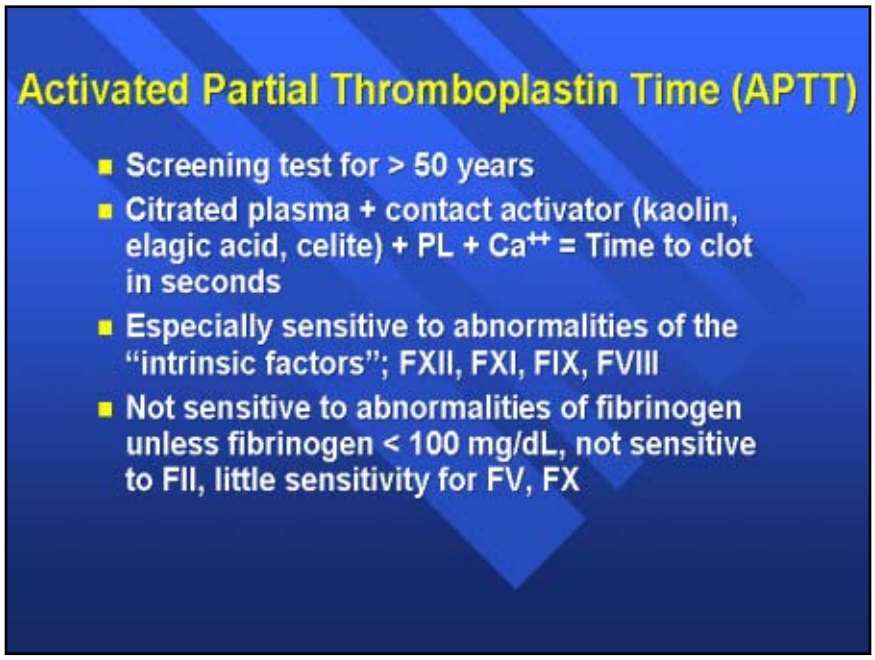
Well, what we're going to be talking about today is that when these screens may not tell you all the information you need to know, and when you have to pursue additional laboratory testing.

Title: Coagulation cascade - In vitro model



Now just as a reminder, this is a cartoon of the PTT and the PT, which is, evaluates the intrinsic and the extrinsic systems, and we'll be talking about this in vitro model of clotting as we continue this morning.

Title: Activated partial thromboplastin time (APTT)



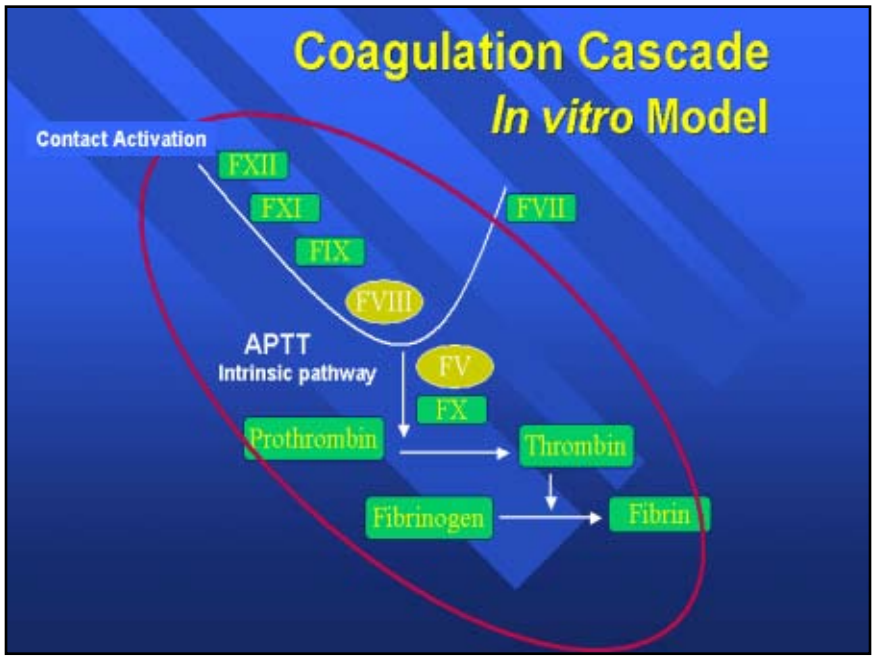
Now the PTT. This test has been a screening test in the coagulation laboratory for over 50 years. The way the test is performed, of course we take citrated plasma, and we add some sort of a contact activator.

This activator can be kaolin, ellagic acid or Celite. And then we add to this phospholipid and calcium. Now, the sensitivity of the PTT reagents depends very much on what activator is used, what phospholipid is used, and what concentration of phospholipid is used.

This is what sets various PTT reagents apart, and determines their responsiveness to not only various factor levels, but also lupus anticoagulants and heparin. Now, the PTT of course is especially sensitive to abnormalities of the intrinsic factors.

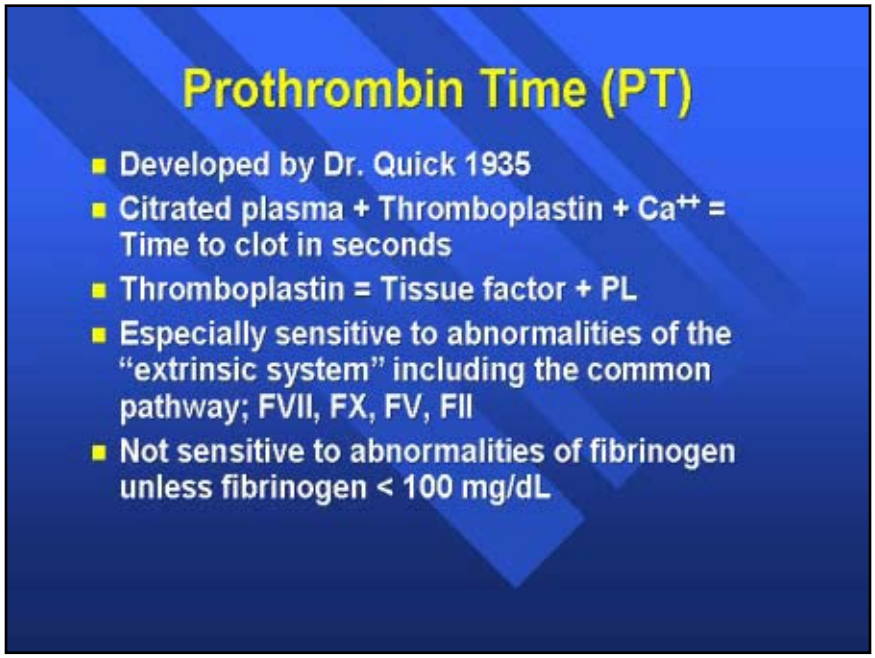
That would be factors XII, XI, IX, and VIII. The PTT is not very sensitive to abnormalities of fibrinogen, factor II, and shows little sensitivity to abnormalities of factor V and factor X.

Title: Coagulation cascade - In vitro model



Now just to reiterate this with a schematic. The PTT is most sensitive to abnormalities of the intrinsic factors, factors XII, XI, IX, and VIII, shows limited sensitivity to V and X, and little sensitivity to abnormalities of prothrombin and fibrinogen.

Title: Prothrombin time (PT)



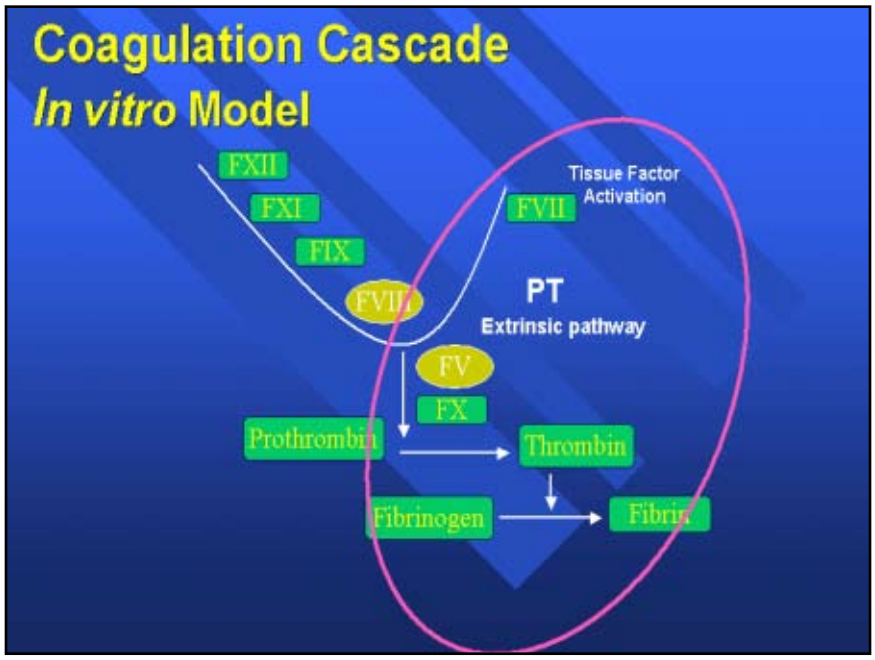
Now the PT again is an even older assay. This was developed by Dr. Quick in 1935. It's a similar process where we take citrated plasma, we add thromboplastin and calcium, and the PT is of course the time to clot in seconds.

Thromboplastin is a mixture of tissue factor and phospholipid. And again, it is this combination that makes our different PT reagents vary between one another. We can use different forms of thromboplastin, either animal forms or human forms of thromboplastin.

And we can certainly have different forms of phospholipid, different concentrations of phospholipid. So these, this is what sets the various PT reagents apart. The PT of course is especially sensitive to abnormalities of the extrinsic system that would be factor VII, and the common pathway, which would be factors II, V, and X.

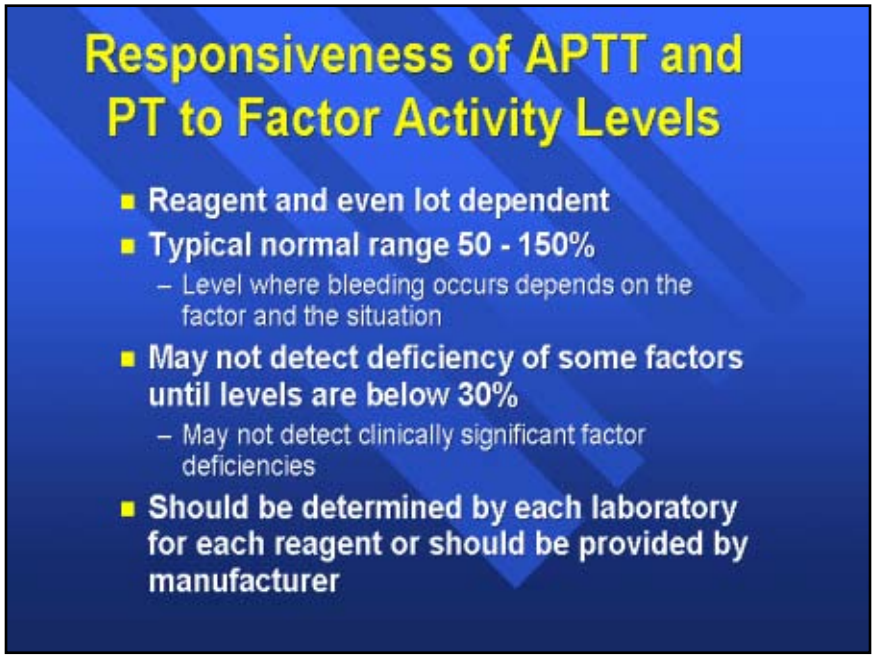
The PT is not sensitive to abnormalities of fibrinogen unless the fibrinogen level is really quite low, less than 100 mg/dL.

Title: Coagulation cascade - In vitro model



Now again just to reiterate, the only factor that is unique to the PT system is factor VII. And the PT is especially sensitive to abnormalities of factor VII, factor V, factor X, and it's also fairly sensitive for abnormalities of factor II typically, not as sensitive to abnormalities of fibrinogen.

Title: Responsiveness of APTT and PT to factor activity levels



Now one of the things that we introduced already was responsiveness. And what we're going to talk about is responsiveness of PTT and PT reagents to various factor levels.

And as I mentioned, this responsiveness is very reagent dependent, and it also varies between lots of the same reagent. Keep in mind of course that if we look at a patient's factor levels, if you've got a normal factor level, your factor activity is somewhere between 50% and 150%.

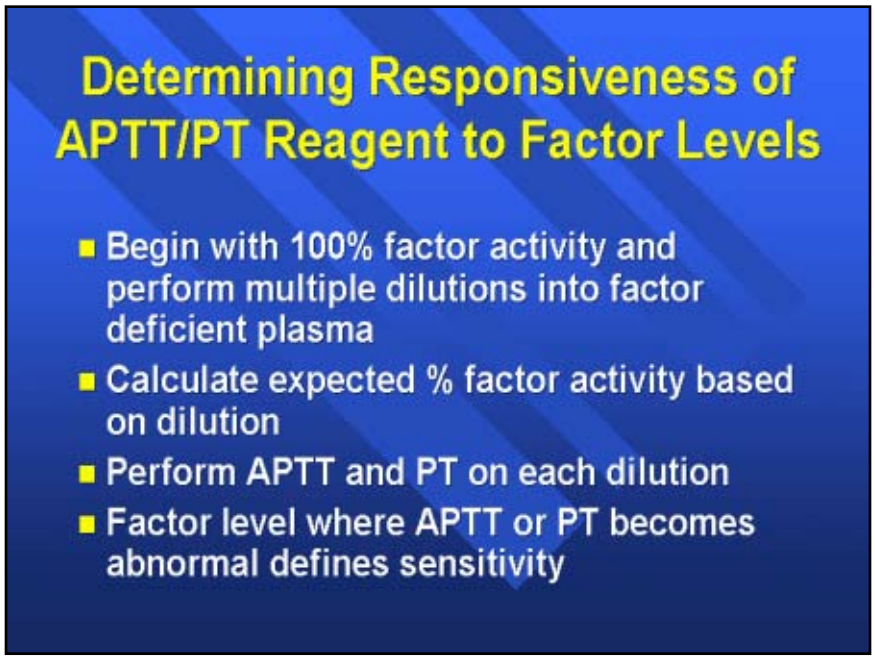
So it's really a very broad range. The level where bleeding occurs with each factor depends on the factor and on the clinical situation. So of course if the patient is not stressed, you don't require as much factor as a patient who is going to have to suffer or going to undergo an operative procedure, where you need more factor to protect that patient.

Now the PT and the PTT may not detect deficiencies of some factors until those factors fall below about 30%, which is below a level that may be required for that patient to achieve hemostasis, especially if that patient will undergo a surgical procedure.

Therefore, the PT and the PTT may not detect clinically significant factor deficiencies. Now what I would recommend is that each laboratory should have a good understanding of the responsiveness of their PT and PTT reagent. At our laboratory, we undergo studies to determine responsiveness of each lot of reagent that we bring in-house.

And I certainly appreciate that not every laboratory can do this. If you can't perform this in-house, and I'll tell you how to do this, you should talk to your reagent manufacturer, and ask them to provide this information to you, so that you have a better understanding of the capability of your reagent.

Title: Determining responsiveness of levels APTT/PT reagent to factor levels

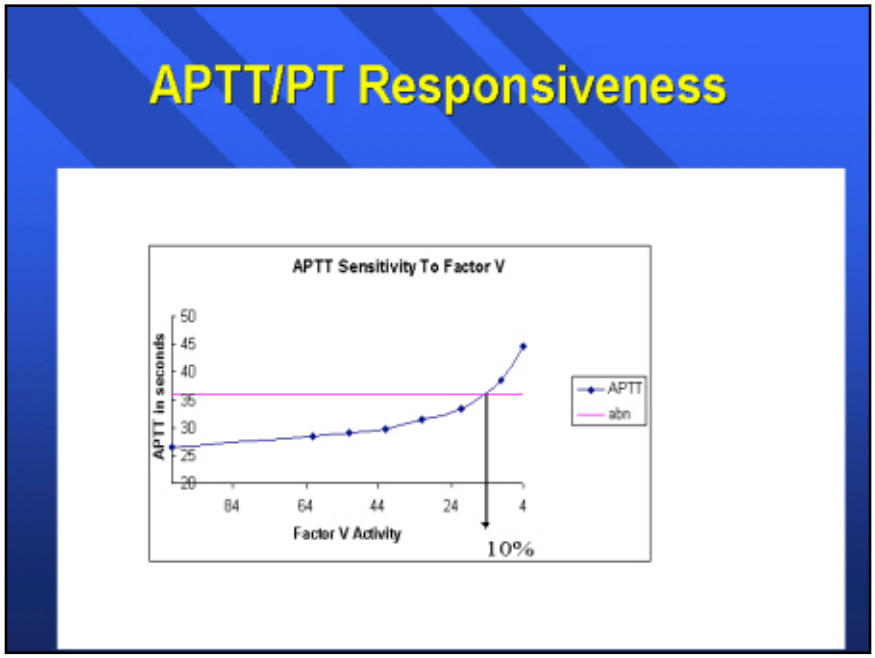


Now, how do we perform our responsiveness studies? What we do is we take a normal plasma that has somewhere around 100% factor activity, and then we perform dilutions into factor deficient plasma.

So let's say for instance we are going to evaluate the factor V sensitivity of a reagent. We take normal plasma, and then we perform dilutions with factor V deficient plasma. Based on those dilutions, we know what the expected factor activity will be.

So if we start out with 100%, a one to two is 50%, one to four 25%, etc. So we calculate the expected factor activity based on the dilution, and then in each of those dilutions, we perform a PTT and a PT assay. The factor level where the PTT or the PT becomes abnormal defines the sensitivity of that reagent for that factor.

Title: APTT/PT responsiveness

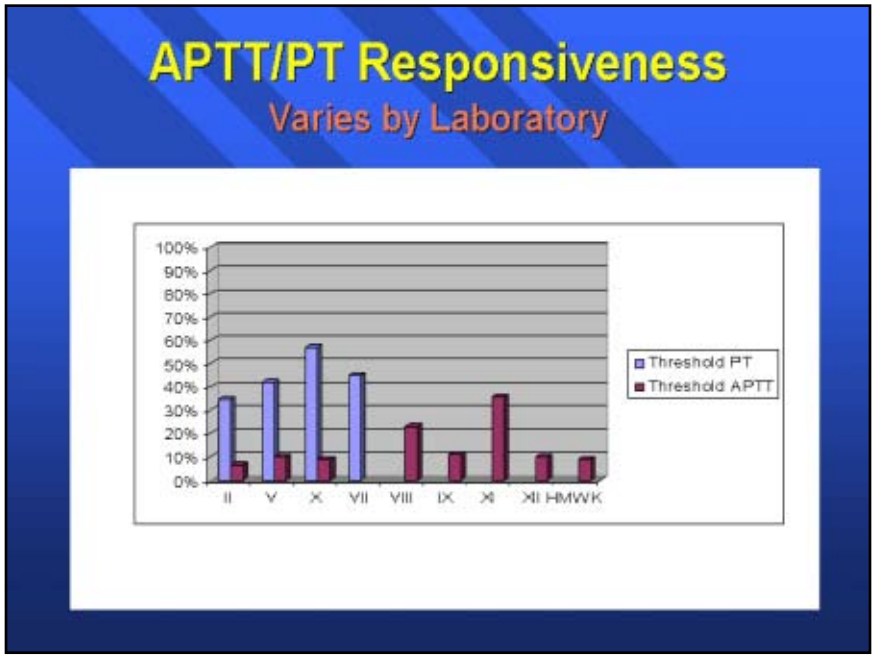


All right, here's an example. In this example, we are looking at the factor V sensitivity of our PTT reagent. So we've taken a normal, a

sample, and we've diluted it into factor V deficient plasma, and at each dilution we've performed a PTT assay.

Our PTT upper limit of normal is 36 seconds, and at that point where the PTT becomes abnormal, which for factor V is 10%, defines the responsiveness of our reagent to factor V activity.

Title: APTT/PT responsiveness - Varies by laboratory



Now what I've depicted here is our responsiveness for the PT and the PTT reagents for the various factors. You can see that the purple-colored figures represent the threshold PT, and the more maroon the PTT. Now as we mentioned, the PT will detect abnormalities of factor VII, as well as the common factors, the common pathway factors II, V, and X.

Most PT reagents are really quite sensitive in that they can detect clinically significant abnormalities of each of these factors. As you can see, most PT reagents have sensitivity in the 30% to 40% to 50% range. So if a factor VII, V, II or X falls below 40% to 50%, you will see this reflected in an abnormal PT.

Let's talk about PTT reagents. PTT reagents of course will detect an abnormality in any of the factors except factor VII. Now the critical factors, and the factors that we talked about where the PTT is most sensitive, are the intrinsic factors, factors VIII, IX, and XI.

Now with our current lot of reagent, I really think it's astounding to note that a factor VIII level has to be below about 22% in our laboratory before we get an elevation of the PTT. And look at the factor IX sensitivity, it's around 10%.

Factor IX is characteristically one of the hardest factors to achieve sensitivity of in the PTT reagent. So you can clearly see that you might miss clinically significant abnormalities if you rely just on a normal PT or PTT. Now typically factor XI sensitivity for most of the reagents is quite good, and here it's about 33% or so.

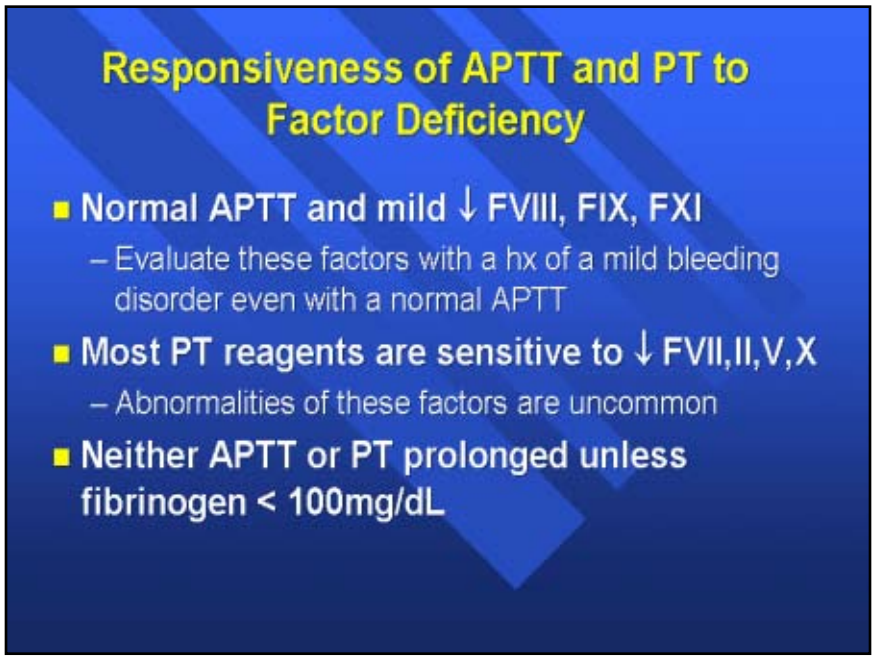
Sensitivity is typically not very good to factor XII and high molecular weight kininogen, but we are delighted to see that, because we don't want to detect abnormalities in these factors because deficiencies of these factors are not clinically significant.

Now let's take a look at the sensitivity to the common pathway factors. The PTT reagent typically is not, most PTT reagents typically are not sensitive to abnormalities in the common pathway factors. Look at this. Your factor II has to be around 5% before it will elevate some PTT reagents.

Therefore, when you are evaluating a patient, when you're looking at an abnormal PT, it's important to keep in mind that if the patient has an abnormal PT and a normal PTT, they certainly can have an abnormality of factor VII, but don't forget about evaluating the common pathway factors as well, because your PT will be more able to detect these abnormalities than will a PTT.

Okay. So this is something that we perform on every lot of reagent that we bring in-house. And this is something that your manufacturer, reagent manufacturer can provide to you to give you an idea of how that reagent performs.

Title: Responsiveness of APTT and PT to factor deficiency

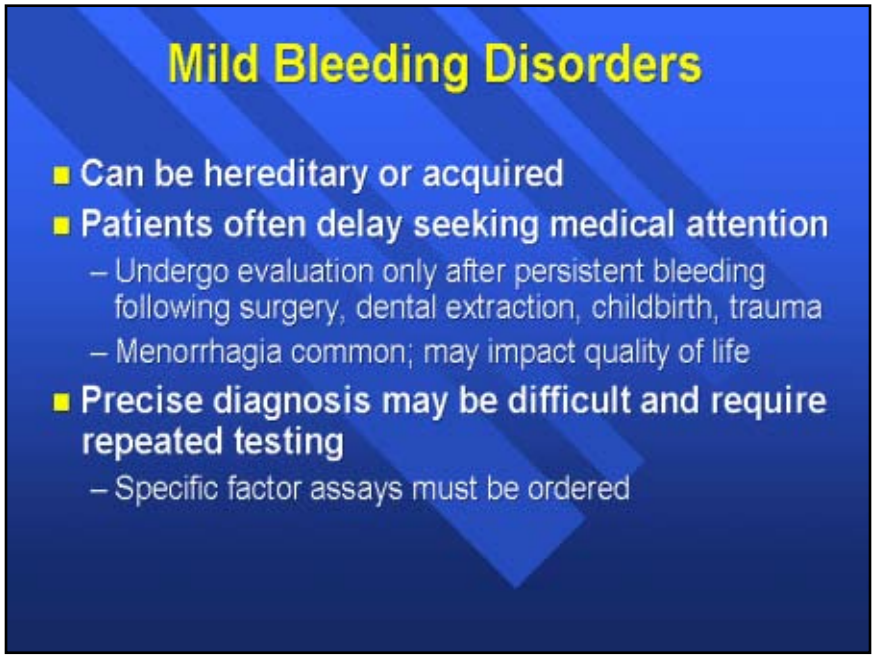


All right. So based on this you can certainly understand that patients may have a normal PTT with mild deficiencies of factors VIII, IX or XI. Therefore, if you have a patient with a history of bleeding and a normal PTT, it's important to evaluate these factors.

As we mentioned, most PT reagents are fairly sensitive to abnormalities of the common pathway. So these pathways, these factors do not really need to be evaluated unless you've got an abnormality of the PT. Also, neither the PT nor the PTT are very sensitive to abnormalities of fibrinogen, unless the fibrinogen is below about a 100.

And that's a level at which patients might experience bleeding, especially if they are subjected to a surgical procedure. And I should've shown you the data from my laboratory, but I forgot to include that. That's why a hemostatic screen typically includes a fibrinogen activity, so that you don't miss any abnormalities in fibrinogen.

Title: Mild bleeding disorders



We're going to spend just a minute talking about mild bleeding disorders. Mild bleeding disorders can be either hereditary or acquired. The most interesting thing about mild bleeding disorders is that most, or many patients delay seeking medical attention until they undergo some sort of a procedure where they suffer persistent bleeding.

This can occur following surgery, dental extraction, childbirth. And it's interesting because families that suffer mild bleeding disorders often don't consider their bleeding abnormal, because it's normal for their family members.

And this is particularly true of women. And when women suffer a hereditary mild bleeding disorder, they almost always suffer menorrhagia. And the most interesting thing is that when a young woman learns about menstruation, she typically learns if from her mother.

And if she has inherited this bleeding disorder from her mother, her mother may tell her what to expect in regards to a normal menstrual period, which is not at all normal for the rest of us, but it's normal for that family. And so they these families don't often understand that they in fact have a mild bleeding disorder.

And another interesting piece of information, the Center for Disease Control performed a survey a number of years ago in Georgia. And what they did is they surveyed gynecologists. And they asked gynecologists, how do you evaluate women with menorrhagia, and how often do you pursue an underlying bleeding abnormality.

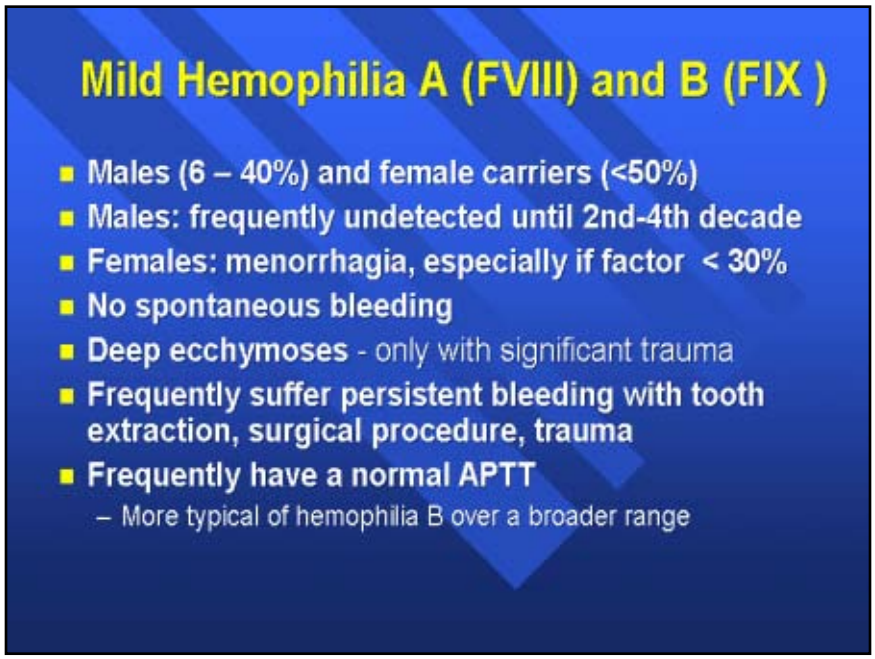
And the answer was when a woman presents with menorrhagia, only 4% of the time do they evaluate that woman for an underlying bleeding abnormality. In fact, when women suffer menorrhagia, up to 40% have an underlying bleeding abnormality.

So not only is this a diagnosis that's often missed in the family, but it's often also missed in the gynecologist's office as well. And it's also been shown very interesting that women that suffer mild bleeding disorders really have a significant impact on the quality of life due to menorrhagia.

To this end I should mention the CDC is initiating a project called Project Red Flag. And it's a project to try to increase awareness, so that we can improve quality of life for this population.

Now, part of the problem with a mild bleeding disorder is not just that it may go unrecognized, is that it's often difficult to diagnose, because people don't always understand the limitations of the PT and PTT assays, and the fact that specific factor assays must be ordered.

Title: Mild hemophilia A (FVIII) and B (FIX)



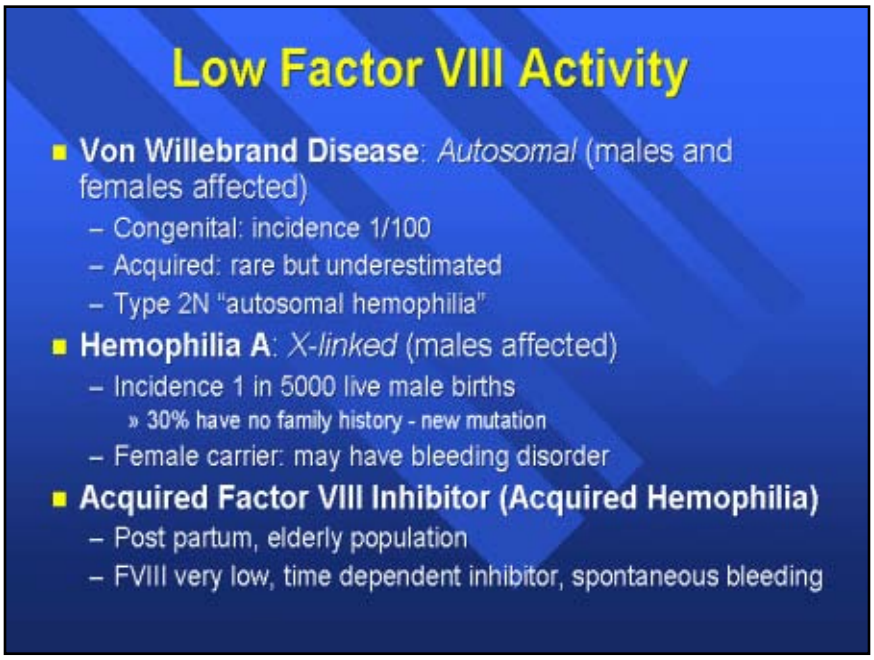
And so what we're going to talk about now are some of the mild bleeding disorders just as a very quick review.

Mild hemophilia A, a factor VIII deficiency, hemophilia B, a factor IX deficiency. This can occur in males, and they typically have factor levels in the range of 6% to 40%. So remember you might be missing those patients between 20 and 40, right, or female carriers who have less than 50%.

Males who suffer mild hemophilia frequently go undetected until some time between the second and the fourth decade. Females typically suffer menorrhagia, especially if their factor level is below 30%. And in fact there's been a push lately to call female carriers of hemophilia mild hemophiliacs.

And this is to help us remember that fact that carriers can bleed. Now typically mild hemophiliacs suffer no spontaneous bleeding. They don't suffer deep ecchymosis unless there is some significant trauma, but this is the population that will bleed if provoked. Okay. This population also frequently has a normal PTT, this is especially true of factor IX deficiency.

Title: Low factor VIII activity



Now if we talk about low factor VIII deficiency or low factor VIII levels, pardon me, low factor VIII levels are really very common. Low factor VIII levels can occur with von Willebrand's disease.

Von Willebrand's disease is an autosomally inherited disorder, and therefore males and females are affected. The incidence is actually quite common, 1 in 100, a very common disorder. One of the things we're going to talk about are some variants that aren't often considered.

Acquired von Willebrand's disease. Acquired von Willebrand's disease, it's considered to be rare, but I tell you it's underreported, underestimated, and underevaluated. And then there's also another form of von Willebrand's disease we'll talk about which is type 2N, which is known as autosomal hemophilia.

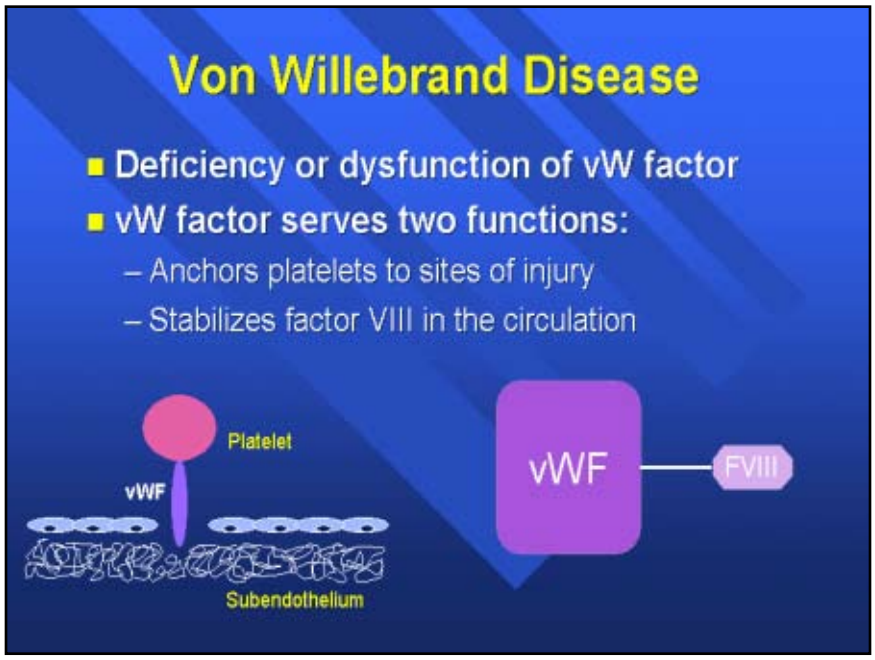
Now another cause of a low factor VIII level of course is hemophilia. Hemophilia A is X linked, therefore males are typically affected. The incidence is really quite high, 1 in 5,000. It is important for us to keep in mind a little known fact, 30% of individuals with hemophilia have no family history.

I'll never forget a patient that I had at the VA when I started who had no family history of bleeding, but bled tremendously. And he had gone to Korea, and suffered terrible hemorrhagic episodes, and he was in fact a severe hemophiliac, which represented a new mutation.

So it's important to keep this in mind, and also female carriers may have a bleeding disorder. And the other cause for a low factor VIII level is acquired hemophilia due to an antibody or inhibitor. This typically occurs in a postpartum time period or in an elderly population.

And typically these patients have a very low factor VIII level. So you won't miss this with a PTT. These are time-dependent inhibitors, and patients typically have such a low factor VIII level that they suffer spontaneous bleeding, but I just wanted to mention that for the sake of completeness.

Title: Von Willebrand disease



Let's very quickly review von Willebrand factor. Von Willebrand's disease is due to a deficiency or a dysfunction of von Willebrand factor. Just as a reminder, the von Willebrand factor has two very important functions. The first thing von Willebrand factor does, is it binds to the platelet, and it takes that platelet and binds it to the site of injury.

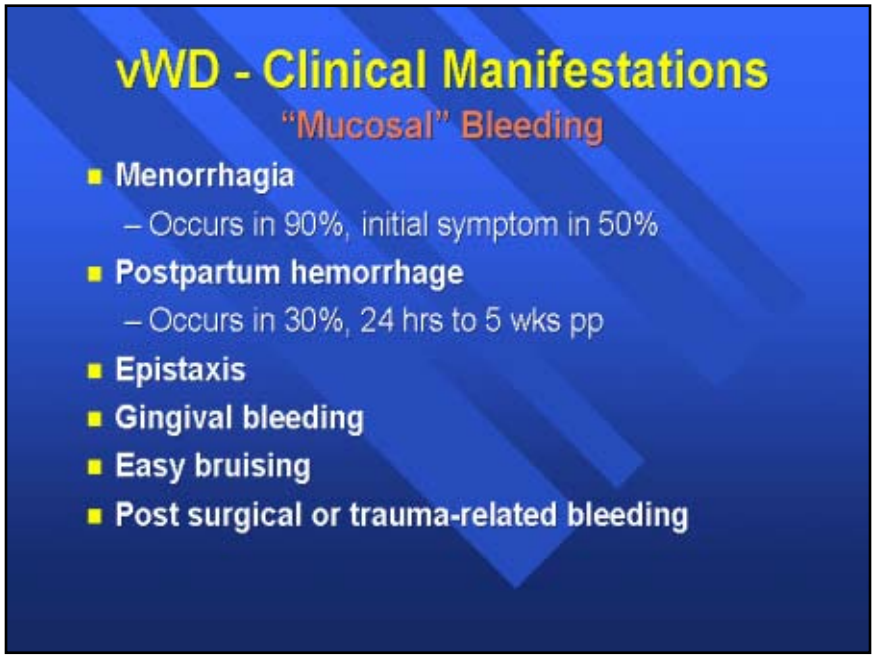
And this is really very important because otherwise platelets would be washed away from the site of injury. So you have to have some way to form a stable platelet plug while the blood is flowing out of the vasculature.

And that's von Willebrand factor. It's an anchor. So it helps the platelets aggregate. It helps the platelets localize, so they can aggregate. The other thing von Willebrand factor does is it stabilizes factor VIII in the circulation. Von Willebrand factor is a very large protein and factor VIII is a relatively small protein.

Factor VIII would be lost in the circulation, would have a very short half-life if it didn't have something to hook on to and stabilize it in the circulation, and that happens to be von Willebrand factor. If your von Willebrand factor is decreased in quantity, so is your factor VIII.

Because factor VIII has nothing to hold onto. So that's why we see decreased factor VIII levels when patients have von Willebrand's disease, some forms of von Willebrand's disease, and have decreased von Willebrand factor levels.

Title: vWD - Clinical manifestations - ''Mucosal" bleeding



Now what are the typical clinical manifestations of von Willebrand's disease? I want to mention this because this is the typical, these

are the symptoms typical of what's called mucosal bleeding. Mucosal bleeding represents a defect in primary hemostasis, either platelet number, platelet function or von Willebrand factor.

Okay. Menorrhagia is extraordinarily common, 90% of women with von Willebrand's disease suffer menorrhagia. This is the presenting symptom in about 50%. Postpartum hemorrhage is also very common, and postpartum hemorrhage can occur anywhere from 24 hours to 5 weeks postpartum.

Epistaxis or nosebleeds, gingival bleeding, gum bleeding, easy bruising, postsurgical bleeding or posttraumatic bleeding. These are the typical symptoms.

Title: Von Willebrand disease - The most common hereditary disorder of coagulation

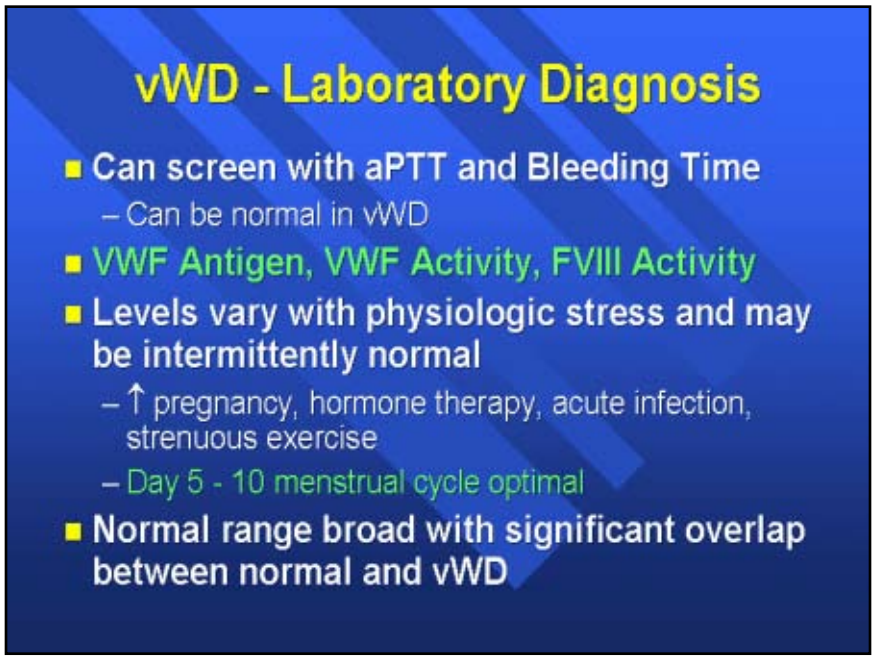
Von Willebrand's disease is the most common hereditary disorder of coagulation with a prevalence of about 1%. It's also a difficult diagnosis to make in the laboratory for a number of reasons.

Clinical signs and symptoms of von Willebrand's disease can be very heterogeneous. And so it can be, diagnosis can be problematic. Once the diagnosis is made, however, the therapies are actually fairly easy to introduce.

And for women who suffer von Willebrand's disease and suffer menorrhagia, these women can actually use nasal Stimate or nasal DDAVP prior to their menstrual period, and relieve some of the terrible bleeding symptoms that they suffer.

So you can use DDAVP, which causes endogenous release of your von Willebrand factor or a plasma product, one of which is Humate-P, which is pasteurized factor VIII plus von Willebrand factor complex.

Title: vWD - Laboratory diagnosis



Now laboratory diagnosis. Some individuals, some physicians still screen for von Willebrand's disease with a bleeding time and a PTT.

This is really not the way to go. Your PTT, I guarantee you, can be normal with von Willebrand's disease. What should be done is an evaluation of von Willebrand factor antigen, probably with activity and a factor VIII activity. Part of the problem with diagnosis is that each and every one of these is an acute phase reactive protein.

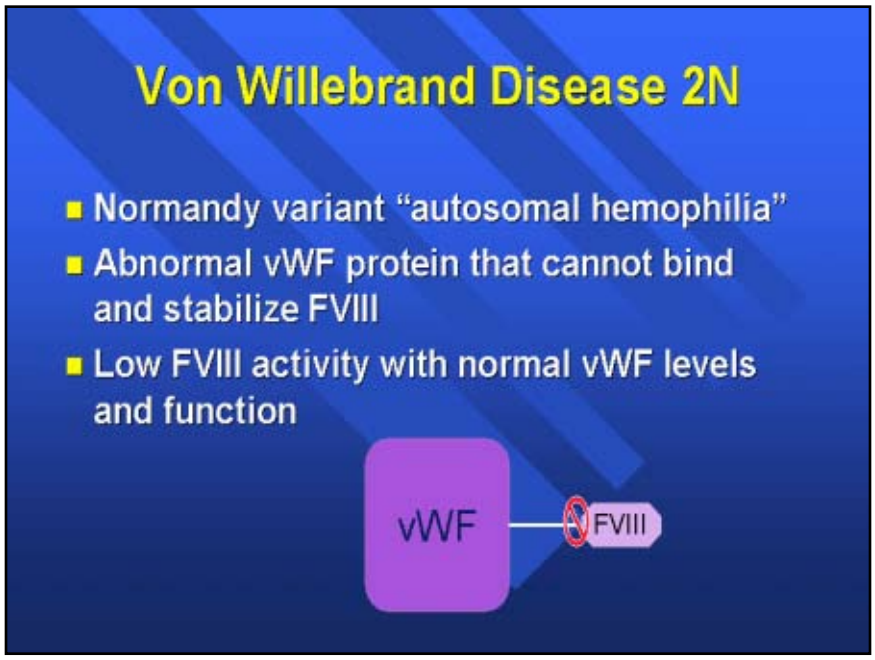
So if your patient is stressed or ill, these levels will increase physiologically. These levels also increase typically in response to estrogen. So they increase when women are put on hormone-replacement therapy or oral contraceptive pills more commonly.

And they increase with pregnancy. So very typical scenario is a woman suffers menorrhagia. She sees her gynecologist. The gynecologist puts her on birth control pills or oral contraceptive therapy to help control the bleeding, and then the gynecologist says oh maybe I should screen her for von Willebrand's disease.

Well they may miss the diagnosis, because the therapy may increase levels into the normal range, which is in fact why the woman may not suffer as much bleeding, let alone that the atrophy induced, of the endometrium induced by the oral contraceptive agents.

Because the von Willebrand levels may fluctuate with hormone therapy if the woman is being evaluated during her reproductive years, the best time to evaluate for von Willebrand's disease is day 5 to 10 of the menstrual cycle. Another problem is that there's a broad overlap between the normal range and those that suffer von Willebrand's disease. And it makes diagnosis difficult.

Title: Von Willebrand disease 2N



A form of von Willebrand's disease that is poorly appreciated that I want to mention is type 2N, and N stands for Normandy, the location in France where this variant was identified. This is also known as autosomal hemophilia.

Type 2N von Willebrand's disease is an abnormality of the von Willebrand factor protein or the von Willebrand protein. And what is abnormal is it's ability to bind to factor VIII. So because it can't bind factor VIII, the half-life of factor VIII in the circulation is short.

The factor VIII is cleared. These patients typically have a low factor VIII level and a normal von Willebrand factor activity and antigen. We have made a concerted effort in our laboratory to look for these patients, and we have found a number of patients with type 2N von Willebrand disease in the past year.

And the way that this can be screened for is there's actually a binding assay to look for the ability of the patient's factor VIII to bind to factor, the patient's von Willebrand factor to bind factor VIII. So autosomal hemophilia.

Title: Acquired von Willebrand Disease

Now another poorly appreciated disorder is acquired von Willebrand's disease. It interests me on a personal basis that you know when I first began in coagulation, I would never have thought about von Willebrand's disease in a 70-year-old patient who has a new onset of a bleeding diathesis.

And that just goes to show that I never really appreciated the fact that so many of the bleeding disorders can be acquired.

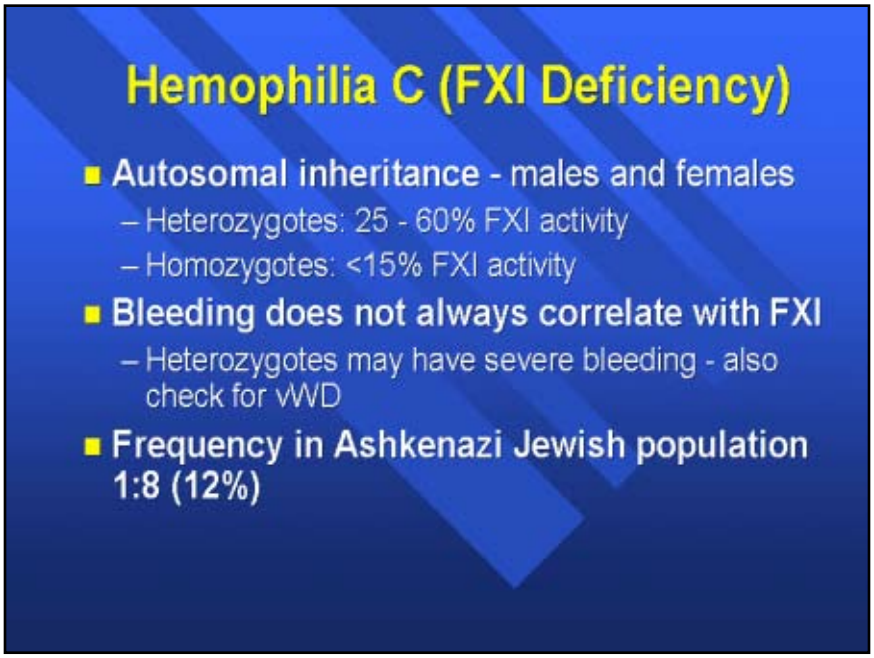
And acquired von Willebrand's disease is actually not that uncommon. When we look for it, we see it. And these patients typically present with new onset bleeding. They have no family history. Laboratory and, if you use laboratory studies and clinical appearance, they are otherwise very similar to congenital von Willebrand's disease.

So the disorders associated with acquired von Willebrand's disease include clonal hematoproliferative disorders, lymphoma, leukemia, and of course these are very common disorders in our elderly population. A variety of malignancies, most commonly renal tumors, Wilms' tumor, autoimmune diseases.

If a patient is developing autoantibodies, they can certainly develop an antibody to their von Willebrand factor. And then finally, cardiac defects. So you might see acquired von Willebrand's disease in a pediatric population who has a VSD or an ASD defect. Similar clinically to hereditary von Willebrand's disease, you should screen with a von Willebrand factor antigen activity and VIII activity.

Patients can have either type 1 or type 2 patterns, and when we talk about type 1 and type 2 in deficiencies and coagulation, type 1 is a quantitative abnormality so we have decreased antigen and activity, and type 2 abnormalities are qualitative, where antigen levels may be normal, but function is diminished.

Title: Hemophilia C (FXI deficiency)



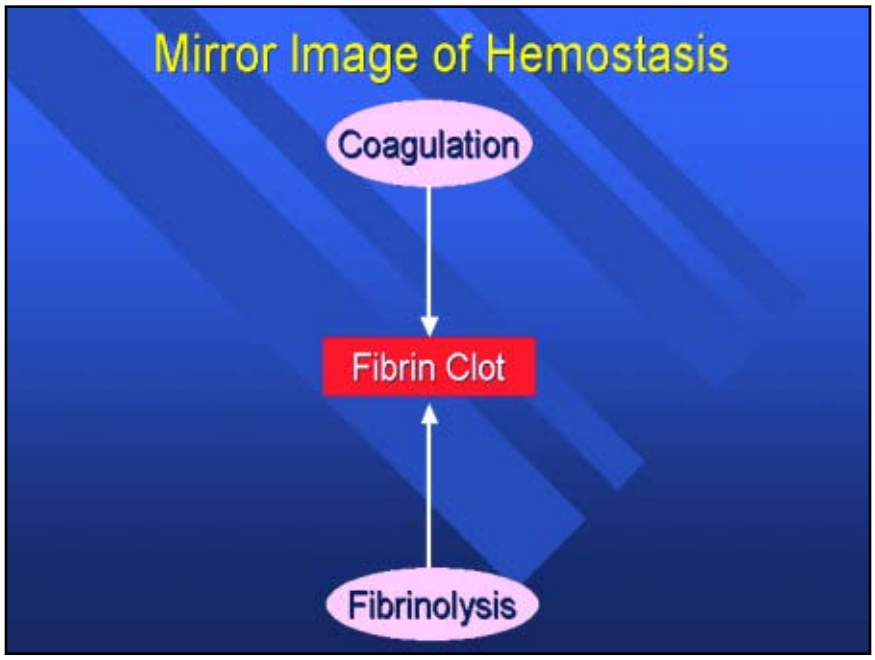
All right now, one of the other abnormalities that's sometimes poorly appreciated is something called hemophilia C or factor XI deficiency.

Factor XI deficiency has an autosomal inheritance, therefore males and females can be affected. Heterozygous typically have values in the range of about 25% to 60%, whereas homozygous have about less than 15% factor XI activity. Interesting that bleeding does not always correlate with the levels of factor XI.

Some patients with what appears to be a heterozygous deficiency may actually suffer a severe bleeding diathesis. And what this suggests to me is that there is probably something else going on, and in fact if you evaluate this population, you will see that a certain proportion of these patients with the heterozygous levels of factor XI may in fact also be suffering from von Willebrand's disease.

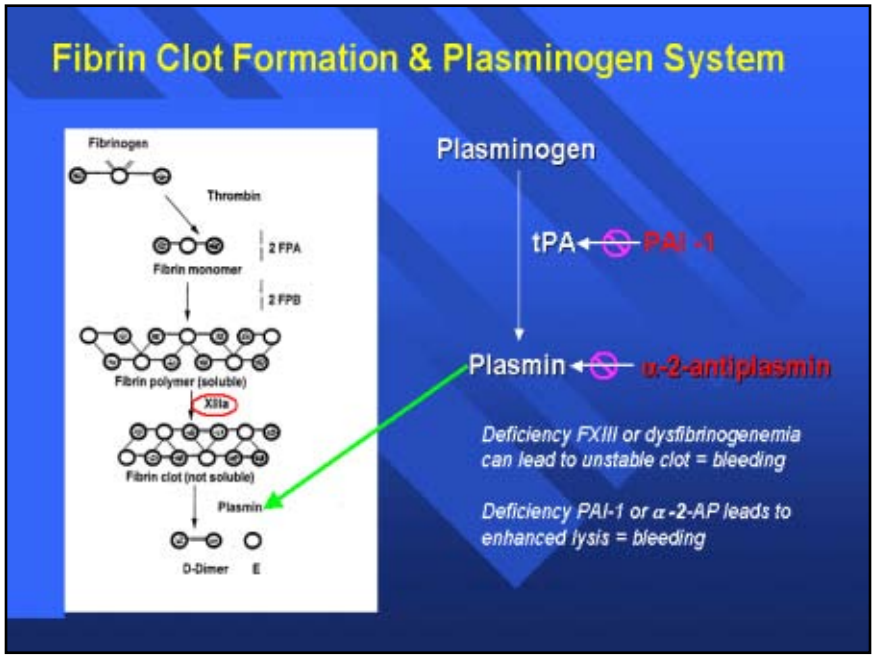
This is also true of hemophilia A and less commonly of hemophilia B. So if your factor level does not seem to fit the bleeding manifestations don't just, don't forgo the workup of other disorders that may be associated. And because von Willebrand's disease is so common, these patients with hemophilia may also suffer von Willebrand's disease.

Title: Mirror image of hemostasis



Now we've been talking about mostly the system where we have formation of the fibrin clot, and one of the ideas that I want to present to you is something that I refer to as the mirror image of hemostasis. When we talk about hemostasis, we talk about clot formation and clot lysis, because that's really the balance that you're dealing with. If you form a clot, you have to lyse a clot.

Title: Fibrin clot formation & plasminogen system



Now if we look at the fibrinolytic system, which is really better known as the plasminogen system, there are a couple of factors there I want to talk about.

Prior to delving into the plasminogen system I do want to talk a little bit more about fibrin clot formation, because that's usually poorly addressed. As fibrinogen is converted to fibrin through thrombin, we have the development of a fibrin monomer, all right.

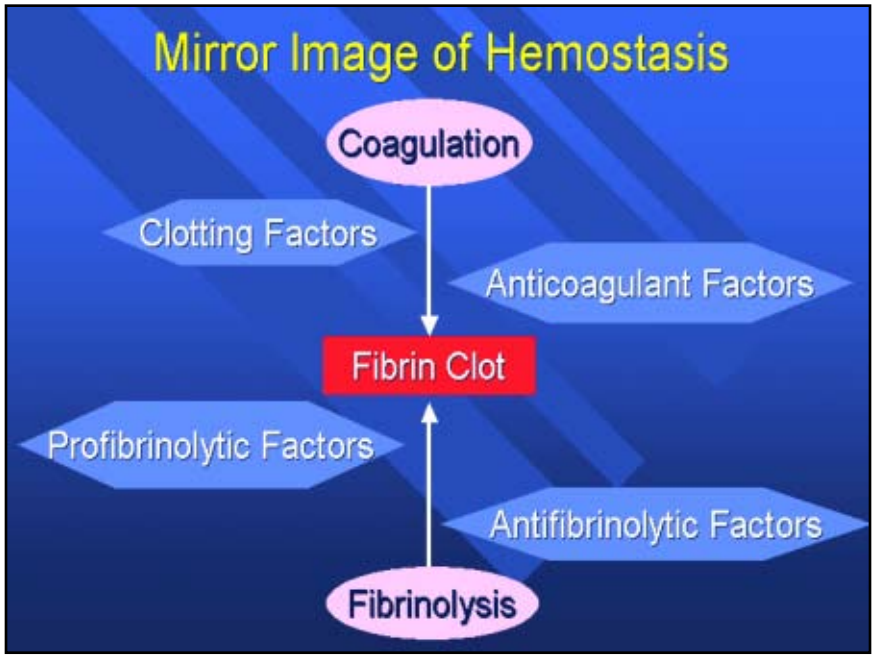
And what happens is that the fibrin monomers will align themselves electrostatically, okay. So by electric charge, they will form an aggregate. And this helps to form the fibrin clot. This is not a very stable relationship, however. And this monomeric, this aggregate of monomers must be stabilized and that occurs through factor XIII.

And with factor XIII, we form covalent bonds between fibrin monomers. And that's what forms a stable fibrin clot. Now we have to have a means to break down a clot, and as I mentioned earlier, that occurs through the plasminogen system. Plasminogen circulates as an inactive zymogen that must be activated, and is activated through TPA, tissue plasminogen activator, and that forms plasmin.

Plasmin is an extremely potent enzyme, almost like trypsin, and trypsin is our most common digestive enzyme. Plasmin will digest the fibrin clot. It will also digest just about anything else that's in its pathway including other factors as well.

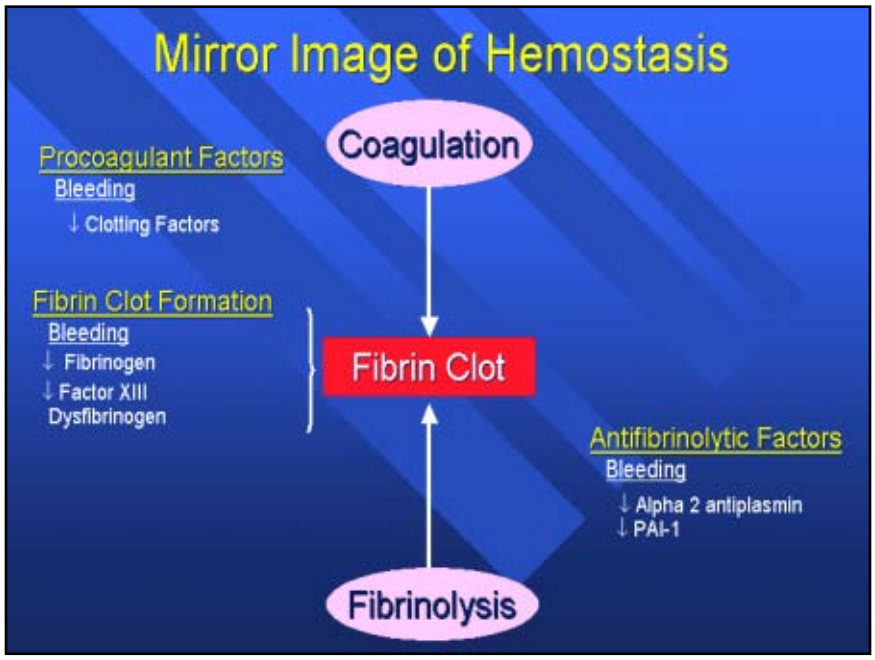
So plasmin must be very tightly regulated, and it's regulated by alpha 2-antiplasmin, which binds plasmin and inactivates it. Now TPA also is tightly regulated by another protein called plasminogen activator inhibitor-1. So just like the coagulation scheme, the fibrinolytic scheme is complex. We have profibrinolytic agents and antifibrinolytic agents, like the procoagulants and anticoagulants.

Title: Mirror image of hemostasis - Clotting factors



So as you can see, we've got clotting factors that lead to fibrin clot formation, anticoagulant factors, protein C, protein S, antithrombin that inhibit clot formation. We have profibrinolytic factors that promote lysis and antifibrinolytic factors that prevent lysis.

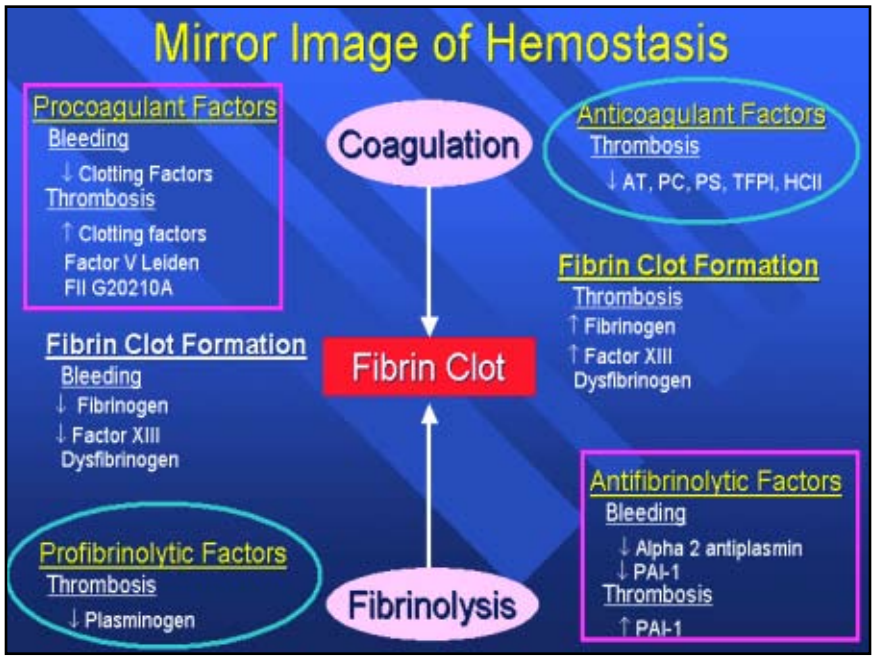
Title: Mirror image of hemostasis - Procoagulant factors



If we are looking for a bleeding diathesis, it's important to look in all aspects of the hemostatic system. So we can have bleeding because the patient is deficient in clotting factors, because the patient cannot form a stable fibrin clot, because they may not have enough fibrinogen, they may not have enough factor XIII or they may have an abnormal fibrinogen, a dysfibrinogen that impairs the alignment of the monomers.

Likewise, if we have a patient that is deficient in their antifibrinolytic factors, this promotes enhanced lysis, and these patients are also at a risk of bleeding.

Title: Mirror image of hemostasis - Anticoagulant factors

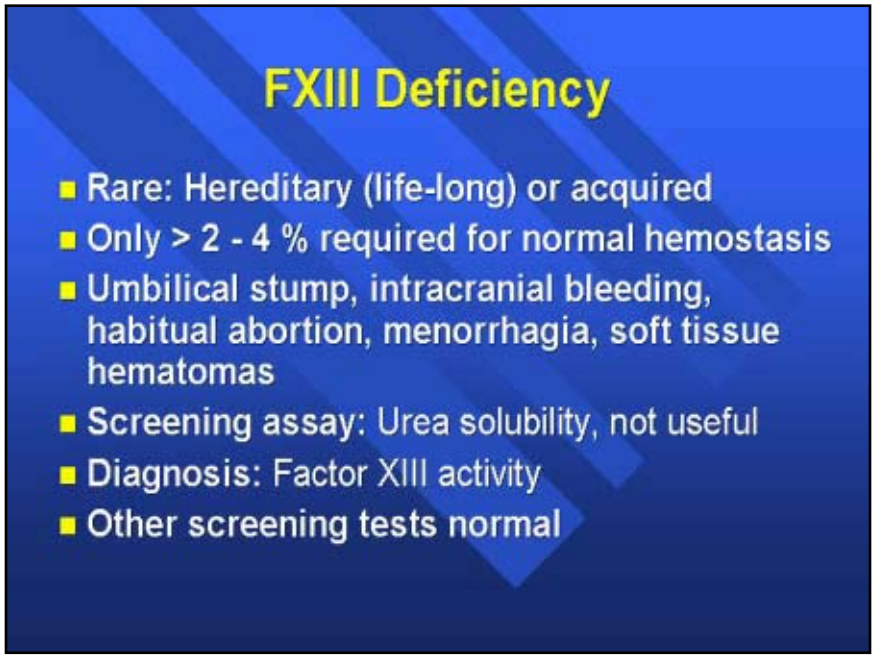


Now I use this very same scheme when I think about thrombosis. And I mentioned, these are mirror image systems. So if you have a patient with bleeding, they can have diminished clotting factors or diminished antifibrinolytic factors.

Now it's the same thing with thrombosis. You may have thrombosis because of diminished anticoagulant factors or diminished profibrinolytic factors. So they are exact opposites of one another on either side of the fibrin clot formation.

So actually I've got this diagram next to my desk, and when a physician calls me and we talk about bleeding and thrombosis, I generally glance at this and make certain that I've covered all aspects of hemostasis. So that no disorder is missed.

Title: FXIII deficiency



Now, factor XIII deficiency. I have to admit I didn't have a lot of respect for factor XIII deficiency. I didn't realize how common this abnormality actually can be.

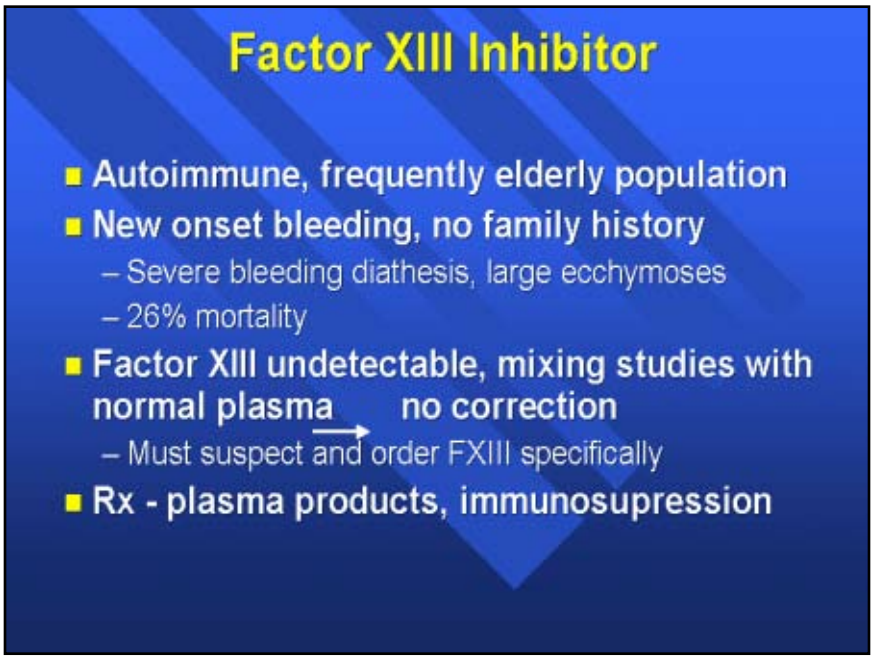
And maybe it's because I'm at a laboratory where I see the most unusual of the most unusual, but nonetheless this is an important consideration. It is rare in the whole scheme of things, but when patients suffer factor XIII deficiency it is certainly life threatening.

We only need about 2% to 4% of factor XIII for normal hemostasis, so only those patients that are homozygous deficient in factor XIII suffer abnormalities. But these babies typically begin to bleed very soon after birth, and typically present with bleeding from the umbilical stump. If the diagnosis is missed, they'd suffer intracranial hemorrhage, which can be a cause of demise in these patients.

Women who somehow make it to adulthood suffer habitual abortion, of course menorrhagia, and soft tissue hematomas. When I was taught about factor XIII deficiency, I was taught that you should screen with a urea solubility assay, where you take a patient's clot and you add 5 mL of urea, and you see how soluble it is.

This actually is a very poor screen, and it should be discontinued. A urea solubility will only detect abnormalities when that factor XIII falls below about 2%. So you really should perform a factor XIII activity. A factor XIII abnormality will not be detected through an abnormal PT or a PTT.

Title: Factor XIII inhibitor



Now some of the appreciation that I have gained for factor XIII is through the identification of inhibitors to factor XIII.

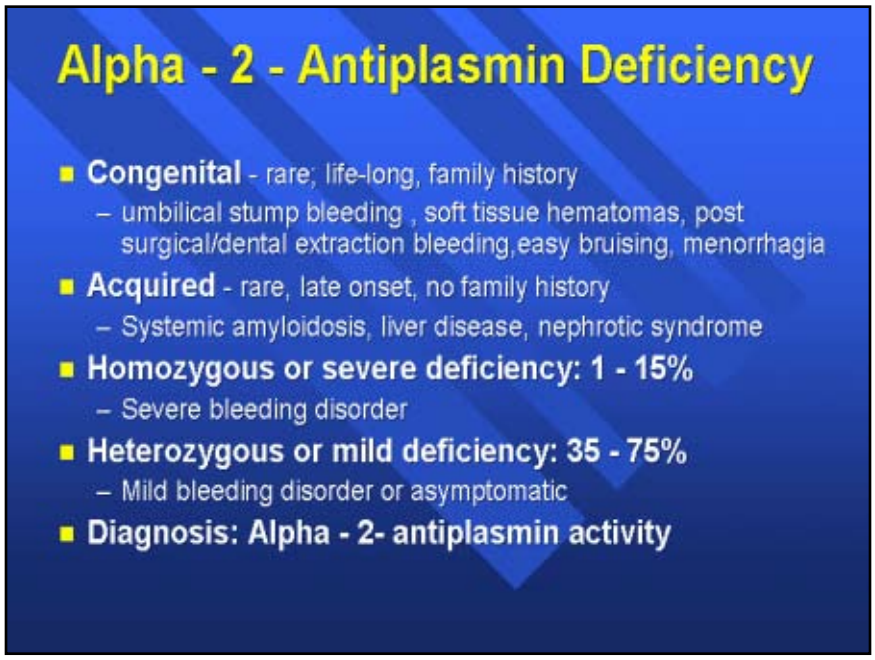
Patients who have an autoimmune disorder or who are on certain therapies may develop antibodies to factor XIII. This occurs most commonly in an elderly population. These patients typically present with new onset bleeding and typically develop very large ecchymoses.

We've diagnosed four factor XIII inhibitors in the past year. And as physicians are becoming more aware of this, they're beginning to identify these patients and make the diagnosis. So we always think about factor XIII when we've got an elderly patient who has a new onset bleeding diathesis.

Of course these patients are going to have a normal PT and PTT. If this diagnosis is missed, it's got about a 26% mortality. The patients that we've seen have actually done quite well with therapy, which includes plasma products and immunosuppressive therapy.

And then what we do is we perform a mixing study, and the initial factor XIII activity is undetectable. And then we add normal plasma, and we don't see the correction that we would expect to see with the addition of normal plasma.

Title: Alpha - 2 - Antiplasmin deficiency



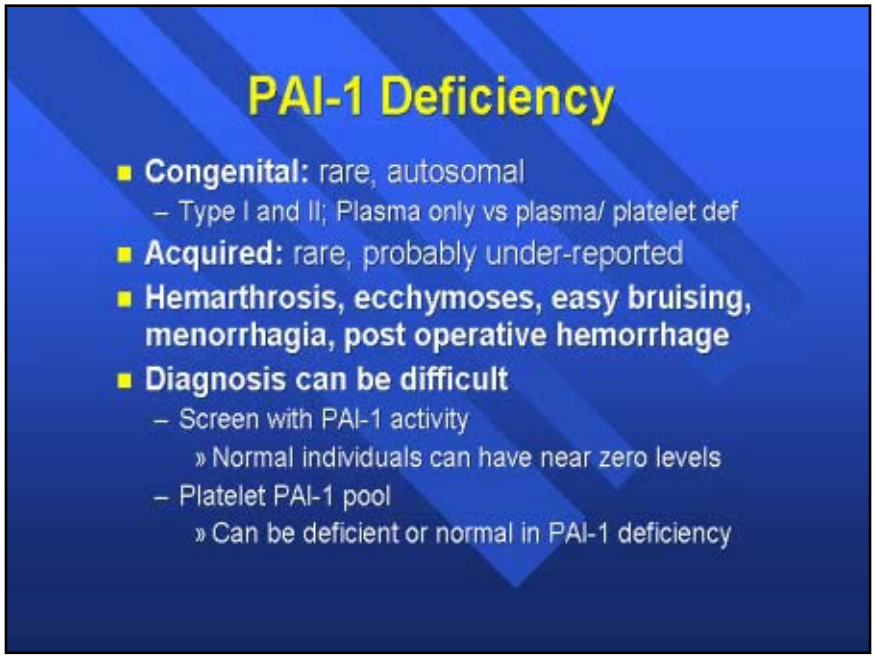
All right, alpha 2-antiplasmin deficiency. Again this is not very common. In fact it's a relatively rare abnormality. I don't believe I've seen this nearly as often as I've seen a factor XIII deficiency.

The clinical presentation is very similar to a factor XIII deficiency where the bleeding begins just after birth, with bleeding from the umbilical stump, very common to experience soft tissue hematomas, postsurgical, dental extraction bleeding, easy bruising. Alpha 2-antiplasmin deficiency can also be acquired, and this should always be thought about in a patient with amyloidosis.

When we have patients with amyloidosis, most of us are trained to think about factor X deficiency. Well, it's not always just factor X deficiency; these patients can also be deficient in alpha 2-antiplasmin. This also occurs of course with severe liver disease and nephrotic syndrome.

Homozygous deficiency 1% to 15%, they have a severe bleeding disorder. Heterozygous deficiency 35% to 75%, alpha 2-antiplasmin levels may have a mild bleeding disorder. The only way to evaluate this is to measure the alpha 2-antiplasmin activity in the patient's plasma.

Title: PAI-1 deficiency



All right, the next deficiency, and the last deficiency we'll talk about is the deficiency of plasminogen activator inhibitor-1, PAI-1. This is a very difficult diagnosis to make, in my opinion. Congenital PAI-1 deficiency is rare. It's got an autosomal mode of inheritance.

Just like our other deficiencies, you can have type 1 and type 2 deficiencies of PAI. Meaning that type 1 deficiency you don't have enough protein, type 2 deficiency you've got the protein, but it doesn't function, and we'll talk about this platelet area later.

PAI-1 deficiency can also be acquired, and I believe personally that this is a frequently missed diagnosis. Because there really isn't a good assay at this point to look for a PAI-1 inhibitor. These patients typically suffer hemarthrosis, ecchymosis, easy bruising, and they may also present with umbilical stump bleeding.

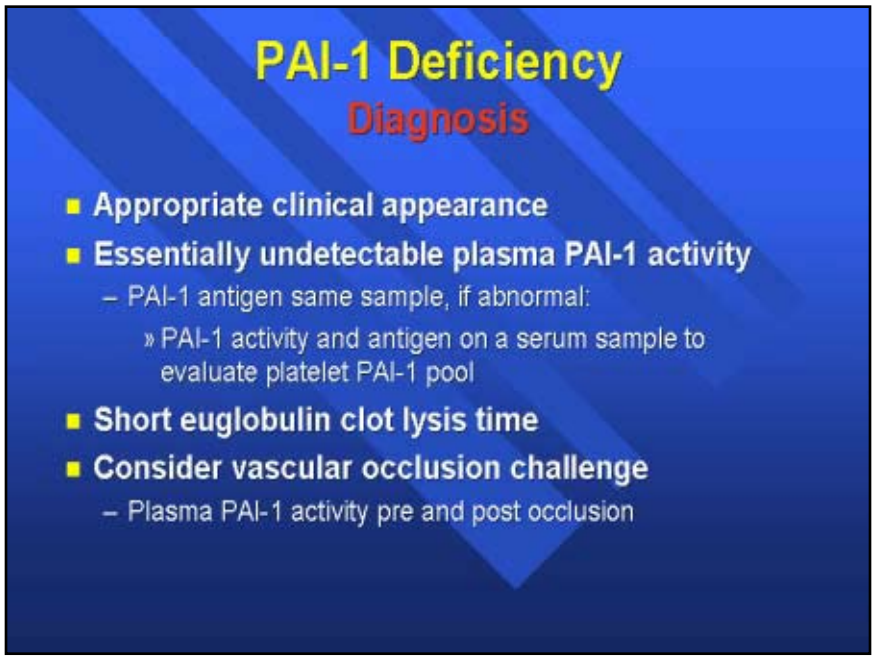
Now the diagnosis can be very difficult. Patients should be screened with the PAI-1 activity assay, but the problem is that normals can have near zero levels, so normals have levels below our lower limit of quantitation of the assay. This is a huge problem. This is probably one of the most frequent phone calls I get.

So one of the things I recommend is that if you're evaluating for a PAI-1 deficiency, have your patient drawn in the morning. PAI-1 shows a diurnal variation. It's at its highest level in the morning hours, and then decreases throughout the day.

This is probably in fact why most myocardial infarctions or heart attacks occur in the morning, is because this is the point at which PAI-1 is at its highest level. Another problem with PAI is that we have a pool of PAI in our plasma, and we also have a pool in our platelets.

Now when patients are PAI-1 deficient, they may have a deficiency of their plasma compartment only or they may have a deficiency of their platelet pool and their plasma compartment, and so it makes diagnosis very difficult.

Title: PAI-1 deficiency - Diagnosis



When you make the diagnosis, it's very important to have the appropriate clinical presentation, a bleeding diathesis, the patient should have essentially undetectable PAI activity levels.

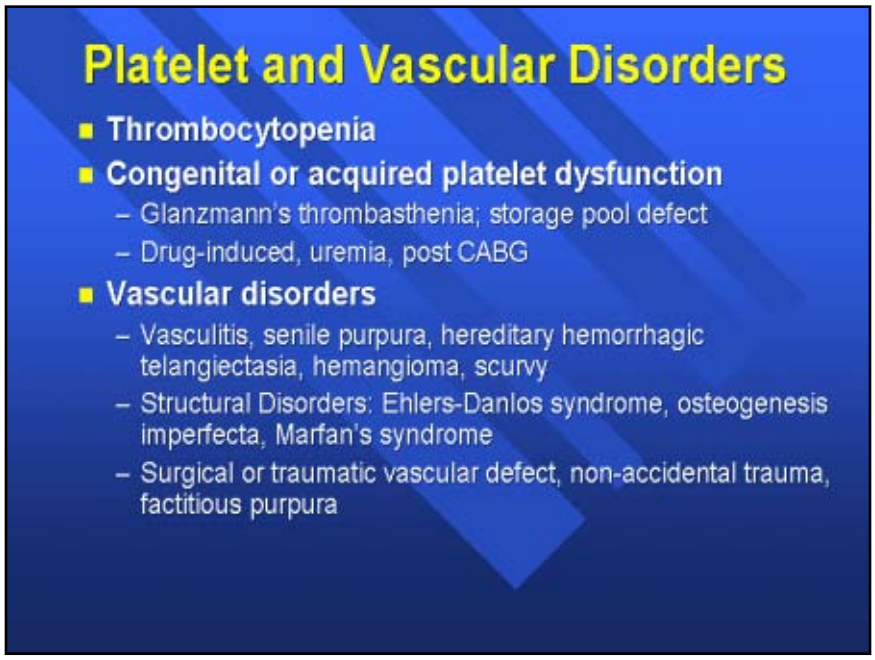
If you have, if you have a low PAI activity level and you're not certain if this truly is PAI deficiency or if this is normal because we know normals can have near zero levels, then what we recommend is that a PAI-1 antigen be performed on the same sample.

PAI-1 antigen levels are typically higher than activity levels, because of the PAI-1 that is typically released from the platelet. If your PAI-1 antigen level on the same sample is abnormal, then what we would recommend is a serum sample to follow up.

A serum sample, because of the way that the serum clots, causes activation of platelets and all of the platelet PAI is released. If you've got no PAI in a serum sample, then essentially there is no doubt that patient is PAI deficient. Other than that, the diagnosis is very difficult to make, because we know that patients may have normal platelet PAI but abnormal plasma PAI.

A patient should have an abnormal euglobulin clot lysis, and if you're really having a hard time I recommend some sort of a venous occlusion test. Because with venous occlusion, we release tPA due to venous occlusion, and that should cause PAI levels to increase. So if you do not have an increase in PAI levels pre and post venous occlusion that would support a diagnosis as well.

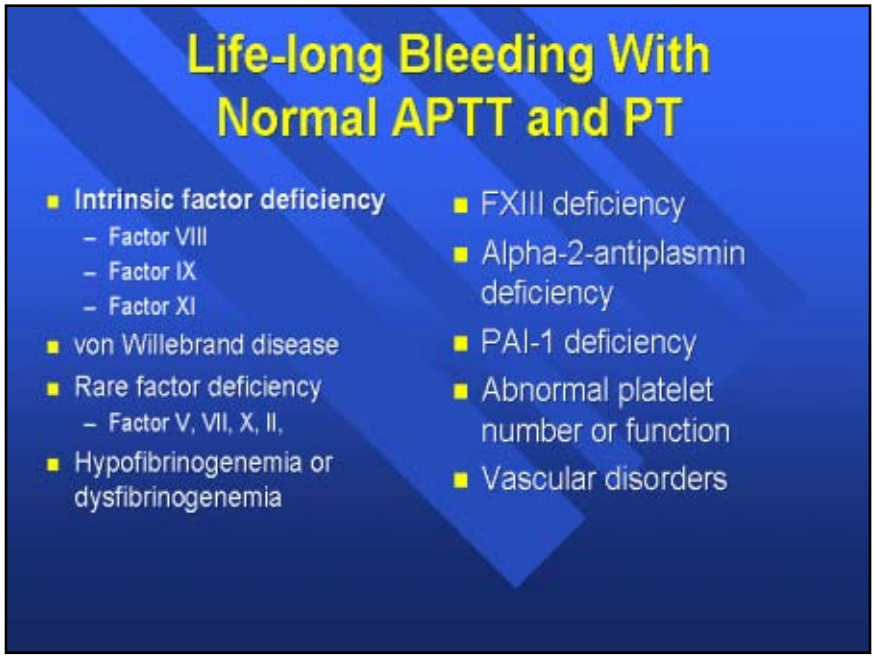
Title: Platelet and vascular disorders



All right, now we really don't have time to discuss platelet and vascular defects that may be associated with the bleeding diathesis, but just keep in mind that patients can certainly suffer thrombocytopenia, congenital or acquired platelet dysfunction, and have a bleeding diathesis with a normal PT and PTT.

And there are a whole variety of vascular disorders that may be associated with a bleeding diathesis, and of course these can't be detected with a PT and PTT assay.

Title: Life-long bleeding with normal APTT and PT

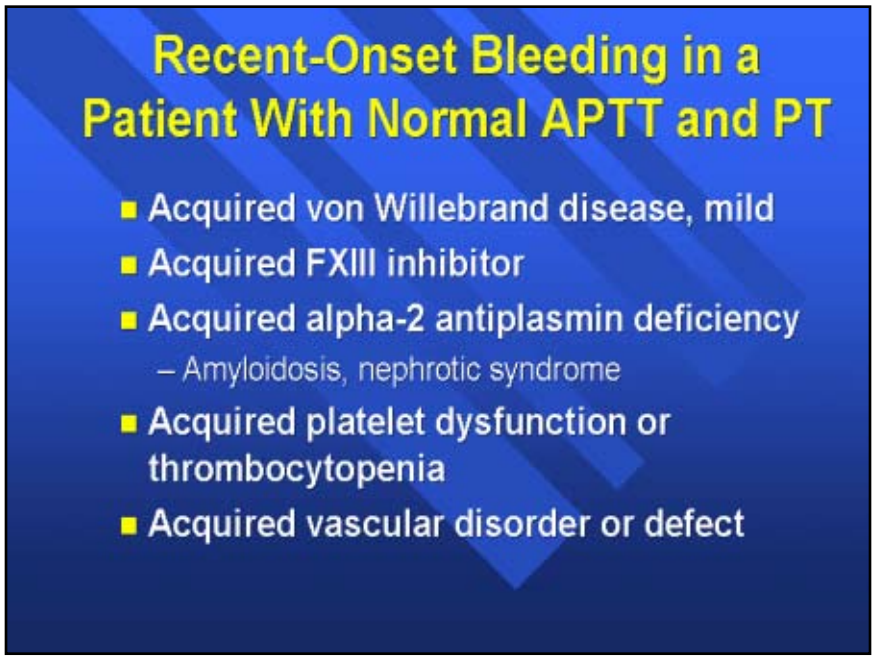


Now this is a table that I've developed to keep in mind the various disorders that you should consider when a patient has a lifelong bleeding diathesis with a normal PT and PTT.

These patients should be evaluated for abnormalities of factors VIII, IX, and XI. Be certain to look for von Willebrand's disease. You can have rare deficiencies in the common pathway factors and factor VII, but these aren't very common. A fibrinogen activity should be evaluated to make certain the patient doesn't have hypo or dysfibrinogenemia.

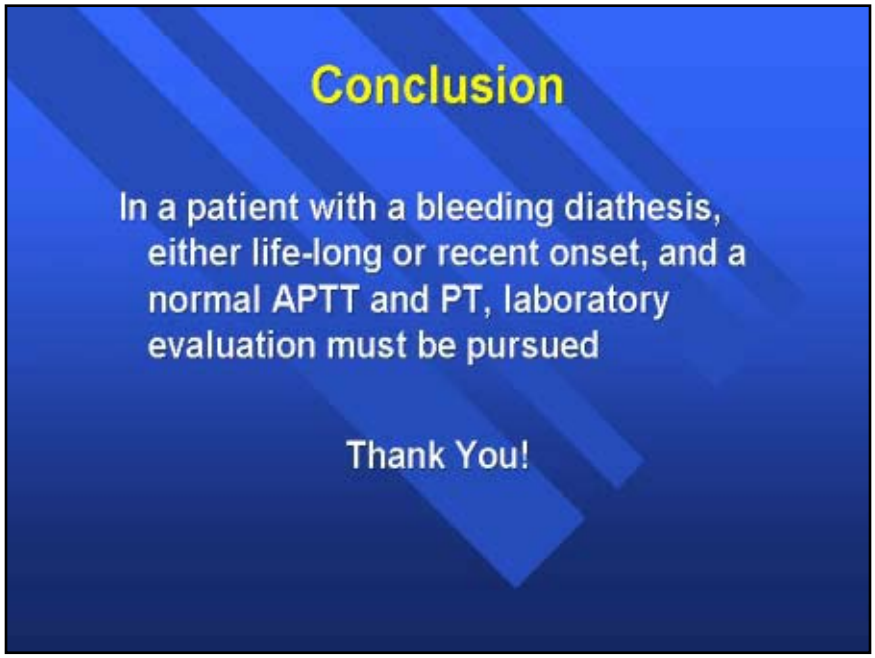
Remember to evaluate for factor XIII deficiency, alpha 2-antiplasmindeficiency, PAI-1 deficiency, and then of course don't forget about platelet disorders or vascular disorders.

Title: Recent-onset bleeding in a patient with normal APTT and PT



But what about a patient with recent onset bleeding and a normal PT and PTT, then you should begin to think about the acquired disorders, acquired on Willebrand's disease, acquired factor XIII inhibitor, acquired alpha2-antiplasmin deficiency, and then of course acquired abnormalities of platelets or the vasculature.

Title: Conclusion



So with that, the very last slide. In conclusion, in a patient with a bleeding diathesis, either lifelong or recent onset and a normal PT and PTT, laboratory evaluation must be pursued.