

# Von Willebrand Disease, Diagnosis & Treatment

## Craig Kessler, MD

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Title: The diagnosis and treatment of Von Willebrand Disease

### THE DIAGNOSIS AND TREATMENT OF VON WILLEBRAND DISEASE

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In any case, I want to thank Stago for their kind invitation to speak to you today about von Willebrand disease. And I hope that over the next hour or so I'll be able to discuss, I'll be able to convey to you some of the basic physiology of the von Willebrand factor protein.

I'd like to examine some of the clinical aspects of von Willebrand disease, and try to provide you with an understanding of the diagnostic approach to von Willebrand disease, and how you can distinguish between von Willebrand disease and hemophilia A, particularly in the laboratory, as well as clinically.

And then discuss with you a little bit about some of the treatment options for von Willebrand disease. And, since most of you are laboratorians, you'll be, you'll be in constant contact with plasma samples from individuals with von Willebrand disease, because as you are no doubt aware, von Willebrand disease is the most common inherited coagulation disorder.

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Title: Overview of von Willebrand Disease (vWD)

### Overview of von Willebrand Disease (vWD)

- **Most common inherited coagulation disorder with a 1-3% prevalence**
  - Equally affects men and women
  - Affects all ethnic groups equally
  - Prevalence of vWD patients needing treatment ranges between 7-10/million with highest prevalence in Scandinavia
  - Characterized by deficiency of von Willebrand factor (vWF) primarily and by deficiency of factor VIII coagulant activity secondarily
  - Associated with quantitative and/or qualitative defects of the vWF protein
- **Historical definition**
  - Excessive bleeding time and normal platelet count in males and females within a family

*Rizza CE. In: Haemophilia and Other Inherited Bleeding Disorders. 1997:87-113.*

And whereas our prior speaker indicated that there were a number of individuals in this room who probably have a factor V Leiden gene polymorphism, there are going to also be a large number, a significant number of individuals in this room who have von Willebrand disease.

And I don't know if the Stago folks told you that we're doing gene testing and laboratory testing on everybody for free at lunch time to be able to diagnose all of these common bleeding abnormalities.

The issue of von Willebrand disease, as we'll see in a few minutes, is that it's very prevalent, but very rarely is it picked up, particularly in individuals who have mild deficiencies, until individuals are surgically challenged, and then it becomes a major issue.

Well, von Willebrand disease is an autosomal inherited abnormality. That means it is going to affect men equally as women. It affects all ethnic groups equally, although I must say that perhaps there's an increased incidence that's recognized in Caucasian populations, and particularly Scandinavian populations, but I think that that is really more related to the fact that the, what is called the founder's effect.

That is, the disease was first recognized in Scandinavia as we'll discuss in a few minutes, and therefore I think that the tendency to diagnose and to be aware of the possible diagnosis of von Willebrand disease is much higher than it is in other areas of the world.

But there is no ethnicity. So whether it's an Asian population, African American, Hispanic, whatever, the prevalence should be about the same. And, the prevalence of the disease is somewhere between 7 and 10 per million population, with the highest prevalence as I said in the Scandinavian countries.

Now, von Willebrand disease is characterized by, as you would imagine, a deficiency of von Willebrand factor protein activities, and surprisingly perhaps to some people, a decrease in factor VIII coagulant activity also. And the reason for this is quite easy to understand.

Von Willebrand factor, which is a very large protein as you'll see in a few minutes, circulates in tandem with factor VIII coagulant protein. And essentially, the von Willebrand factor protein provides stability to the factor VIII coagulant protein and keeps the factor VIII protein within the plasma circulation.

Now individuals who have von Willebrand disease, and who have a quantitative deficiency of von Willebrand factor protein will therefore not be able to keep factor VIII in the circulation. So therefore, the factor VIII leaks out of the blood vessels, and you essentially have a quantitative factor VIII deficiency with your factor VIII assays.

This is not related at all to a synthetic defect of factor VIII production by the body. It only has to do with the fact that you can't keep the factor VIII in the circulation when you don't have the von Willebrand factor protein to function as a chaperon, so to speak, for the factor VIII coagulant protein.

Now, individuals can have von Willebrand disease from a clinical perspective in two ways. You can have either a quantitative defect in the von Willebrand factor protein or you can have a qualitative defect in the von Willebrand factor protein. We will discuss both of those types of problems in the next few minutes.

Now, historically, von Willebrand disease was diagnosed in individuals who presented with excessive bleeding times, normal platelet counts, and often the individuals were females, and the disease was originally called female hemophilia, because this is not a sex-linked recessive disease, this was an autosomal disease.

And as an autosomal disease when a physician saw a woman who came in with a low factor VIII activity but with a prolonged bleeding time, this woman was diagnosed with female hemophilia.

## History of vWD

- 1926: Professor Erik von Willebrand describes severe mucocutaneous bleeding in a 5 yr old Finnish girl from the Åland Islands; 4 sisters with hemorrhagic deaths before the age of 4; proband died at 13 with her 4th menstrual period
- “Pseudohemophilia” with autosomal pattern of inheritance
- 1928: Dr. George Minot describes similar disease in 5 pts
- 1971: the deficient protein was discovered and termed factor VIII-related antigen because it co-purified with factor VIII (FVIII)
- 1976: Zimmerman recognized FVIII:Ag to be a distinct molecular entity and renamed it vWF protein

Rizza CR. In *Haemophilia and Other Inherited Bleeding Disorders*. 1997:87-113, Ruggen ZM, Zimmerman TS. *Blood*. 1987;70:895-904.

In actuality, we were able to learn eventually that there is a difference between the factor VIII molecule and the von Willebrand factor protein. Now, von Willebrand disease was first diagnosed and recognized in 1926 by Professor von Willebrand, and Professor von Willebrand was a German hematologist.

And he was referred a case of a 5-year-old Finnish girl who had severe mucocutaneous bleeding. She was from a place that I'll show you on the map in a second, called the Åland Islands. This lady, this girl, this young girl had four sisters who also had hemorrhagic disease and in fact her four sisters died.

And her four sisters died (of hemorrhagic complications) before the age of 4 years of old, 4 years of age, and the young girl who was referred to Professor von Willebrand died at 13 following her fourth menstrual period with intractable bleeding.

So this woman or this girl obviously had severe von Willebrand disease, but it gives you an idea that we're not, that in spite of the fact that there's so many people in the population that actually have von Willebrand disease, that there are varying degrees of severity, and the most severe type certainly has high morbidity if it's not recognized and diagnosed properly.

Now, this was called pseudohemophilia as I alluded to earlier, because it was an autosomal pattern occurring in women. Now, just to show you that timing is everything.

In 1928, a hematologist at the University of North Carolina, a pathologist, Dr. George Minot, also described von, this similar bleeding history in an individual cohort of five patients. But because he was two years later than Professor von Willebrand, it was called von Willebrand disease and not Minot disease.

In 1971, it was the first time that we really appreciated that there is a difference between factor VIII coagulant protein, and what was then called factor VIII-related protein, which turned out to be von Willebrand factor protein.

And, Ted Zimmerman working with Oscar Ratnoff at the Cleveland Clinic were the two people who were most instrumental in indicating that there were two proteins with separate inheritance patterns, and that you could have one disease without the other or both simultaneously. Okay.

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**Title: Finland by virtual Finland**



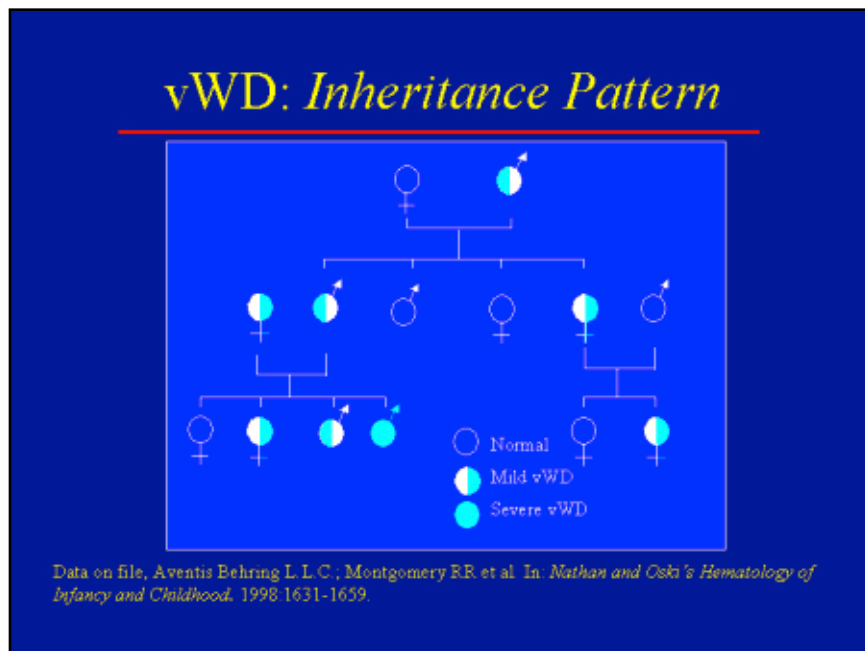
Well, this is a map of Scandinavia. I hope that you can see this from the back. This is Finland over here, Sweden over here, and here are the Aland Islands right off the coast, the southern coast of Sweden. Now, the Aland Islands are beautiful, and the Aland Islands interestingly enough are mainly now resort areas for individuals from Finland.

In fact, only Finnish citizens can own land on the Aland Islands, and so that sort of gives you a picture then that there may be some consanguinity perhaps in the area, which might be responsible for the fact that there was such severity in the von Willebrand disease that was first diagnosed on the Aland Islands.

Now it's interesting that even though Professor von Willebrand was the individual who established the diagnosis of von Willebrand in this 5-year-old Finnish girl, he himself had never visited the Aland Islands.

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**Title: vWD: Inheritance pattern**

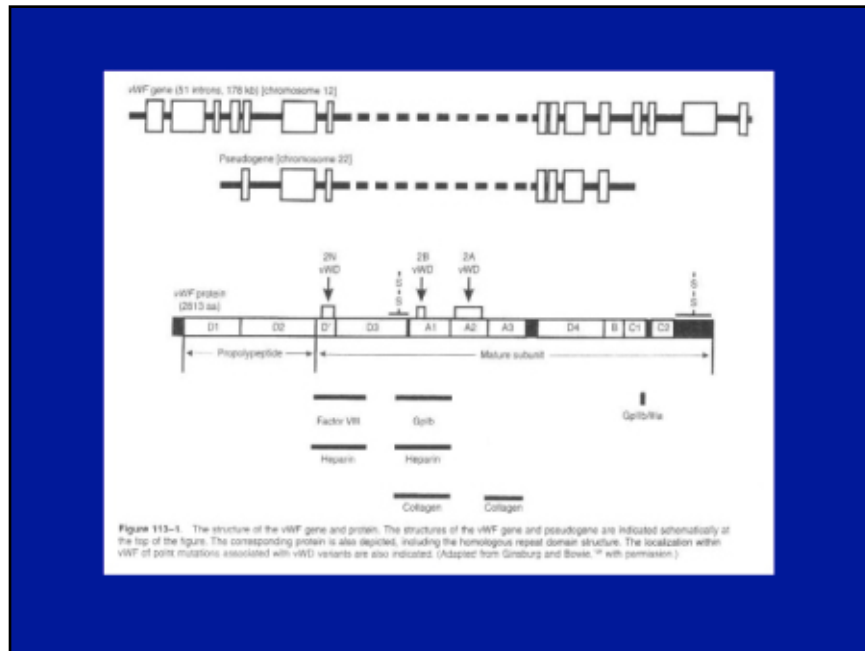


Data on file, Aventis Behring L.L.C.; Montgomery R.R. et al. In: Nathan and Oski's Hematology of Infancy and Childhood, 1998:1631-1659.

Now, the inheritance pattern of von Willebrand disease as I indicated to you earlier, is an autosomal pattern. So that as you can see on this classic diagram for von Willebrand disease, you'll see the disease in both males and females usually in equal prevalence. And of course, then the gene can be present in either parent.

Now, in severe von Willebrand disease, it's probably more of an autosomal recessive disorder than the mild types of von Willebrand and the variant von Willebrand's disease categories that we'll discuss, which are typically just autosomal dominant.

## Title: Figure 113-1 The structure of the vWF gene and protein



Now, the von Willebrand factor gene has been isolated. And it's been isolated on chromosome 12, and this gives you sort of an idea of what the gene looks like. It has 51 introns. It has a molecular weight of about 178 kilodaltons.

By the way, this is important from a medical standpoint, because you may wonder well why with all the gene therapy that we are discussing these days, why not begin to develop gene therapy to reverse or correct von Willebrand disease in von Willebrand patients?

And the reason is that a 178 kilobases constitute a very large molecule, and in fact you'll see in a few minutes that the von Willebrand molecule is the largest protein which circulates in plasma, and that is a very good Jeopardy question I guess. It happens to be the largest protein in the circulation.

And the reason for this is that it polymerizes from a base unit of about 250,000 up to 20 million. Now, you can see on this bottom depiction of the actual von Willebrand gene that there are numerous areas of the gene that are very important.

Now these are called domains within the gene, and the molecule itself, and you can see that there is a D domain and an A domain, and a B and a C domain. And these domains actually have homology with each other, so that you can actually take apart the molecule, and you can see the similarity of the domains to each other.

And you can see over here that parts of the molecule are really devoted to certain functions and interactions with other proteins in the circulation. So that for instance the von Willebrand gene has a defect at the D portion over here, which is responsible for a variant type of von Willebrand disease called 2 Normandy.

And as you know 2 Normandy is the von Willebrand disease, which is associated with ineffective interactions between the factor VIII molecule and the von Willebrand factor protein. And we will discuss this in a few minutes.

So if you have a gene defect at this area, then you will not be able to form the complex between the factor VIII and with its chaperon, the von Willebrand factor protein. Then there are other areas of defects that are responsible for the other variants of von Willebrand disease. The von Willebrand 2B variants and the von Willebrand 2A variants.

And then you can see also that areas of the von Willebrand factor protein interact with other circulating proteins in the plasma. And von Willebrand factor protein is what we call an integrin. And an integrin protein is sort of a sticky adhesive-like protein. And there are numerous other sticky proteins in the circulation that von Willebrand factor interacts with. These include predominantly heparin and also fibrinogen.

And so, von Willebrand factor does interact with heparin, so that if you for instance had to make the diagnosis of von Willebrand disease in an individual who was fully heparinized realizing that the ristocetin cofactor by itself is not necessarily interfered with by heparin, but in actuality full doses of heparin may interfere with your ristocetin assays.

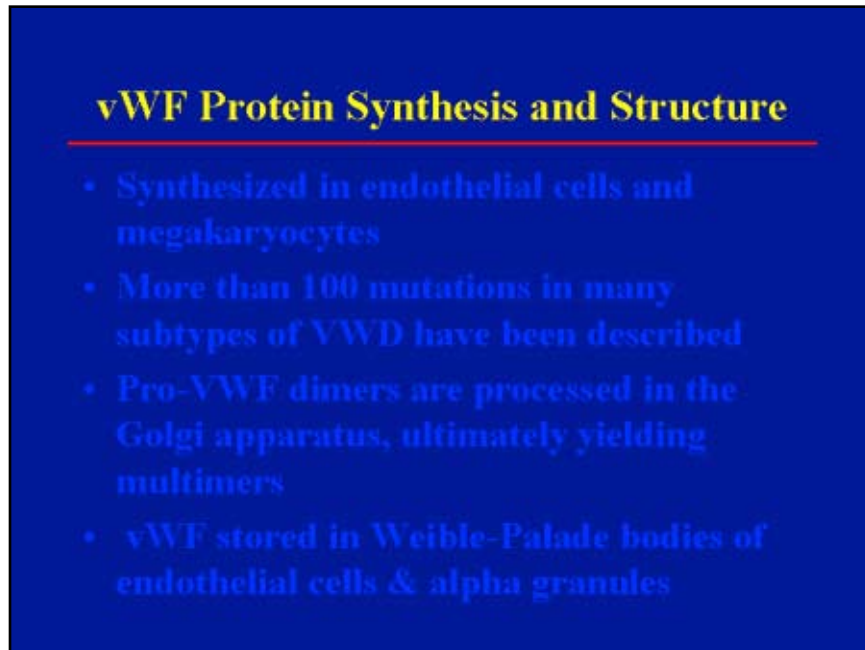
So that's one thing to remember when you get a patient. Now, of course, not a large number of von Willebrand patients will be on heparin, but I'll show you when we discuss the treatment of von Willebrand disease that those individuals even though they may have a bleeding defect, once you begin to replace their von Willebrand factor, they become for all intents and purposes normal individuals from the coagulation perspective.

And in fact, these individuals may become hypercoagulable in a paradoxical fashion that I'll discuss a little later. So you may see individuals who have von Willebrand disease on heparin in the future. Heparin also because of its stickiness will interact obviously with platelets.

It can interact with two specific receptors on the platelet surface that we'll see in a few minutes. One is a rheologically-sensitive receptor, the glycoprotein Ib, and the other is less rheologically sensitive, which is called glycoprotein IIb/IIIa. And it also of course interacts with collagen as a way of bridging between the damaged endothelial lining of the blood vessel with the circulating platelets.

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**Title: vWF protein synthesis and structure**



**vWF Protein Synthesis and Structure**

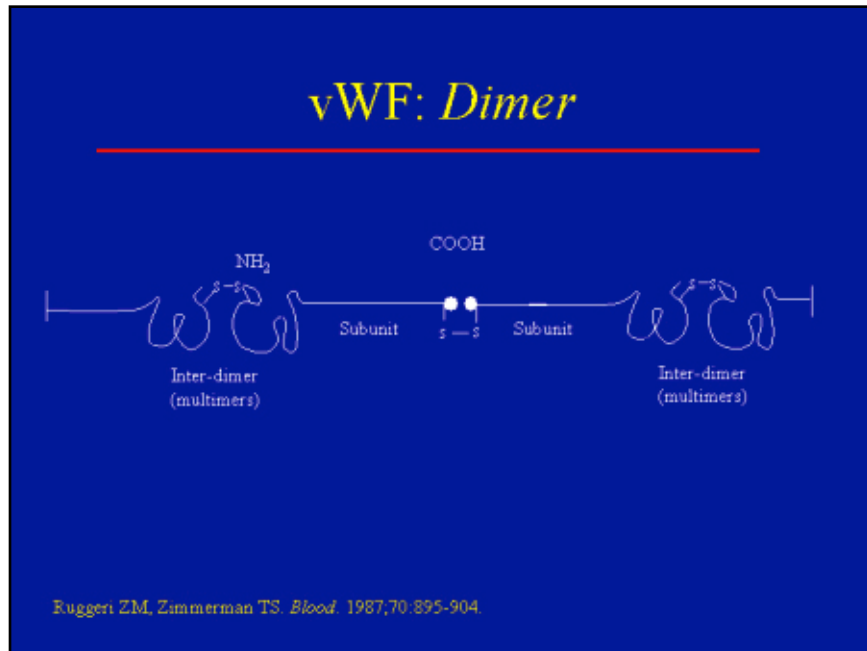
- Synthesized in endothelial cells and megakaryocytes
- More than 100 mutations in many subtypes of VWD have been described
- Pro-VWF dimers are processed in the Golgi apparatus, ultimately yielding multimers
- vWF stored in Weibel-Palade bodies of endothelial cells & alpha granules

Well, von Willebrand factor protein is synthesized in the endothelial cell on the inner lining of the blood vessel, and it's also interestingly enough manufactured in the megakaryocyte.

Now, one of the difficult, I think, issues with the diagnosis of von Willebrand disease, perhaps more so from the genetic counseling perspective or the intrauterine or the intrauterine diagnosis of von Willebrand disease, is the fact that there have been so many mutations that are associated with producing von Willebrand disease that it's difficult to be able to produce a laboratory assay that would be easily available to use for genetic counseling and predicting whether or not the child, the unborn child of a mother who has von Willebrand or father with von Willebrand disease, whether or not the baby itself has von Willebrand disease. The von Willebrand disease is, I'm sorry the von Willebrand factor protein is synthesized then in the endothelial cell or the megakaryocyte, and in the endothelial cell it's stored in what are called the Weibel-Palade bodies.

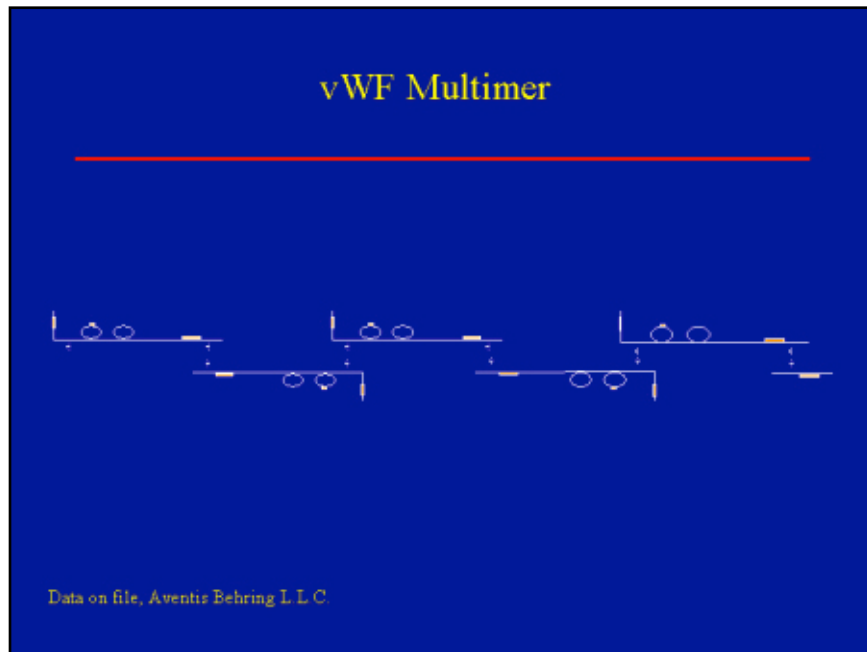
In the platelet, it is stored in the alpha granules of the platelet. So that you can almost appreciate then, that if you focus coagulation by virtue of platelet plug formation at areas of endothelial damage, then you actually then focus a large concentration of von Willebrand factor protein at the site of blood vessel damage, which is exactly what you want to do, so that you don't have disseminated intravascular coagulation, but you're able to get your platelet plug, and then humoral hemostasis to be localized at the site of blood vessel injury.

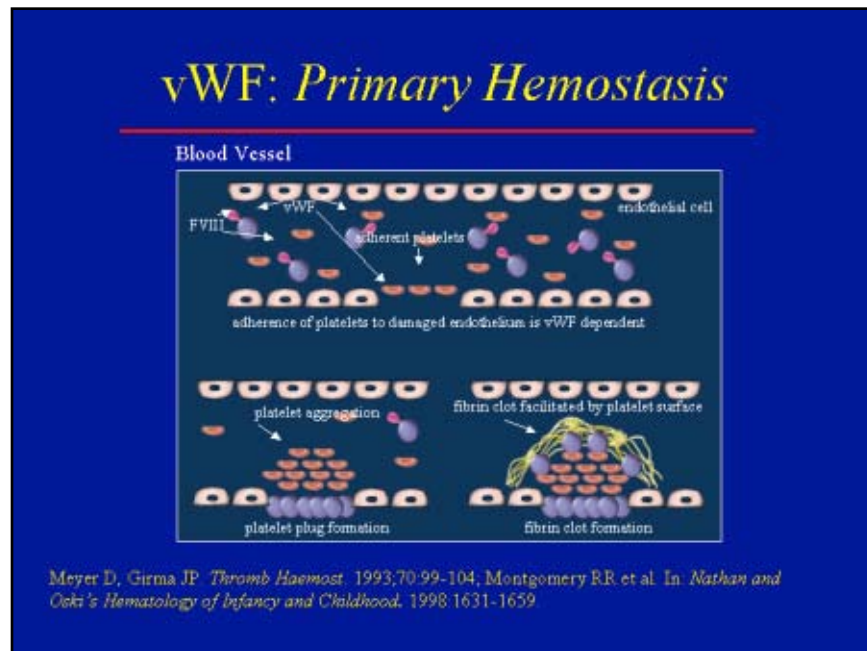
**Title: vWF: Dimer**



Now the von Willebrand factor protein itself as I indicated to you before has this, has a subunit structure, and the subunit structure will form dimers, and then these dimers will polymerize in such a way so that you'll then begin to go from a molecule of 250,000 daltons up to 20 million.

**Title: vWF multimer**





So how does von Willebrand factor actually work in vivo? Well, it's primarily related to the phenomenon of primary hemostasis so that in this cartoon as you can see, you have the schema of the endothelial cells lining the blood vessel. You have the blood as you can see flowing through the lumen of the blood vessel.

You have a damaged area of endothelial cells. And then when the endothelial cells are damaged and the blood vessel itself is denuded, you will essentially then have subendothelial components that are exposed then to circulating proteins and platelets within the blood, within the circulating blood itself.

So what happens then is that the first line of defense is to form a monolayer of platelets at the site of blood vessel injury, and this is mediated then by the ability of von Willebrand factor protein to stick to the subendothelial collagen component of the subendothelial matrix, and then form the bridge to circulating platelets.

And then eventually when the platelet plug is formed, it recruits, the platelet becomes activated, and then the platelets begin to recruit other platelets in the environment to stick to that monolayer until the platelet plug is formed.

Now the initial platelet plug or the initial platelet monolayer formation is really rheologically dependent, that means that the ability of the factor VIII to stick to the, I'm sorry the von Willebrand factor to stick to the platelet is dependent on the sheer forces of the blood as it goes through the blood vessel.

It just so happens that the platelets, which you know are plentiful in the blood stream, are extremely light compared to the red cells and the white cells.

So that in a stream of blood flow, say moving in this direction, because the platelets are so light compared to the red cells and the white cells, the platelets are pushed away from the center of the blood vessel against the blood vessel wall, and in very small blood vessels where the sheer rate is quite high, it forces the platelets against the areas of damaged endothelium.

And therefore, in the presence of von Willebrand factor and the presence of intact glycoprotein Ib on the platelet surface, the platelet will adhere directly to that collagen with the von Willebrand factor as a bridge between the collagen and the platelet.

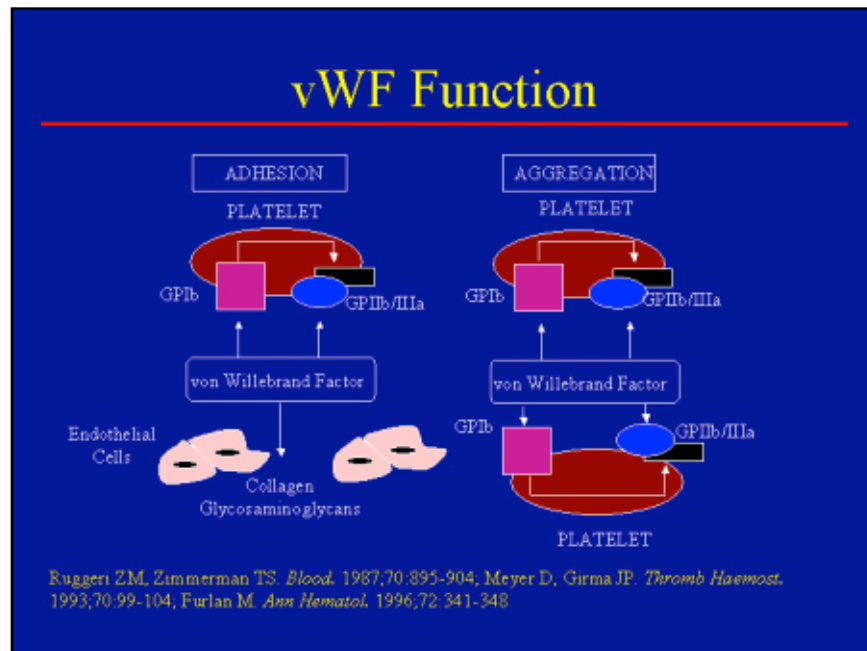
Now, once the monolayer is formed and the recruitment of other platelets in the environment occurs, that is von Willebrand factor interaction with another type of membrane glycoprotein on the platelet called IIb/IIIa.

And, IIb/IIIa is a very interesting glycoprotein because it has now been genetically produced in a, in a monoclonal antibody fashion, in fact by Barry Coller who is now at the Rockefeller Institute, and Dr. Coller developed this monoclonal antibody which has become a very important type of therapeutic drug for individuals who have undergone angioplasties, because it has been appreciated that the von Willebrand factor protein is very important in the development of re-thrombosis in the coronary vessel after angioplasty.

And if you want to prevent the blood clot from re-forming in the coronary vessel then if you interfere with the IIb/IIIa interaction with von Willebrand factor, then you can actually prevent platelet-platelet interactions which form the primary basis of the re-thrombosis.

So this is not irrelevant pharmacology or physiology here, and I'm sure that in some of your hospitals where angioplasties are done frequently and the monoclonal IIb/IIIa antibodies are used, that you'll begin to see a large number of individuals who actually develop a side effect of this drug, which is thrombocytopenia. So I'm sure you'll see that in a few minutes.

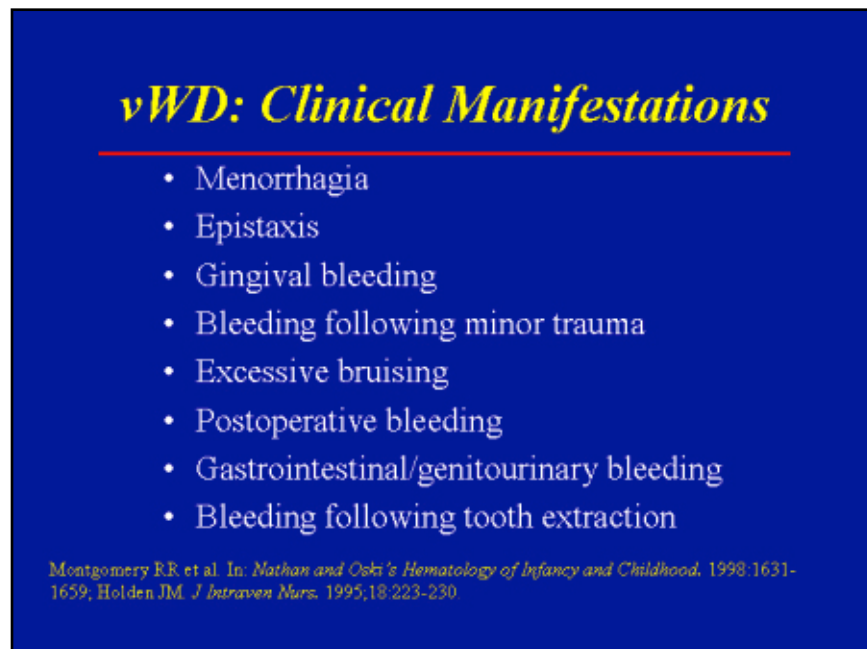




And this just provides a reiterating cartoon over here. Because I think repetition is important in visualizing what's happening here with the von Willebrand factor protein. So the first event is a monolayer of platelet adhesion to the damaged blood vessel, and this is mediated with a glycoprotein Ib interaction with von Willebrand factor protein, which in itself sticks to collagen and bridges the platelet to the collagen.

The second event is platelet aggregation, which is dependent on von Willebrand factor bridging between two platelets mediated by the glycoprotein IIb/IIIa glycoprotein marker and receptor site.

Title: vWD: Clinical manifestations



Now, let's go back to the clinical manifestations of von Willebrand disease. Von Willebrand disease then is a platelet abnormality by virtue of what we've already discussed, and it is also a clotting factor disease deficiency based on the fact, again, that many of these patients have factor VIII deficiencies in addition to the von Willebrand factor protein deficiency.

My cartoons I think have illustrated perhaps why individuals who have von Willebrand disease may have prolongations in their bleeding times. Because when you do a bleeding time in your laboratory, you have a standardized incision so to speak in microvasculature.

Well, you need to have the platelet adherence with eventual platelet plug formation in order to stop the bleeding with your bleeding time standardized laceration to that blood vessel. In individuals who have von Willebrand factor deficiency then you cannot get the

adherence to actually be efficient enough to stop the bleeding within the normal ranges of the bleeding time.

So, the same thing happens in vivo with other areas of mucosal surface and endothelium. For instance, when we talk about the platelet bleeding prototype, we always think of bleeding from mucosal surfaces.

Now, mucosal surface bleeding is not so mysterious when you begin to think that mucosal cells also release a large number of fibrinolytic types of enzymes, so that even if you're able to produce a platelet plug and then a fibrin clot, the cells on mucosal surfaces contain a large number of fibrinolytic types of enzymes, which will break down the clot almost as rapidly as those clots occur.

That's one of the reasons why you'll see in my discussion on therapy that we want to shut down the fibrinolytic pathways somewhat by using antifibrinolytic agents in conjunction with some of the replacement therapies that we use in individuals who have von Willebrand disease.

Well, one of the obvious mucosal surfaces, and perhaps explaining why family cohorts such as the original Proband family from the Aland islands died because of menses, menstrual bleeding is the fact that menorrhagia or uterine bleeding is a very common manifestation of von Willebrand disease.

Similarly epistaxis from, which is nose bleeding; gingival bleeding, gum bleeding; bleeding following minor trauma; excessive bruising; postop bleeding; GI and GU bleeding, obviously two other mucosal surfaces; and bleeding following tooth extractions. Obviously, then this is very characteristic of all types of platelet types of bleeding manifestations.

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**Title: Diagnosing vWD: Initial evaluation**

### **Diagnosing vWD: Initial Evaluation**

- Comprehensive personal and family history is critical
- Clinical history/laboratory evaluation
  - Initial lab may reveal normal or variably abnormal results (ie, platelet count, bleeding time, PT, aPTT, vWF Ag and Activity)
  - BT abnormal in only about 50%
  - Mucocutaneous bleeding/menorrhagia, etc.

Rizza CR. In *Haemophilia and Other Inherited Bleeding Disorders* 1997 87-113, Montgomery RR et al. In *Nathan and Oski's Hematology of Infancy and Childhood*. 1998 1631-1659.

Now, when we see patients in our office, and we begin to explore the etiology of hemorrhagic diseases that might explain why the patient is an easy bruiser or abnormal bleeder, the first thing to do is to discuss with the patient his family history of bleeding.

And as I indicated before, this is very critical because if you find an autosomal pattern of bleeding, it puts you in a different direction than if you see something which is sex linked, which puts you in a different direction. Now the initial lab may be normal in von Willebrand disease depending on the severity of the disease.

It can also be variable as I'll show you in a few minutes because von Willebrand factor just like factor VIII is what we call an acute phase reactant, and therefore in situations in which there are inflammatory stresses to the body such as cancer, infection, etc., these protein, the protein synthesis of factor VIII and von Willebrand factor proteins will be accelerated so that you may actually mask the deficiencies.

In addition to that, platelet aggregation studies in von Willebrand disease are always normal except for the ristocetin-induced platelet aggregation studies. But you may see variability in those assays, as well as in the PTT, depending on what the factor VIII activity is, and the von Willebrand factor antigen levels as well.

The bleeding time interestingly enough is only abnormal in about 50% of von Willebrand disease patients, always accelerated in individuals, I'm sorry always abnormal in individuals with severe von Willebrand disease. And quite truthfully, as a clinician, I will only do the bleeding time once in a von Willebrand patient, and that's to try to make the diagnosis.

Once I establish a diagnosis, I never need the bleeding time to be done again. It doesn't really help. It doesn't always correlate with your replacement therapy. It doesn't correlate with bleeding propensity.

And the other reason is that it's so technician dependent on its results, and there are so few people who do bleeding times on a regular

basis that to me this is an outmoded approach to the diagnosis of von Willebrand disease, and clearly we need more standardized, and perhaps mechanistic types of assays to establish the in vitro bleeding time perhaps rather than the kind of bleeding times that we're all used to.

It always drives me crazy when even in my own laboratory where I have some degree of control I think, that the, that when I have the request of all of the technicians in the laboratory who want to rotate from hematology, to coag, to chemistry, to whatever, that everybody wants to do bleeding times, and you just cannot do bleeding times and get accurate results when individuals do only a few bleeding times during their careers.

What's even worse is that the surgeons still have this compelling need to have a bleeding time result particularly before tonsillectomies, and I've never been able to figure that one out personally, but particularly before tonsillectomies, and they usually forget to order it, and then they send a medical student in to do the bleeding time, and then what happens is you get this, as you know this stat call at 8:00 in the morning, we need to get this patient to surgery in a half an hour, he's got prolonged bleeding time, the medical student did it, and figure it out for us, does this patient have von Willebrand disease or whatever?

So in my own mind, I think the bleeding time is going to slowly leave the laboratory, and hopefully we'll be able to develop new techniques that will give us the same information in a more standardized and accurate manner.

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**Title: Diagnosing vWD: Laboratory testing**

**Diagnosing vWD: Laboratory Testing**

- Ristocetin cofactor activity (vWF:RCof)
- FVIII clotting activity (FVIII:C)
- vWF antigen assay (vWF:Ag)
- vWF multimer analysis
- Ristocetin-induced platelet agglutination (RIPA)
- Blood type
- Collagen binding assay
- Ex vivo bleeding times (PFA-100, Clot Signature Assay)
- Conventional bleeding time (Ivy, Duke, etc.)
- Platelet count

Montgomery RR et al. In: *Nathan and Oski's Hematology of Infancy and Childhood*, 1998 1631-1659, Kleinert D. In: *Congenital Bleeding Disorders: Principles & Practices*, 2000 9-15.

So, how do we make the diagnosis of von Willebrand disease using the laboratory? Well, the prototypic types of tests as you know are the ristocetin cofactor activities, and this is a test which is readily available as you know. And ristocetin cofactor activity was actually developed in the following way.

Ristocetin as you may know is an aminoglycoside antibiotic, and when this antibiotic was initiated in clinical trials, it happened to be in Melbourne, Australia, it was noted that 98 out of 100 patients in the hospital developed thrombocytopenia.

And they couldn't understand what was happening here, obviously you don't want any antibiotic that is going to cause thrombocytopenia, so some clever hematologists decided to take a look at this in the laboratory using platelet aggregation assays.

And they found out that there were only two patients, actually there were three patients in the hospital whose platelets did not spontaneously aggregate in the presence of ristocetin. Two of those patients had von Willebrand disease, and one of the patients had Bernard-Soulier syndrome, which is a glycoprotein Ib deficiency which is inherited.

Well, there you go, so you need, so you see that von Willebrand factor is necessary for platelet agglutination in the presence of ristocetin. If you lack the von Willebrand factor or you lack the receptor on the surface of the platelet where von Willebrand factor interacts, then you do not get ristocetin-induced platelet aggregation.

And although ristocetin is never going to be used as an antibiotic, it certainly has been very useful to be used as a diagnostic tool for von Willebrand disease. Well, the, that provides us with some information about the functional aspect of von Willebrand factor protein.

But we can also quantitate von Willebrand factor using an antigen assay, and that's also very important because as we will see in a few minutes, although there should be a 1:1 correlation of von Willebrand factor activity to von Willebrand factor antigen levels, in individuals where there is discordance between the antigen and the activity, then you should suspect the presence of a quantitative, I'm sorry of a qualitative abnormality in von Willebrand disease, and that satisfies the definition for the variants of von Willebrand disease. The ristocetin-induced platelet aggregation assay is just a platelet, is a patient's platelet-rich plasma to which you add ristocetin.

Whereas the ristocetin cofactor activity you know is where you have the formal and fixed platelets, and you add the, those formal and fixed platelets to the patient's platelet-poor plasma, and then add ristocetin exogenously, and so that assay actually tells you what the patient's von Willebrand factor protein is doing by itself, whereas the ristocetin-induced platelet aggregation study gives you an idea of what the platelet function of that von Willebrand patient is like. Now, the factor VIII clotting assay is also important in defining the severity of the disease. And also blood type will be important as I'll show you in a second.

The collagen binding assay is not readily available in most laboratories, and in actuality correlates very closely with the ristocetin cofactor activity. But it provides you, if you do have a collagen binding assay, it provides you with the ability to take a look at another one of the functions of the von Willebrand factor protein capacity.

And there are some individuals who have defective von Willebrand interaction with collagen, but yet not have necessarily ristocetin cofactor abnormalities. Some people have felt that perhaps the collagen binding assay is more sensitive than the ristocetin cofactor activity, particularly in mild von Willebrand patients.

The bleeding time we've discussed, but the ex vivo bleeding time is what I have alluded to. And there are certainly the PFA-100 machine, and I don't know how many of you have access to the PFA-100 machine, but the PFA-100 machine has taken over in many laboratories for the typical bleeding time assays, the Ivy bleeding time technique that we had been using previously.

My own personal feeling is that the PFA-100 is not very helpful for most patients who have mild von Willebrand disease, and is very easy to detect in those individuals who have severe von Willebrand disease, but in my own laboratory, I do not use the PFA-100 except for research purposes.

There is another assay called the clot signature analyzer. This I'll show you some slides of where, we actually just submitted, and a paper will be coming out in a journal very shortly looking at this assay which is no longer available, unfortunately, because the company developing it went into bankruptcy.

But I think that in actuality, this is the prototype of the future, and that's the reason I'm going to show you a little bit about this. And then of course platelet count is important, because there are some variants in von Willebrand disease that are associated with thrombocytopenia.

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**Title: vWD: Influence of ABO blood type**

<u>ABO Blood Type</u>	<u>Lower vWF Limit</u>
Type O	35.6 U/dL (mean 74.8 U/dL)
Type A	48.0 U/dL (mean 105.9 U/dL)
Type B	56.8 U/dL (mean 116.9 U/dL)
Type AB	63.8 U/dL (mean 123.3 U/dL)

Attributable to the /Secretor locus that supplies the oligosaccharide antigens of the Lewis blood group system

Should adjusted means be used routinely to dx vWD? No studies; controversial

Gill JC et al. *Blood*. 1987;69:1691-1695

Now what is the importance of the blood group in the diagnosis of von Willebrand disease? Well, it appears that blood type O individuals will run a lower von Willebrand factor activity, and I might add a decreased factor VIII activity too, compared to individuals who have other types of red cell antigens.

And this is very important because many coagulation laboratories are establishing normal curves for type O patients. That is, if you have a type O patient who runs a 15% or more lower von Willebrand factor activity than everybody else, do those patients have von Willebrand disease or do those patients have type O blood with low von Willebrand factor protein?

Now, a large number of people are spending a large portion of their careers trying to define this. And essentially from the pragmatic perspective and from the treatment perspective, it really doesn't matter, because many of these type O red blood cell group individuals with low von Willebrand factor activities will bleed.

So in essence, it doesn't matter whether they're type O, what really matters is what their von Willebrand activity is and what their clinical history is in order to determine how you treat them or prophylax them in anticipation of surgery. But it is very important to

remember then that type O blood individuals will run lower than normal ranges of von Willebrand activities.

Now, why is this? Well, it is probably related to the fact that in order to actually develop blood groups it really is dependent on other enzyme systems to put oligosaccharides on to surfaces of proteins. And so, it appears then that type O blood individuals can't modify their protein with the carbohydrates in such a way to make it a more functionally effective protein.

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**Title: Laboratory values: Modulating conditions**

### Laboratory Values: Modulating Conditions

- Conditions associated with higher vWF levels
  - Race - vWF levels about 15% higher in African Americans
  - Chronic inflammation, acute infection, acute trauma
  - Pregnancy, oral estrogen replacement, or BCP use
  - Age                                 Diabetes
  - Malignancy                         Stress
  - Surgery                               Exercise
- Conditions associated with reduced vWF levels
  - Hypothyroidism

Data on file, Aventis Behring L.L.C., Montgomery RR et al. In *Nathan and Celi's Hematology of Infancy and Childhood*. 1998:1631-1639, Gill JC et al. *Blood*. 1987;69:1691-1695.

Now, we haven't discovered yet what the enzyme is that's missing, but I think that it's only a matter of time before we do discover this. So what other variables will affect the von Willebrand factor activities? Well, race does appear to be somewhat important, because the von Willebrand factor activities are higher in African Americans than they are in Caucasians.

The chronic inflammatory states certainly show the von Willebrand factor as an acute phase reactant. So that individuals who are infected will have higher than their baseline levels. Pregnancy, estrogens, rather involved with oral contraceptives, oral estrogen replacement therapy.

Age, malignancy, surgery, diabetes, stress, exercise, all of these influence von Willebrand activities, as well as endocrine abnormalities such as hypothyroidism, which sort of down regulates the body's metabolism, and its ability to produce proteins at their normal rate.

I can remember that I had a patient who presented to me who I was virtually positive had von Willebrand disease, but I could never make the diagnosis on her because her von Willebrand activities were always well into the normal range.

And then one time, and this is pretty typical that sometimes in von Willebrand disease patients you have to screen them frequently, may-be three or four times in order to make the diagnosis, that one time the patient's laboratory values were clearly indicative of von Willebrand factor deficiency, and I asked her what was different this time versus the other time.

She said well, I'm on vacation, and therefore I slept late this morning, and I took the subway into your office, whereas typically what I did was I used to jog into your office and have the blood work done.

And so every time she jogged, as an acute phase reactant her von Willebrand factor would go up, and then the day she slept late is the day that I was able to make the diagnosis.

It's also important if you begin to take a look at the von Willebrand diagnosis in females that you will get different levels of von Willebrand activity depending on the part of the menstrual cycle that you draw the blood from.

So if you correlate the von Willebrand factor with the menstrual cycle, you will see that the highest von Willebrand factor will be obtained at the point of the menstrual cycle where the estrogen levels are the highest. So therefore, the best time to try to diagnose von Willebrand disease, mild von Willebrand disease in a female is at the beginning of the cycle, because that's when estrogens are the lowest.

If you wait until right before she has her period then that's the time that you may not be able to make the diagnosis as easily because estrogens are high.

**Classification of vWD Based on Laboratory Findings**

Type	FVIII	vWF:Ag	vWF:RCof	RIPA*	Multimeric Pattern
1	↓	↓	↓	± ↓	all sizes present
2A	normal	↓	↓↓↓	↓↓	absence of large & med forms
2B	normal	± ↓	± ↓	normal	absence of large forms
2M	normal	↓	↓↓↓	↓	all sizes present
2N	↓↓	↓	↓	normal	all sizes present
3	absent	absent	absent	absent	absent
Platelet-type	↓	↓	↓	cryo ↑	absence of large forms

\*RIPA = ristocetin-induced platelet agglutination

Montgomery RR et al. In: *Nathan and Oski's Hematology of Infancy and Childhood*, 1998:1631-1659; Sadler JE. *Thromb Haemost*. 1994;71:520-525

Now, we classify von Willebrand disease based on the clinical finding, so that we call, we classify von Willebrand disease into type 1, 2, and 3. And there's then the variant platelet-type von Willebrand disease as well.

Now, type 1 is associated with decreased factor VIII, decreased von Willebrand activities, decreased ristocetin-induced platelet aggregation, but when you do the SDS polyacrylamide gel electrophoresis on von Willebrand factor protein, then all of the multimers are present, albeit in reduced form, in reduced amounts.

The variants of von Willebrand disease are those in which the factor VIII activities will probably be normal, may be a low normal, but there is going to be a discordance between the 1:1 decreases, the ratio decreases over von Willebrand antigen and the ristocetin cofactor, so that the von Willebrand antigen will be higher than the ristocetin cofactor activities.

And when you see this discordance, then you should be alerted to a possible variant of von Willebrand disease.

The ristocetin cofactor and the ristocetin-induced platelet aggregation assay is critical in the differentiation of the different types of variants of von Willebrand disease, because in type 2A von Willebrand disease there is decreased ristocetin aggregation, whereas in 2B not only is there normal ristocetin-induced platelet aggregation, but there is a hyperaggregability effect, and I'll show that to you in a minute.

When you do the SDS polyacrylamide gel electrophoresis of these molecules in the type 2A, you miss the highest and intermediate multimers, in type 2B you only miss the highest, I'm sorry the highest molecular weight multimers.

The highest molecular weight multimers of von Willebrand factor are those that are up at around 20 million; those are the workhorses of the von Willebrand factor protein.

So if you're missing those, you really miss the essence of the function of the von Willebrand factor. Then there's another type called 2M, and most of you probably do not diagnose this very frequently in your laboratories. Again, there may be some discordance, but this is mainly a platelet abnormality.

All sizes are present in the multimeric analysis, and then 2 Normandy, what I spoke to you a few minutes ago, where the gene defect is actually at the site of factor VIII binding to the von Willebrand factor protein. The abnormality is actually in the von Willebrand portion binding to factor VIII.

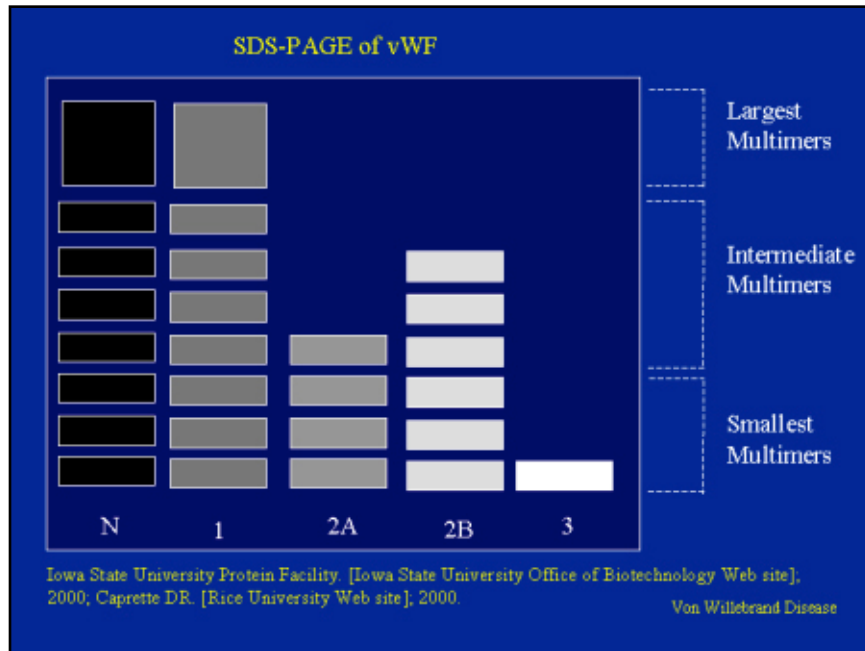
There have now been other abnormalities in which there has been an abnormality of factor VIII binding to the von Willebrand factor protein, but if it's the defect on the von Willebrand portion, this is 2 Normandy.

And again, the factor VIII is greatly reduced, the von Willebrand factor antigen and activity may be only mildly reduced, ristocetin-induced aggregation is normal, and the multimeric analysis is also normal.

And then type 3, which is probably the autosomal recessive type of von Willebrand disease is extremely severe, and these individuals look like the worst of both possible problems. They look like hemophiliacs.

That is, they'll have bulky bleeds at the muscles, joints, whatever, and they'll also have the severe platelet abnormality bleeding with very severe mucocutaneous bleeding as well, you can't even detect any von Willebrand antigen in these individuals, and therefore you can't do any multimeric analysis.

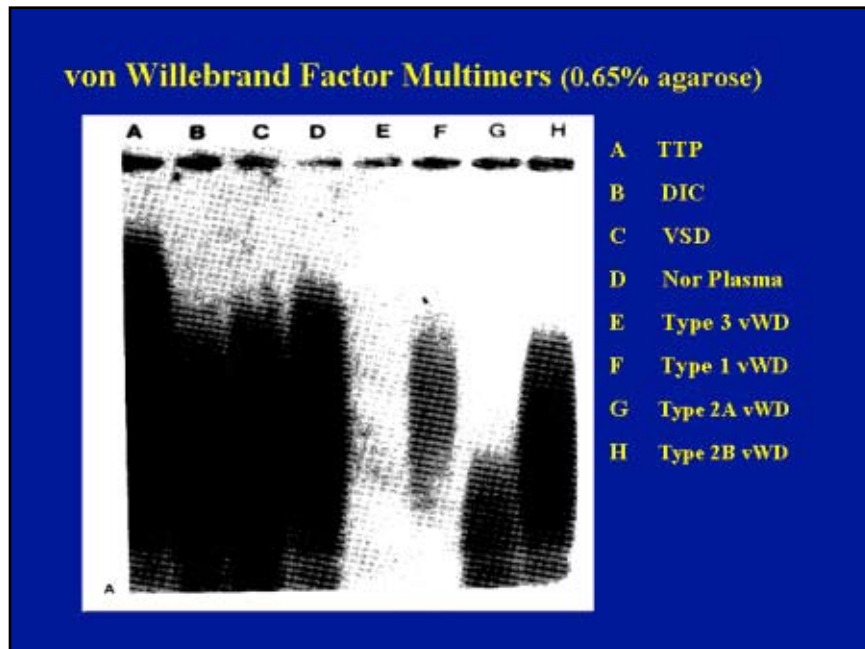
And of course, their von Willebrand activities with ristocetin are going to be greatly reduced. I'm not going to go into the platelet-type right now, that'll just confuse you.



So if you do SDS polyacrylamide gel electrophoresis for von Willebrand factor, this is a cartoon of what the normal multimeric pattern will be, again the smallest multimer of about 250,000 going up to 20 million. In individuals with type 1, they have normal composition, but there is a quantitative deficiency.

In individuals with type 2A, these are the individuals who have decreased ristocetin-induced platelet aggregation, these individuals are lacking the intermediate and highest molecular weight multimers. The type 2B are missing the highest molecular weight multimers, and the type 3 are missing all of the molecular weight multimers.

**Title: von Willebrand factor multimers (0.65% agarose)**



Now if, this is actually what the gels look like, and what I want to point out is another feature of how this assay may actually be very helpful to you, because as you probably can see over here in lane A, there appears to be a von Willebrand factor protein, which appears even larger than what you actually see in normal individuals who do not have von Willebrand disease here in lane B.

Now, when von Willebrand factor protein is synthesized in the endothelial cell, it is actually processed before it gets into the circulation, and the way it's processed is with an enzyme, which is called von Willebrand factor protease activity.

Now, this is a cleavage enzyme, and so what happens is that when you have this type of von Willebrand factor synthesized in the endothelial cell, the von Willebrand factor cleavage protease will cleave off this amount of the von Willebrand factor protein, so this is what you see circulating in the plasma.

Well, individuals who have this type of pattern then have defective von Willebrand factor cleavage protease activity, and these are the types of individuals who would develop thrombotic thrombocytopenic purpura, TTP.

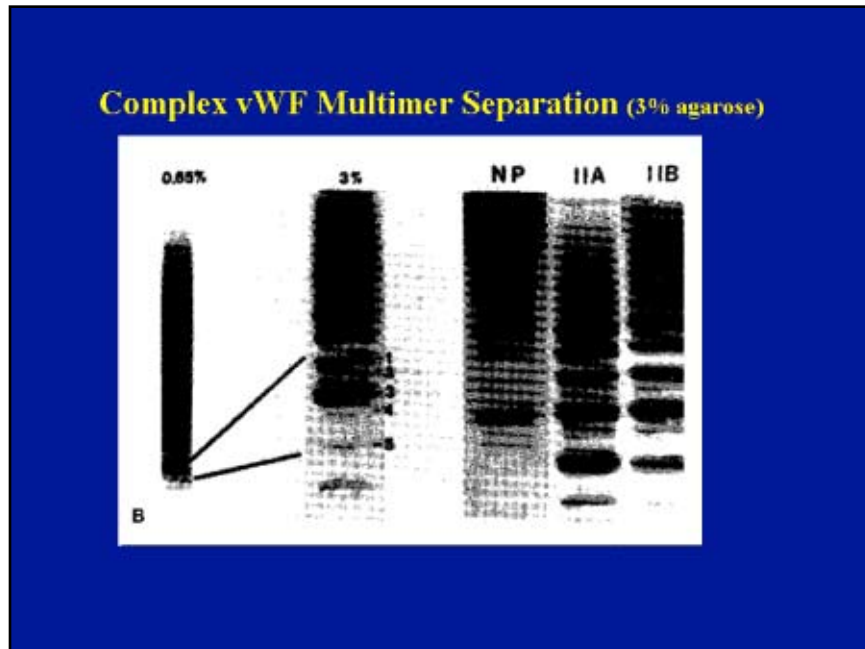
And as you know, this is now genetically been determined as an abnormality in the ADAMs 13 gene, and so now we are developing assays for ADAMs 13 inhibition, because in individuals who don't inherit this abnormality of TTP, you can acquire it, and you acquire it by virtue of developing autoantibodies that are directed against the von Willebrand factor cleavage protease, which prevents then this portion of the von Willebrand factor protein from being cleaved to circulate in this form.

So that essentially realizing that the highest molecular weight multimers are the workhorse of the von Willebrand factor protein, the portion of the von Willebrand factor protein that causes the platelet-platelet interaction, individuals who have this type of von Willebrand factor protein composition will have in vivo platelet aggregation, and that is the predominant pathophysiology of TTP.

You get microinfarcts in various organs, okay, and thrombocytopenia. I just wanted to point that out as I think an important issue that you'll be seeing in the laboratory very soon, as these kits become commercialized for the diagnosis of TTP.

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**Title: Complex vWF multimer separation (3% agarose)**

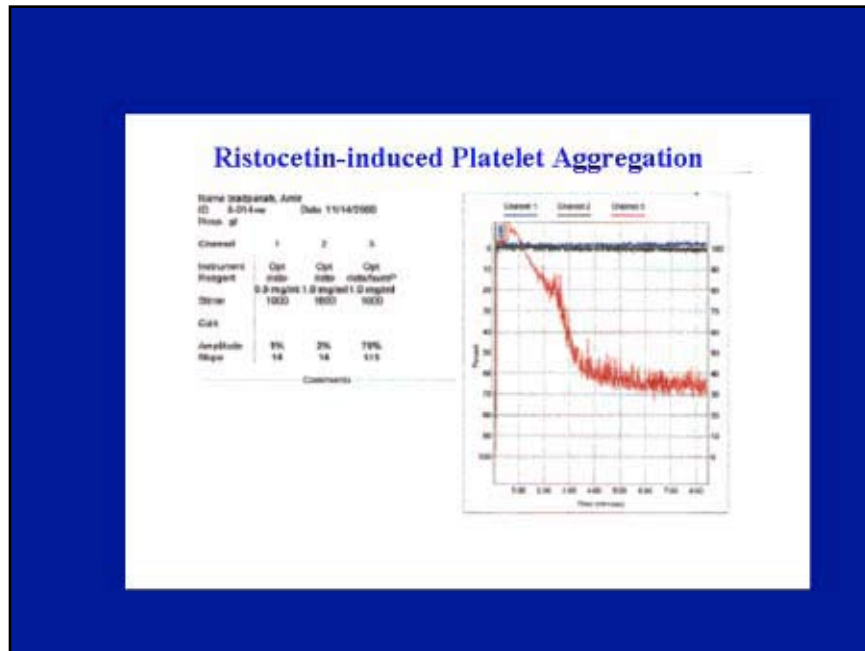


Now there are other ways of taking a look at the multimeric pattern, and this helps you to diagnose individuals with a 2M variant. And this is very highly specialized, and this takes a look then at the von Willebrand factor protein in the middle molecular weight multimeric range.

And in these individuals, you'll see sort of sandwiches that are formed, they're actually called sandwiches over here with a banding, and this helps you also with the diagnosis of von Willebrand disease.



**Title: Ristocetin-induced platelet aggregation**



Well, all of you are familiar with the ristocetin-induced platelet aggregation assay. This is the situation in which you add ristocetin to the platelet-rich plasma from the von Willebrand patient, and in severe cases you see no aggregation at all.

By the way, this is really agglutination rather than aggregation; agglutination are the platelets sticking together, aggregation is once they've stuck together and then they release.

Agglutination is reversible, aggregation is irreversible. Once you get good agglutination, then you can proceed onto aggregation. But this is the aggregation pattern with ristocetin, which is normal. This is the classic type of von Willebrand disease which is severe.

**Title: Table 4. Operating characteristics of common tests used in vWD diagnosis**

Table 4. Operating characteristics of common tests used in vWD diagnosis (from Miller *et al.*, [13]).

Clinical and laboratory parameters	Sensitivity, %	Specificity, %
Symptoms alone	52	76
Bleeding time	29-75	88-97
Factor VIII	37-59	77-97
vWF:Ag	53-81	97
vWF:RCo	50-72	97

Now, I think I'll skip over that. Von Willebrand factor type 1, which is the most common type of abnormality seen in approximately 80% of von Willebrand patients is really mild from the clinical perspective. And typically as I showed before, you'll have 1:1 ratios in the reduction of the von Willebrand antigen and the activity levels.

**vWD: Type 1**

- **Most common form**
  - Approximately 75%-80% of vWD patients
  - Generally mild to moderate in severity
- **Characterized by**
  - Proportionately reduced levels of FVIII, vWF:RCof, and vWF:Ag
  - Functionally and structurally normal vWF
  - Prolonged bleeding time

Rizza CB. In: *Haemophilia and Other Inherited Bleeding Disorders*. 1997:87-113, Montgomery RR et al. In: *Nathan and Oski's Hematology of Infancy and Childhood*. 1998:1631-1659, Holden JM. *J Intraven Nurs*. 1995;18:223-230, Association of Hemophilia Clinic Directors of Canada. *CMAJ*. 1995;153:19-25.

And so this is the abnormality, which is associated with structurally normal von Willebrand factor protein, but just decreased in quantity.

**vWD: Types 2A and 2B**

- **Type 2A**
  - Absence of large and intermediate size vWF multimers
  - 10%-12% of all vWD patients
  - Decreased RIPA
- **Type 2B**
  - ↑ vWF affinity to platelet GPIb & secondary loss of large and intermediate size vWF multimers
  - 3%-5% of all vWD patients
  - Normal or increased RIPA

Montgomery RR et al. In: *Nathan and Oski's Hematology of Infancy and Childhood*. 1998:1631-1659, Mazurier C, Meyer D. *Baillieres Clin Haematol*. 1996;9:229-241, Sadler JE [American Society of Hematology Web site]. 2000, Federici AB, Mannucci PM. *Haemophilia*. 1999;5:27-37.

The variants are slightly different. As I showed you, the type 2A, and this is the intermediate and the highest molecular weight multimers. It occurs in approximately 10% of von Willebrand patients.

These individuals will have decreased ristocetin-induced aggregation, and this is in contrast of the type 2B variant in which patients will have hyperaggregable responses. And in our own laboratory and I suspect that in yours too, you may be doing your ristocetin-induced platelet aggregation with various concentrations of ristocetin.

In my own lab, I do a 0.6 and a 1.2 mg/mL final concentration of ristocetin. Normal individuals who don't have von Willebrand disease will not show any agglutination or aggregation pattern at 0.6, they will at 1.2. Individuals with type 2 von Willebrand, 2B von Willebrand disease will show aggregation at 0.6, and hyperaggregability patterns at 1.2.

So that's one way of distinguishing these. I might add, however, please be careful about your quality control, because platelets are not happy if they are perturbed, and you should be running your platelet aggregations on fresh specimens.

So you don't want the specimens to be sitting out on the bench top for more than an hour or so. They should be capped, because

acidity plays a major role in the ristocetin responses, and if you leave them uncapped, then they'll become more acidic and more perturbable, so that even lower amounts of ristocetin may cause aggregation.

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**Title: vWD: Types 2N and 2M (rare)**

## **vWD: Types 2N and 2M (Rare)**

- Type 2N
  - Also called vWD (Normandy) and autosomal hemophilia
  - 1%-2% of all vWD patients
  - Results when a genetic defect prevents vWF from binding to FVIII
  - Often misdiagnosed as mild hemophilia A
- Type 2M
  - Characterized by decreased binding to platelet GPIb
  - 1%-2% of all vWD patients
  - Normal multimeric pattern

The 2 Normandy as I showed you before have decreased factor VIII, but may not have decreased von Willebrand activities, and certainly the ristocetin-induced aggregation is normal, and these are individuals who are typically classified as mild hemophilia A, and so all of your patients who have mild hemophilia A now should be I think looked at as to rule out 2N.

Because I know in our own population, many of our patients who were previously classified as mild hemophilia A, now that we've got the assay available for 2N actually are 2Ns rather than mild As. And then the type 2M individuals are individuals who had defective ability of the von Willebrand factor to bind to glycoprotein Ib.

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**Title: vWD: Type 3**

## **vWD: Type 3**

- 1%-3% of all vWD patients
- Characterized by virtually no detectable vWF:Ag and FVIII:C
- Patients suffer from severe, spontaneous bleeds
  - Mucosal bleeds are common
  - May experience joint bleeds similar to hemophilia
- Inhibitors to vWF may develop following replacement therapy

Furlan M. *Ann Hematol.* 1996;72:341-348, Montgomery E.F. et al. In: *Nathan and Oski's Hematology of Infancy and Childhood.* 1998:1631-1659, White GC 2d et al. *Am J Hematol.* 1982;13:335-342

Now, type 3 is fortunately rare, 1% to 3% of all von Willebrand patients, and as I said these individuals are the most severe clinically, and are typically easy to diagnose in the laboratory, because you can't see any von Willebrand activities at all.

**Acquired vWD**

- Extremely rare
  - Fewer than 100 well-documented cases
- Causes
  - Circulating inhibitors
  - Adsorption, e.g. essential thrombocythemia
- Associated with an underlying autoimmune disorders, MGUS, lymphoproliferative malignancy, medications (valproate, ciprofloxacin)
- Treatment must target the underlying as well as acute symptoms of bleeding
- vWF protein containing concentrates are often required
- Difficult to diagnose; Mixing studies may be unhelpful

Bloom AL. *Mayo Clin Proc.* 1991;66:743-751. Tefferi A, Nichols WL. *Am J Med.* 1997;103:536-540.

Now, there is a category of individuals who develop autoantibodies to the von Willebrand factor protein.

These patients typically have autoimmune diseases, but they can also have myeloproliferative disorders such as essential thrombocythemia, where the large number of platelets will absorb out the highest molecular weight multimers of the von Willebrand factor protein, and these individuals even though they have millions of platelets, have clinical bleeding which is problematic.

These individuals need to have their platelet count reduced, and if you can get their platelet counts lower, then their von Willebrand factor activities return to normal, but you can also develop autoantibodies directed against von Willebrand factor.

These are often very difficult to diagnose, because you can't do mixing studies necessarily and then do ristocetin aggregation or ristocetin cofactor activities and see the effect of mixing with normal plasma as a way of detecting these. So we really have some difficulty in this rare abnormality.

**Targeted Laboratory Criteria in vWD**

- Normalization of bleeding time
- FVIII: 50%-100%
- vWF:RCof: 50%-100%
- Other: vWF:Ag, collagen binding assay, vWF - FVIII binding, platelet function analyzer (PFA)

Data on file, Aventis Behring L.L.C.

So when we try to develop laboratory criteria definitions for von Willebrand disease, and then we treat these patients, what we want to do is we want to normalize the bleeding time. We want to get the factor VIII activities back to normal. We want to get the ristocetin activities back to normal. And we also want to see that everything else works, that the patient doesn't bleed anymore.

## **Treatment: Mild vWD**

- Mild vWD patients require treatment to correct bleeding time at the time of surgery or trauma
- For oral surgery, patients should receive desmopressin acetate and/or an antifibrinolytic agent
- Desmopressin acetate is indicated for the treatment of menorrhagia

Montgomery R.R. et al. In: *Nathan and Oski's Hematology of Infancy and Childhood*, 1998:1631-1659, Stimate™ (desmopressin acetate) [package insert], Aventis Behring L.L.C., 1999.

So how do we treat the patients? Well, sometimes all we need is an antifibrinolytic agent such as Amicar. Because in mild individuals they may be able to form a blood clot, but then eventually they can form a blood clot, but then the blood clot breaks down on mucosal surfaces, stop the fibrinolytic activity with your antifibrinolytic agent, and they do fine.

And this is what we oftentimes do for patients who have minor dental procedures. In addition to that, we can use a drug called DDAVP or Stimate, which is a vasopressin analog.

And this material seems to push the von Willebrand factor protein out of the endothelial cell from the Weibel-Palade bodies into the circulation, so for a short period of time you actually have enough von Willebrand factor protein to sustain hemostasis for the minor surgical procedure.

And we're actually using DDAVP in women who have severe menorrhagia associated with their von Willebrand disease.

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**Title: Stimate (desmopressin acetate) - A synthetic version of vasopressin**

## **Stimate™ (desmopressin acetate)**

- A synthetic version of vasopressin
- Increases plasma vWF by stimulating its release from intracellular stores; also releases t-PA & ↑ fibrinolysis
- Treatment of choice for type 1 and certain type 2A patients
- Administered intranasally or intravenously
- Side effects include: facial flushing, hyponatremia, headache, nausea, mild abdominal cramps, vulval pain, nasal congestion, rhinitis, nosebleed, sore throat, and alteration in blood pressure, seizure, angina, MI, CVA
- Tachyphylaxis may occur
- Amicar or tranexamic acid useful for mucosal bleeds

Now the problems with Stimate are that although it can increase the von Willebrand factor activities, it also releases tissue plasminogen activator from the endothelial cells, another reason why when you use DDAVP you should use it in concert with the antifibrinolytic agents, which will prevent the t-PA from converting plasminogen to plasmin.

We use it predominantly for mild type 1s, you can use it in some of the 2As. It does not work in very frequently in type 2Bs, and in

fact if you increase the plasma concentration of these abnormal multimeric forms of von Willebrand factor protein in 2Bs after the injection of DDAVP,

you may actually exacerbate the platelet agglutination in vivo, and these patients may get very rapid thrombocytopenias, and they can actually develop strokes and myocardial infarctions if you do that. So the physicians really should be very aware of how to use this drug for their patients with von Willebrand disease.

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**Title: Stimate (desmopressin acetate) - Desmopressin acetate indicated for mild to moderate severity...**

### **Stimate™ (desmopressin acetate)**

- Desmopressin acetate indicated for mild to moderate severity vWD (type 1 and 2A; ?2B) with FVIII levels > 5%
- Useful for treatment of episodes of spontaneous or trauma-induced injuries such as hemarthroses, intramuscular hematomas, mucosal bleeding, or menorrhagia
- Contraindicated in individuals with known hypersensitivity to desmopressin acetate, angina, CAD, seizures, etc.
- All patients should be tested before actual need to assess response with IN and IV forms. Restrict free H<sub>2</sub>O intake to thirst during the stimulation test to avoid hyponatremia
- Useful in qualitative platelet disorders

So we can use this also in individuals to raise the factor VIII levels in these patients. And, DDAVP comes in IV and intranasal forms. Make sure that the patient is getting the right concentration. Most DDAVP is available for enuresis, and that is not a high enough concentration to produce the increased von Willebrand activity. So, unless your patient is also a bed wetter, those things aren't going to help.

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**Title: vWF concentrates: Humate-P & AlphaNate SD**

### **vWF Concentrates: Humate-P & AlphaNate SD**

*Antihemophilic Factor/von Willebrand Factor Complex (Human), Dried, Pasteurized*

- Humate is only FDA-approved factor VIII concentrate for vWD treatment but AlphaNate SD study indicates its usefulness
- Plasma-derived, pasteurized, FVIII concentrate rich in high molecular weight vWF multimers
- Subjected to a rigorous purification process designed to minimize the risk of transmission of certain pathogens such as HIV, HAV, HBV, and HCV (cont)
- Indicated for vWD patients who are unresponsive to desmopressin acetate; for types 2B, 2N, and 3

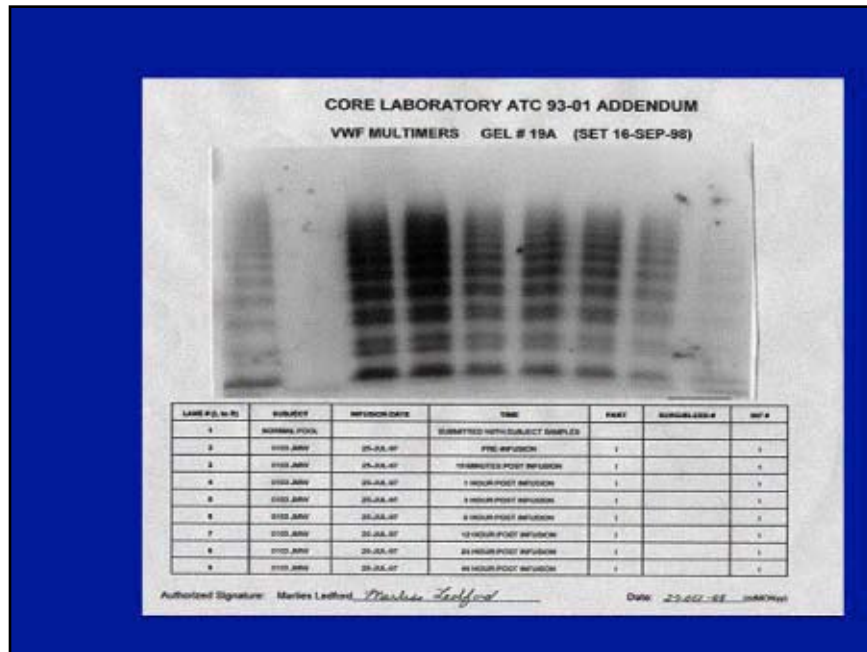
Now, the way that we treat many of the other patients is by replacing them with materials that are plasma derived, and contain von Willebrand factor protein. And we can take advantage of some of the materials that we use for the treatment of hemophilia A, because these are contaminated with von Willebrand factor. These are plasma-derived products.

They are genetically engineered materials, don't contain factor VIII materials, do not contain any von Willebrand factor protein, so

you can't use them in von Willebrand disease, but Humate-P, which is the only licensed product right now in the United States, but AlphaNate has good track record in the literature is also useful for these patients.

And one of the interesting things about von Willebrand disease is that once you give individuals a von Willebrand factor replacement therapy, remember that they may have factor VIII deficiencies, but they are making factor VIII at a normal rate, you replace the von Willebrand factor, and then you may get an overshoot of factor VIII activity 24 to 48 hours later.

**Title: Core laboratory ATC 93-01 Addendum**



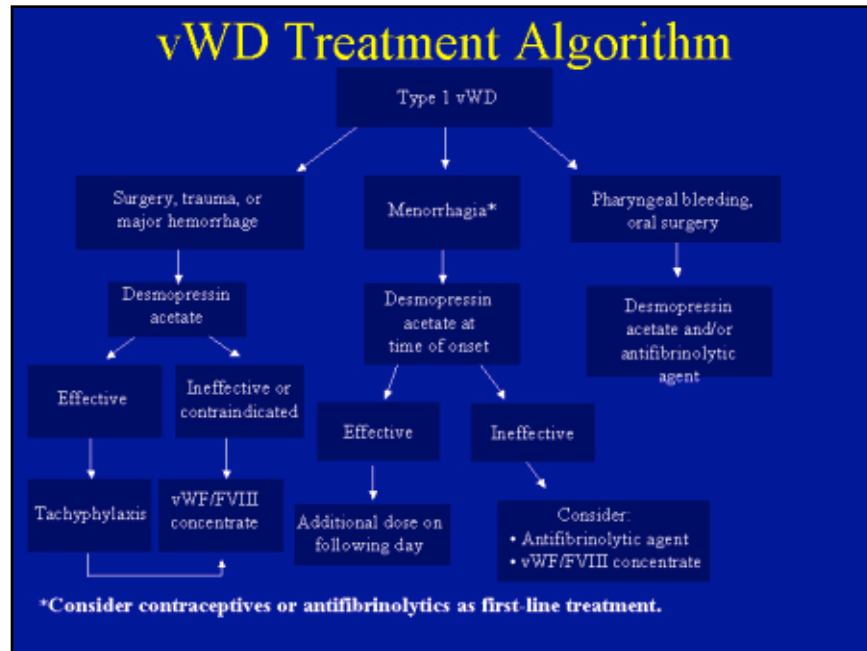
And it is in this situation that we've actually found that individuals can become hypercoagulable in spite of the fact that they have von Willebrand disease, because their factor VIII levels may go up to 250%, 300%, and then those individuals end up with clotting problems ironically, even though they were being treated for their von Willebrand disease.

And therefore, we're learning now how to use these drugs in a little bit more, I think, careful manner, so that 24 hours after they are replaced, we then don't even worry about the von Willebrand factor activities any more, we only follow the factor VIIIs.

Because it appears that bleeding is associated in von Willebrand disease after 24 hours based on the factor VIII assay rather than the von Willebrand activities. Okay? So once you get your platelet plug formed, and you begin your humoral coagulation on top of the platelet plug, then the von Willebrand factor activities are irrelevant from the clinical perspective.

This is just to show you that these products actually do correct the defect. Here's an individual with, actually this is severe von Willebrand disease, nothing in this lane. You give the patient the Humate-P or AlphaNate, you actually replace all the multimers, and then over time you can see that they are eventually degrade.

**Title: vWD treatment algorithm - Type 1 vWD**



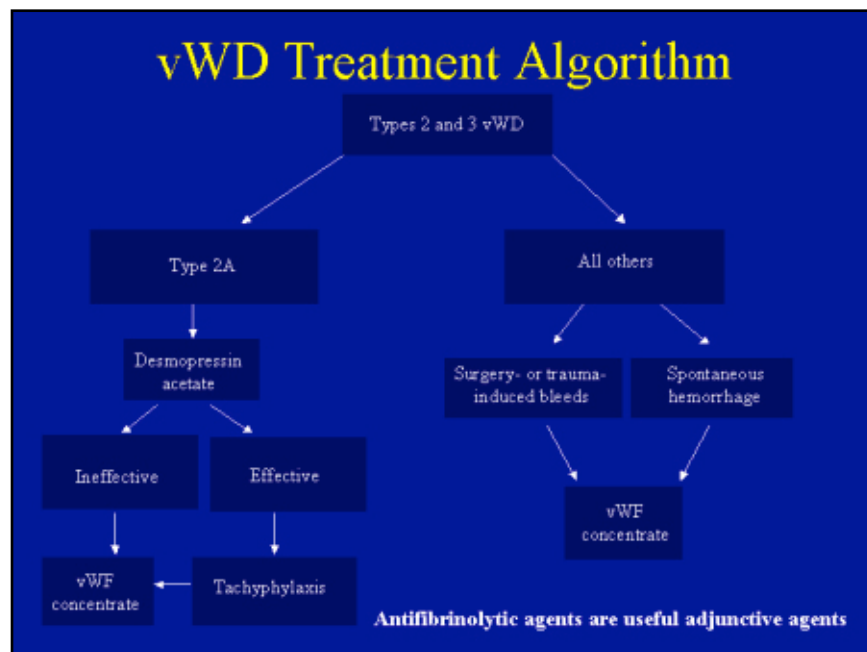
So when we begin to develop algorithms for treatment in type 1 von Willebrand disease, depending on what you're doing, you can use either desmopressin acetate, the DDAVP; if that's ineffective, you have to go to a von Willebrand factor-containing factor VIII concentrate.

Similarly with menorrhagia we do use DDAVP in some of these women. You may need to use both an antifibrinolytic agent and factor VIII concentrate with von Willebrand factor protein.

This is more difficult, to use antifibrinolytic agents in menorrhagia, because they can actually then form clots in the uterus, and then essentially have, essentially uterine cramping as if they were trying to expel a child, it's very painful.

I might also add here if you have renal bleeding, and you use Amicar that you can form clots in the bladder that can't be expelled through the urethra, and sometimes even develop clots in the ureters which provide, which present with stone, renal stone types of symptoms. And then for oral and mild mucosal bleeding desmopressin and Amicar alone or with DDAVP is useful.

**Title: vWD treatment algorithm - Types 2 and 3 vWD**



And then for the variants, you can try DDAVP for 2A, but for the others it is better to think of using something that contains von Willebrand factor protein.



## The Future

- Gene transplantation
- Elimination of transfusion-associated infections
- Develop new products with extended  $t_{1/2}$
- Understand and overcome inhibitor development
- Quality of life issues
  - Elimination of joint morbidity
  - Optimizing the individual's social and academic performance

So, in my last slide where are we going with von Willebrand disease? So that if ever I'm invited back to you, to speak to you, I'll be able to be maybe a little bit more elegant about the treatment of von Willebrand disease. Well, the gene transplant and the ultimate cure for von Willebrand disease I think is many years away.

The size of the gene, the fact that the von Willebrand factor protein is heavily carbohydrate modified, and therefore it is difficult for gene transplants to be performed efficiently, I think this is going to be a long-term goal.

The elimination of the transfusion-associated infections is a major issue, because all of these are plasma-based products, and therefore the issue is going to be that you cannot, you cannot actually prevent parvovirus transmission, and this is very important in pregnant women with von Willebrand disease, where there is vertical transmission of parvovirus from the mother to the unborn child.

You can develop hydrops fetalis and other fatal types of intrauterine abnormalities. Is there a genetically engineered von Willebrand factor protein? There may be in the near future. Although right now the cost benefit doesn't allow for the manufacturers to proceed with this.

Other products where you can modify the circulating survival of von Willebrand factor protein, I think are going to be in the near future. And I think that there are quality of life issues that need to be addressed. And I think the most important issue over here with von Willebrand disease, it is possible that the patients would not have needed to have hysterectomies.

(Question from the audience.) Well, I think that it is difficult to make the diagnosis of von Willebrand. Oh yes the question is, how do you make the diagnosis of von Willebrand disease in women who are on oral estrogen contraceptives, because their von Willebrand factor activities will already be elevated in response to the estrogen, is there a way that you can establish the diagnosis?

And the answer is, no. In many of these women who are on oral contraceptives, they will have their disease masked. But from a practical standpoint, that's all right. Because if their von Willebrand activities are in the normal range on oral contraceptives, estrogen-containing contraceptives, then you don't need to treat them.

They should have normal hemostasis. Now, how long do you need to keep them off of the birth control pill, the estrogen-containing birth control pill before their von Willebrand factor levels come back down to normal?

I would probably recommend at least one menstrual cycle. And this is a very common problem. It is even more common in pregnancy where the obstetrician says I've got a patient over here with von Willebrand disease, and I'm afraid to take her to delivery, because she has von Willebrand disease, help me.

And of course, pregnancy just like with its own estrogen surge similar to the estrogen-containing birth control, these women's von Willebrand activities and their factor VIII's I might add, always look at their factor VIII's too, will be normal, and these patients will not bleed.

However, within a very short time after delivery, their von Willebrand factor proteins and their factor VIII's will drop unless they are nursing. Again, if they are nursing, you maintain the estrogen surge, so that their von Willebrand activities and factor VIII's may remain in the normal range for a long period of time.

Then once they stop nursing, then you'll see it plunge back down to baseline. That's probably the best time to diagnose them after pregnancy. Any other questions.

(Question from audience.) A: Okay the question was, is the normal range of von Willebrand activities different in children than they are in adults? The answer is, no. The newborn, in the newborn perhaps, but not in the, but not in the adult.

Remember that the, that even in newborns there is not a whole lot of difference, mainly because you know babies are, it's pretty stressful to come through the canal, vaginal canal or to be born via C-section, and so, and they're usually crying after they're delivered.

All that stress causes a physiologic elevation of von Willebrand factor activity, probably a protective effect from the baby's perspective. Also a protective effect for the mother for delivery as well, because her von Willebrand activities are the highest that they'll ever be.

I might add too one interesting thing, and that is that Pitocin which is used in the induction of vaginal delivery has a large amount of vasopressin activity, so that you can actually increase the level of factor VIII and von Willebrand factor protein in women who are in labor being induced by Pitocin.

It has the same type of effect as vasopressin does, sort of an interesting side piece of information. Okay, well, thank you very much.