



**Instrumentation  
Laboratory**

A Werfen Company

# **von Willebrand Disease— An Under-diagnosed Bleeding Disorder**

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Program for HealthTrust Members

# Disclosures

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# Learning Objectives

- Learn the clinical significance and incidence of von Willebrand Disease (vWD)
- Discuss the history and discovery of vWD
- Describe the disease pathology and symptoms
- Explore the lab assays available to diagnose vWD
  - Define benefits and drawbacks to each method
  - Discuss when to test/when not to test

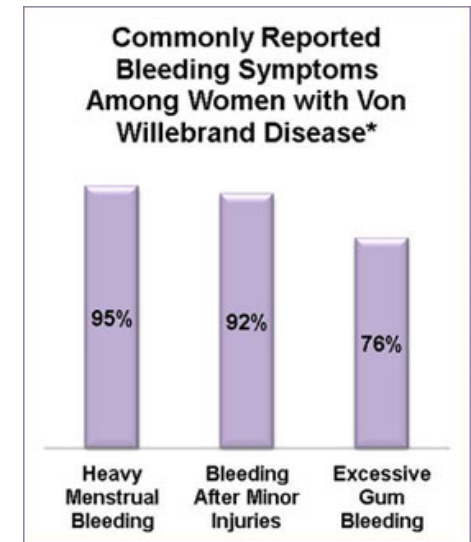
# vWD

- Is the most common hereditary bleeding disorder but may not be detected with routine coagulation screening assays such as the PT and aPTT
  - aPTT may be prolonged if the Factor VIII (FVIII) is decreased enough
  - The incidence is approximately 1-2% of the general population\*
- It is inherited autosomally and affects both males and females equally
- It may also be an acquired disease

\*Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. Blood. 1987 Feb;69(2):454-9.

# vWD in Women

- Females show more bleeding symptoms than men due to menses, pregnancy and the period post-childbirth
- In a CDC survey of 105 women with vWD:
  - Average of 16 years from onset of symptoms to diagnosis
  - Average of 6 bleeding episodes until diagnosis

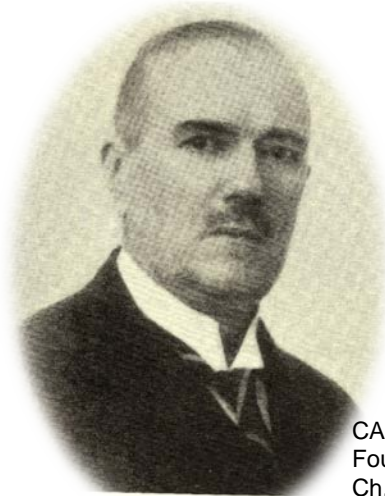


\*Based on a CDC study of 102 women with vWD.

A Kirtava et.al, Trends in clinical management of women with von Willebrand disease: a survey of 75 women enrolled in haemophilia treatment centres in the United States, Haemophilia March 2004; 158-161.

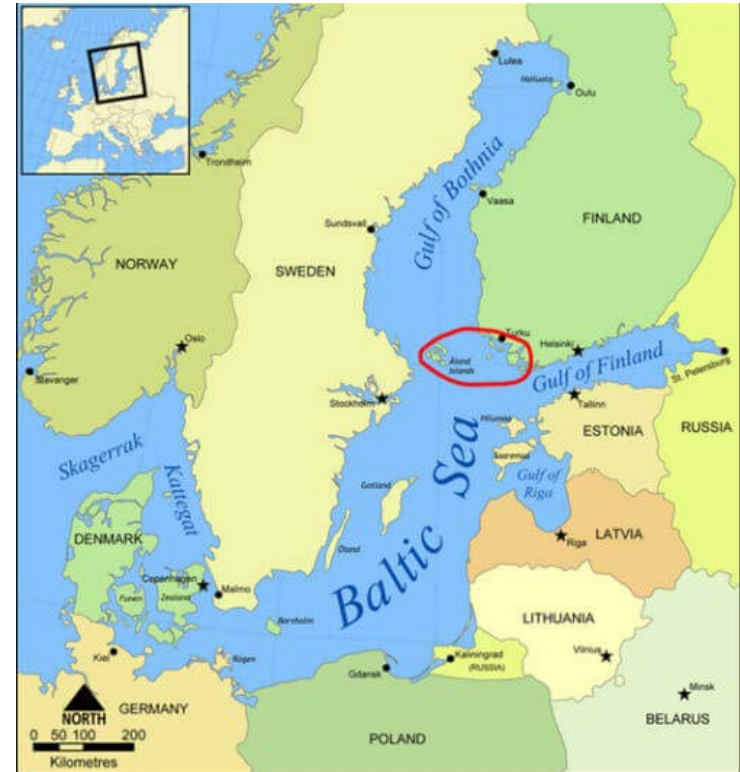
# Discovery of vWD

- Reported in 1924 by Dr. Erik von Willebrand
- Patient: 5-year-old girl from the Åland Islands, between Finland and Sweden



von Willebrand (1924)

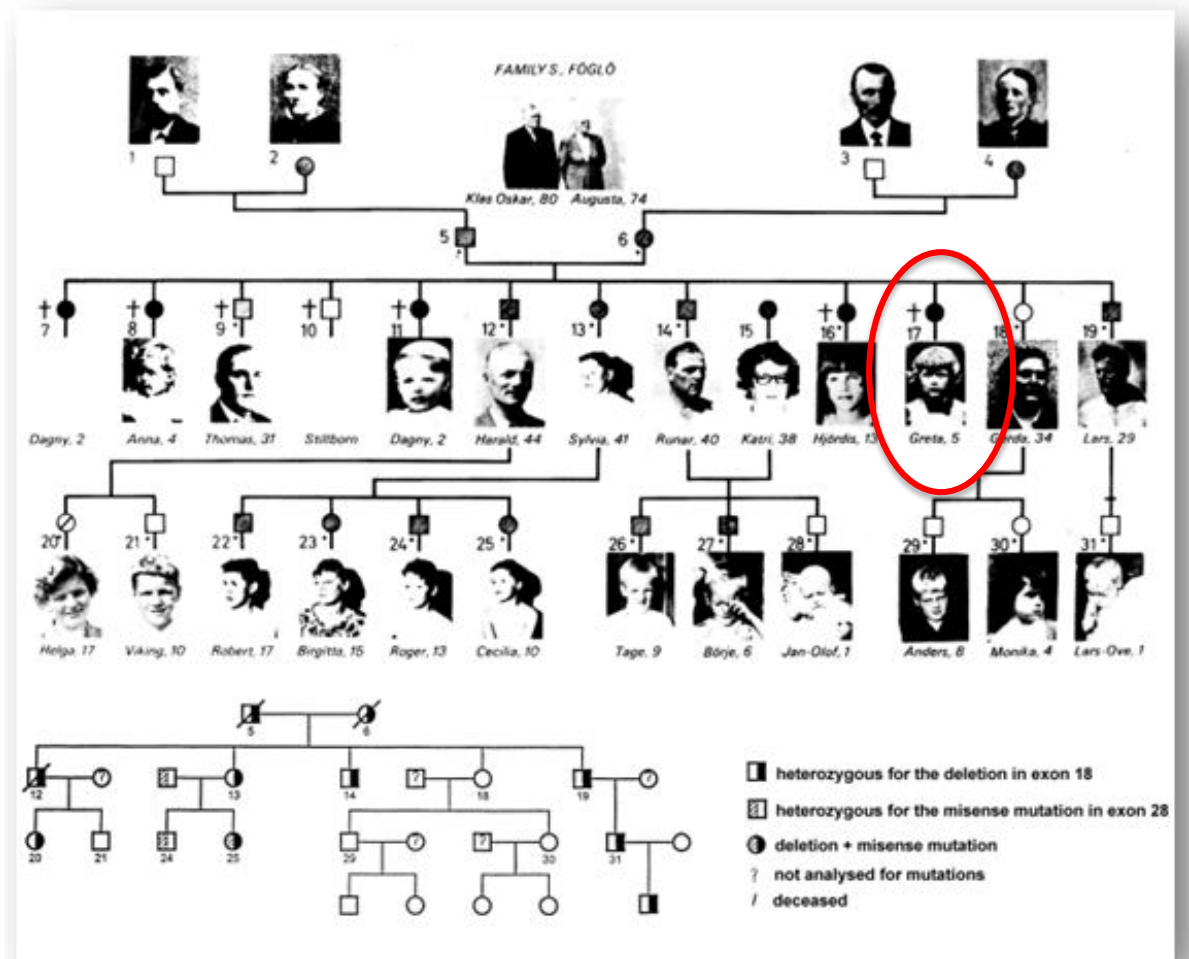
CA Owen Jr., *A History of Blood Coagulation*, Mayo Foundation for Medical Education and Research, 2001. Ch.13, pg 126



# Discovery of vWD

- Family of 66 had 23 bleeders
  - 16 female
  - 7 male

“Hereditary pseudo-hemophilia”



# Discovery of vWD – A Complication in Hemophilia Research

- 1953 – 3 independent laboratories found that patients with VWD lacked FVIII, but Hemophilia A shows sex-linked inheritance while vWD is autosomal
- 1972 – W. Owen and Wagner separated the globulin into 2 parts:
  - FVIII
  - vWF

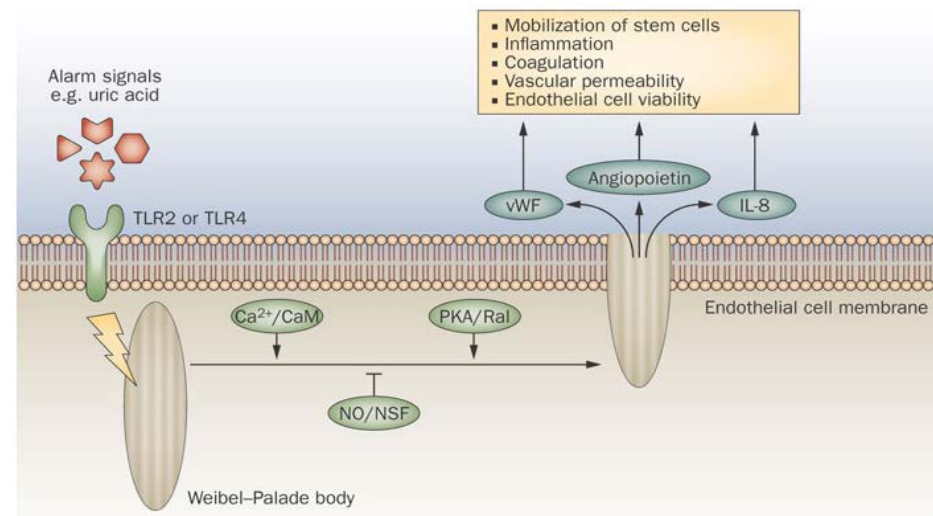


# Clinical Significance of vWD

- Bleeding usually presents as mucosal symptoms:
  - Menorrhagia
  - Epistaxis
  - Bruising
  - Bleeding with dental work
- Usually mild, but can be severe
  - Manifests as spontaneous bleeds and joint bleeds
- More severe with blood type O

# Pathophysiology of vWD

- vWD occurs when there is either a decrease in production of von Willebrand Factor (vWF) or if the vWF does not function properly
- It is produced in the endothelium cells and stored in the Weibel-Palade bodies
- It is also produced in megakaryocytes in bone marrow and stored in alpha granules of platelets



<http://www.nature.com/nrneph/journal/v5/n7/images/nrneph.2009.87-f3.jpg>

# vWD Classification

## Quantitative Subtypes

- Type I
  - Mild decrease in vWF
  - Most common type of vWD
- Type 3
  - Virtually complete absence of vWF

Nichols W et al., The Diagnosis, Evaluation and Management of von Willebrand Disease, NHLBI guideline, Dec 2007.

# vWD Classification

## Qualitative subtypes

- Type 2
  - vWF defect
  - Type 2A – Decrease in platelet adhesion; deficiency of HMW Multimers
  - Type 2B – Increase affinity for platelet GP1b
  - Type 2M – Decrease in platelet adhesion without decrease of HMW Multimers
  - Type 2N – Markedly decrease in binding affinity for Factor VIII

Nichols W et al., The Diagnosis, Evaluation and Management of von Willebrand Disease, NHLBI guideline, Dec 2007.

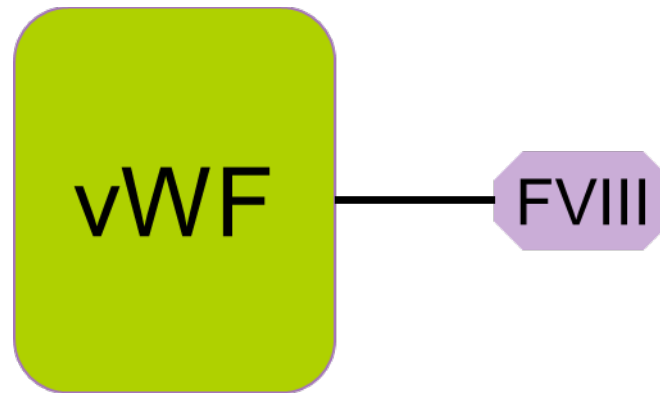
# vWF and Age

- vWF increases with age
- In elderly patients (>65 yrs), FVIII and vWF levels increased with age in Type 1 vWD, sometimes to a normal level, but symptoms did not diminish
- Elderly patients with Type 2 vWD did not show an increase in FVIII and VWF with time, but showed an increase in bleeding symptoms

Sanders YV, et.al., von Willebrand Disease and Aging: An Evolving Phenotype, Jour Thr Hemo, July 2014, 12:1006-1075

# Functions of vWF

- vWF binds to FVIII and prolongs its half-life in circulation



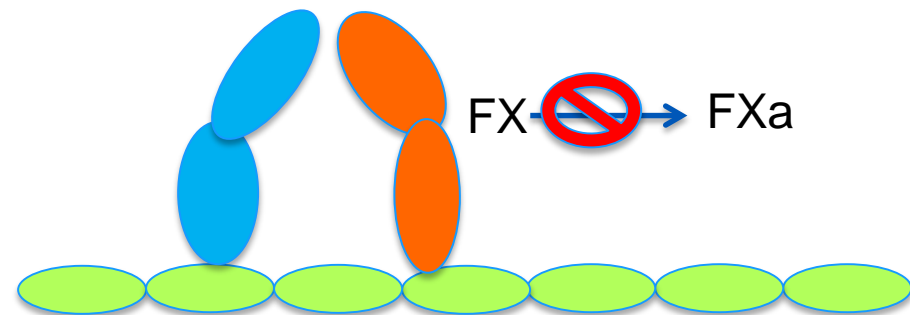
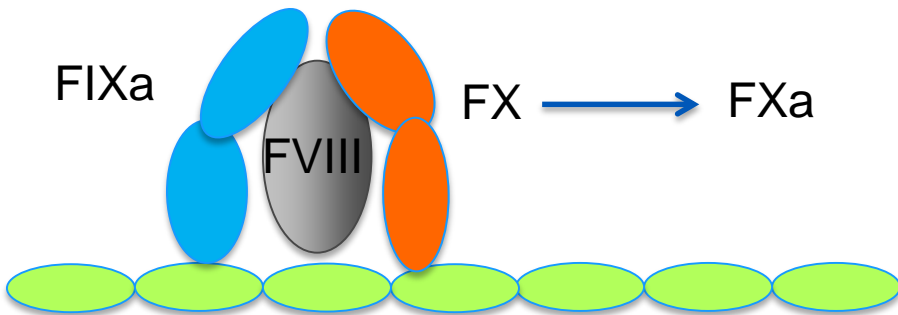
$\downarrow [\text{vWF}] = \downarrow [\text{FVIII}]$

- Half-life of FVIII with vWF = 12 hours
- Half-life of FVIII without vWF = 2 hours

Schwartz RA (2013, Sept 27). Factor VIII. Retrieved from <http://emedicine.medscape.com/article/20319-overview#10104>

# FVIII is a Cofactor in Fibrin Formation

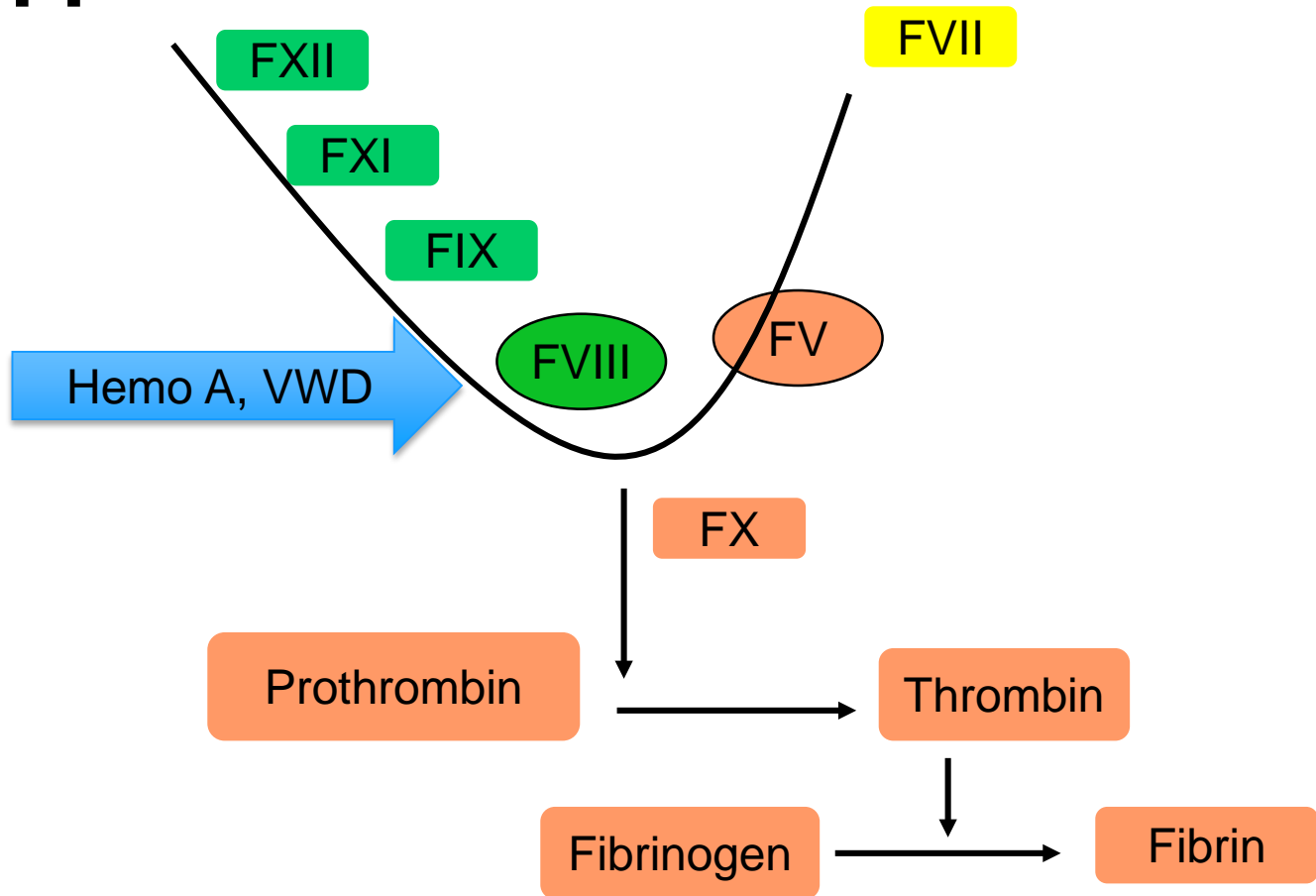
- Without FVIII, the tenase complex is not formed
- Fibrin formation is decreased



# Clotting Cascade

PTT

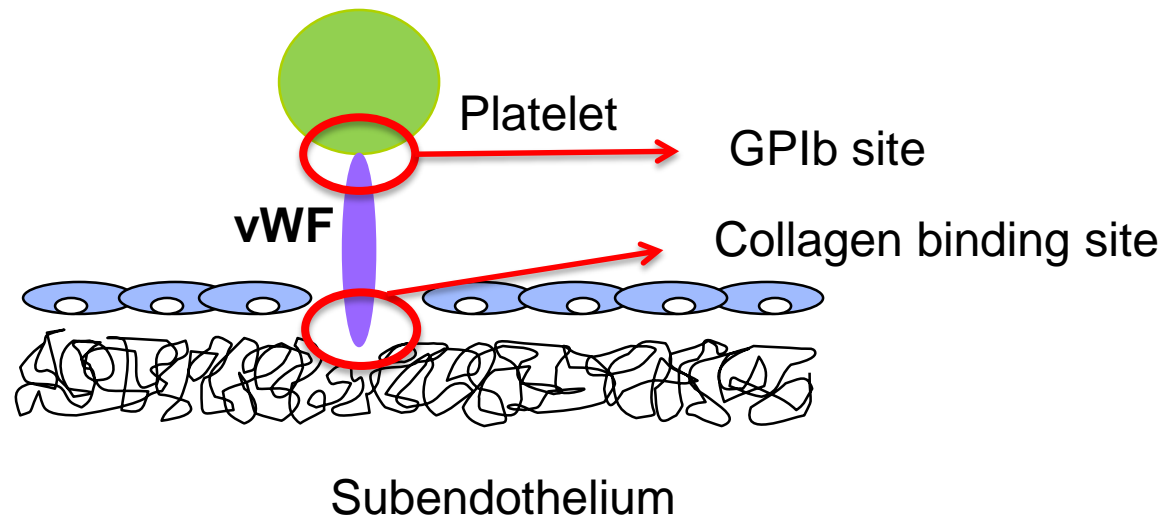
PT



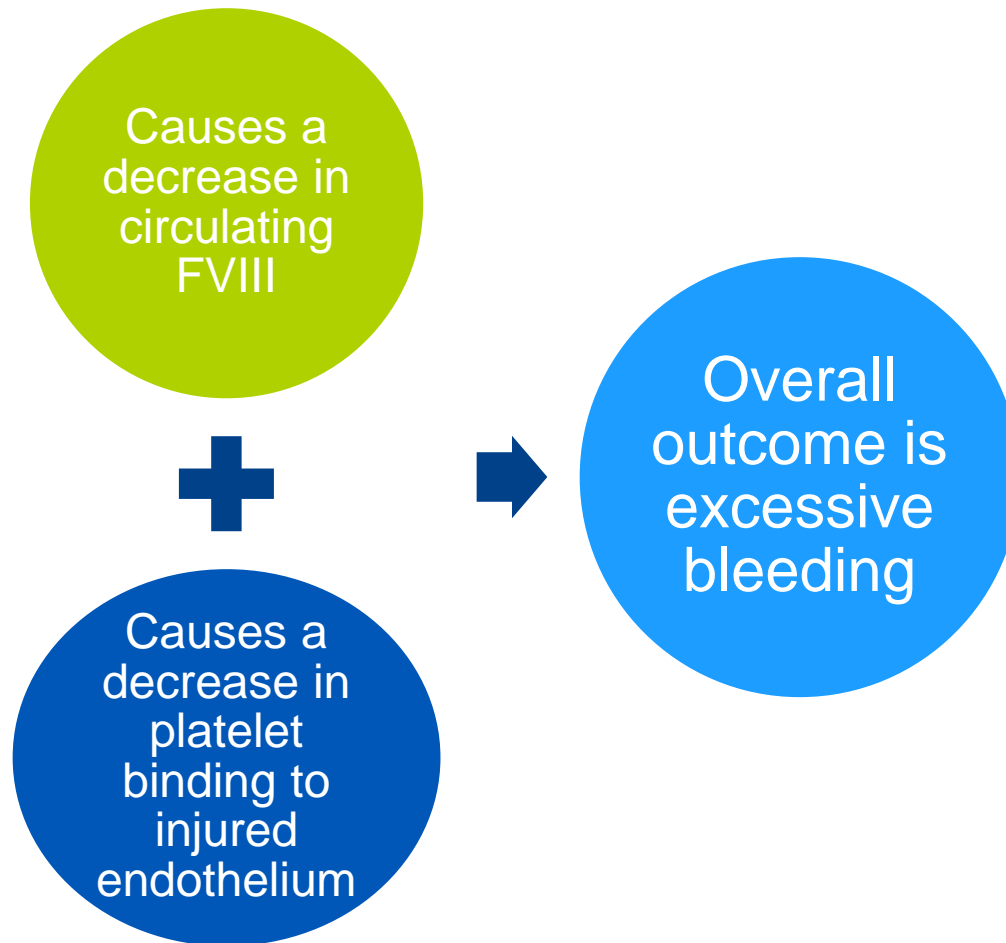


# vWF Binds to Platelets

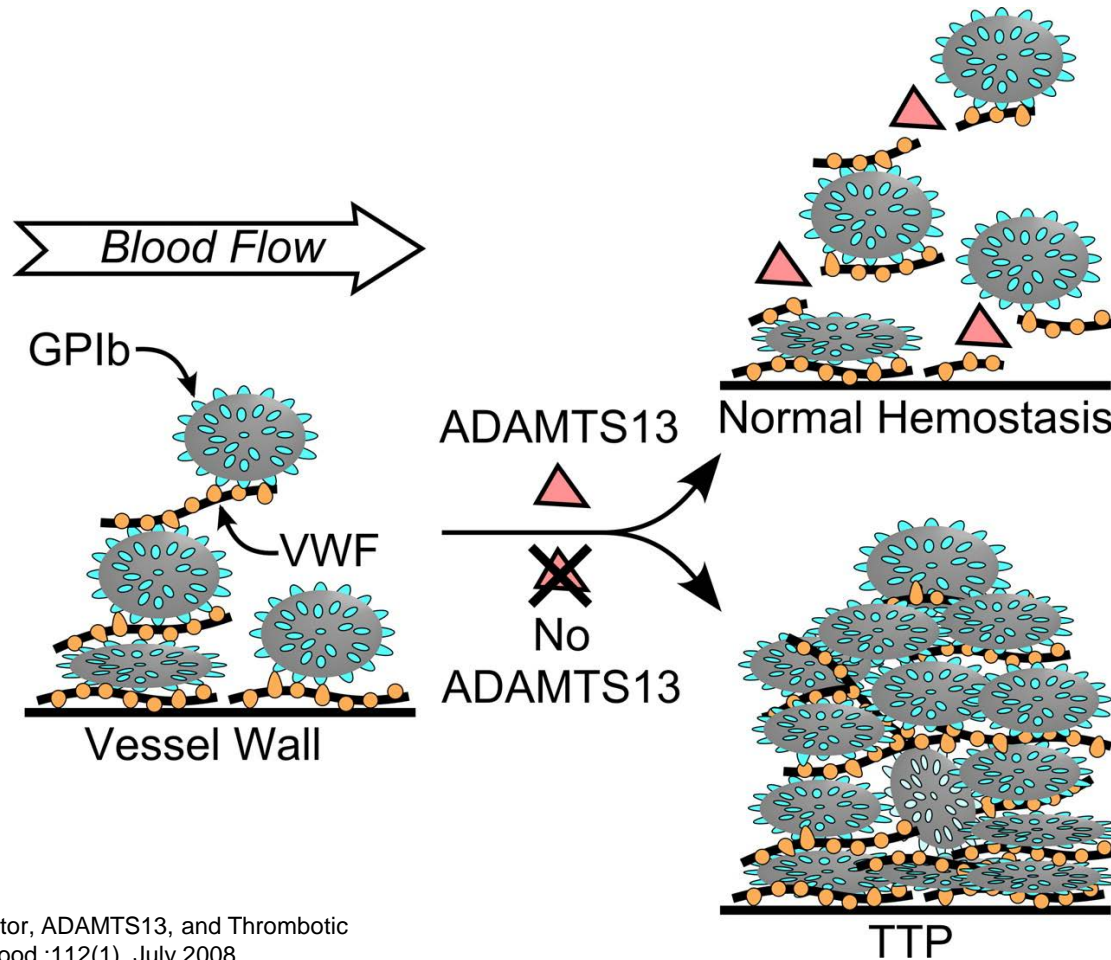
- vWF binds to GPIIb/IIIa on the platelet surface and tethers platelets to the endothelium at the site of injury



# Decreased vWF Activity



# vWF is Regulated by ADAMTS13



Sadler JE, Von Willebrand Factor, ADAMTS13, and Thrombotic Thrombocytopenia Purpura, *Blood* :112(1), July 2008.

# vWD: Clinical Diagnosis

# Diagnosing vWD: NHLBI Guidelines

- Mildly low vWF is common
  - Weak relationship between vWF levels and bleeding manifestations
  - Usually no associated gene abnormality
- Bleeding symptoms are common
  - Prevalence of at least one symptom in survey of healthy control group is at least 25%
- Low vWF and bleeding often associate coincidentally
  - 0.4% of population has at least one bleeding symptom, a positive family history and low vWF levels by chance alone
- Low vWF (between 30 – 50%) confers an increased risk for bleeding and *is not by itself diagnostic for vWD*

Nichols W et al., The Diagnosis, Evaluation and Management of von Willebrand Disease, National Heart, Lung and Blood Institute (NHLBI) guideline, Dec 2007.

# Guidelines for Diagnosis of vWD

- Bleeding is common in healthy persons
- Difficult to distinguish low vWF as the cause of bleeding
- First step:  
    Question the patient

Nichols W et al., The Diagnosis, Evaluation and Management of von Willebrand Disease, NHLBI guideline, Dec 2007.

## Questions to Patient

1. Have you or a blood relative ever needed medical attention for a bleeding problem or been told you have a bleeding disorder or problem:
  - During/after surgery
  - With dental procedures, extractions?
  - With trauma?
  - During childbirth or for heavy menses?
  - Ever had bruises with lumps?
2. Do you have or have you ever had:
  - Liver or kidney disease, a blood or bone marrow disorder; a high or low platelet count?
3. Do you take aspirin, NSAIDs (provide common names), clopidogrel (Plavix™), warfarin, heparin?

## History From Patient

Personal history of VWD

Abnormal laboratory test

Positive family history of a bleeding disorder or bleeding

Patient is concerned about bleeding; patient who has unexplained anemia or history of previous DDAVP use

No

Yes

No evaluation; usual care

Ask questions in Box 1 (p 21) and the 3 questions above (if not already asked), AND obtain history of treatment (e.g., blood transfusion) Examine for signs of bleeding or underlying disease

Negative

Positive

No further evaluation; usual care

Evaluate further: initial laboratory tests and possible referral (figure 4, p 25)

# Obtain Clinical Bleeding History

## Box 1. Suggested Questions for Screening Persons for a Bleeding Disorder

1. Do you have a blood relative who has a bleeding disorder, such as von Willebrand disease or hemophilia?
2. Have you ever had prolonged bleeding from trivial wounds, lasting more than 15 minutes or recurring spontaneously during the 7 days after the wound?
3. Have you ever had heavy, prolonged, or recurrent bleeding after surgical procedures, such as tonsillectomy?
4. Have you ever had bruising, with minimal or no apparent trauma, especially if you could feel a lump under the bruise?
5. Have you ever had a spontaneous nosebleed that required more than 10 minutes to stop or needed medical attention?
6. Have you ever had heavy, prolonged, or recurrent bleeding after dental extractions that required medical attention?
7. Have you ever had blood in your stool, unexplained by a specific anatomic lesion (such as an ulcer in the stomach, or a polyp in the colon), that required medical attention?
8. Have you ever had anemia requiring treatment or received blood transfusion?
9. For women, have you ever had heavy menses, characterized by the presence of clots greater than an inch in diameter and/or changing a pad or tampon more than hourly, or resulting in anemia or low iron level?

Nichols W et al., The Diagnosis, Evaluation and Management of von Willebrand Disease, NHLBI guideline, Dec 2007.



# Bleeding in Healthy Persons and Patients With vWD Overlap Considerably

**Table 7. Common Bleeding Symptoms of Healthy Individuals and Patients Who Have VWD**

Symptoms	Normals (n = 500; <sup>137</sup> n = 341; <sup>+138</sup> n = 88; <sup>++139</sup> n = 60 <sup>++140</sup> ) %	All types VWD (n = 264; <sup>137</sup> n = 1,885 <sup>141</sup> ) %	Type 1 VWD (n = 42; <sup>+142</sup> n = 671 <sup>136</sup> ) %	Type 2 VWD (n = 497 <sup>136</sup> ) %	Type 3 VWD (n = 66; <sup>136</sup> n = 385 <sup>85</sup> ) %
Epistaxis	4.6–22.7	38.1–62.5	53–61	63	66–77
Menorrhagia*	23–68.4	47–60	32	32	56–69
Bleeding after dental extraction	4.8–41.9	28.6–51.5	17–31	39	53–70
Ecchymoses	11.8–50	49.2–50.4	50	N.R.	N.R.
Bleeding from minor cuts or abrasions	0.2–33.3	36	36	40	50
Gingival bleeding	7.4–47.1	26.1–34.8	29–31	35	56
Postoperative bleeding	1.4–28.2	19.5–28	20–47	23	41
Hemarthrosis	0–14.9	6.3–8.3	2–3	4	37–45
Gastrointestinal bleeding	0.6–27.7	14	5	8	20

Nichols W et al., The Diagnosis, Evaluation and Management of von Willebrand Disease, NHLBI guideline, Dec 2007.

# vWD: Laboratory Diagnosis

# vWF Laboratory Testing Caveats

- vWF is an acute phase reactant, increased in times of stress
  - Even the stress of phlebotomy may increase the vWF level
  - Pregnancy or estrogen use
  - Inflammation
  - Exercise
  - Liver disease
  - Vasculitis
  - Thrombotic Thrombocytopenia Purpura/Hemolytic Uremic Syndrome
- Patients with a strong clinical suspicion of vWD may need to have testing repeated multiple times if the initial results are negative.

# Pre-analytical Considerations

- vWF is a labile factor
  - Sample should be centrifuged and tested or the plasma removed and frozen within 4 hrs of collection
  - Whole blood should not be iced or stored refrigerated as this has been shown to cause a loss of high MW multimers in some individuals
- Mixing is critical
  - Frozen plasma should be thawed in a 37°C water bath until completely liquid
  - The sample should be well mixed either by inversion or on a rocker to ensure complete dissolution

vWF is part of the cryoprecipitate fraction so must be redissolved before testing

CLSI Guideline H21-A5, Collection, Transport, and Processing of Blood Specimens  
For Testing of Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, 2008.

# Laboratory Testing for vWD

- Initial screen – rule out other causes for bleeding:
  - PT, aPTT, CBC with platelet count, fibrinogen (or thrombin time)
- If platelet or other factor abnormality is ruled out:
  - FVIII activity
  - vWF activity
  - vWF antigen

# Laboratory Testing for vWD

- Second line testing:
  - vWF multimer analysis
  - Collagen binding activity
  - vWF 2N binding assay
  - Ristocetin induced platelet aggregation (RIPA)

All are lab defined protocols – no FDA cleared assays are available commercially

# vWD Laboratory Diagnosis

- FVIII activity
  - Clot-based activity assay (typically)
  - Usually correlates with vWF Antigen
  - Abnormal FVIII and normal vWF Ag in:
    - Hemophilia A
    - Type 2N vWD

# FVIII Activity Assay



+



aPTT in seconds

Dilution of patient  
plasma

Equal volume FVIII  
deficient plasma



Clotting time in seconds =



Factor activity

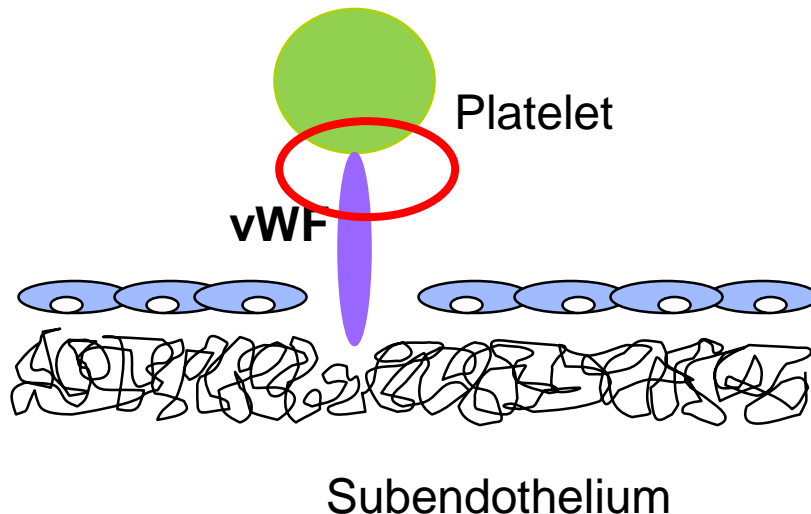


# von Willebrand Factor Assays

- vWF Activity
  - Ristocetin Cofactor activity (not to be confused with RIPA)
  - Collagen binding activity
  - Activity assay
- vWF Antigen
  - Measures quantity of protein using antibody detection methodology
  - Latex immunoassay
  - ELISA method

# von Willebrand Factor activity: vWF:RCO

- Ristocetin Cofactor activity



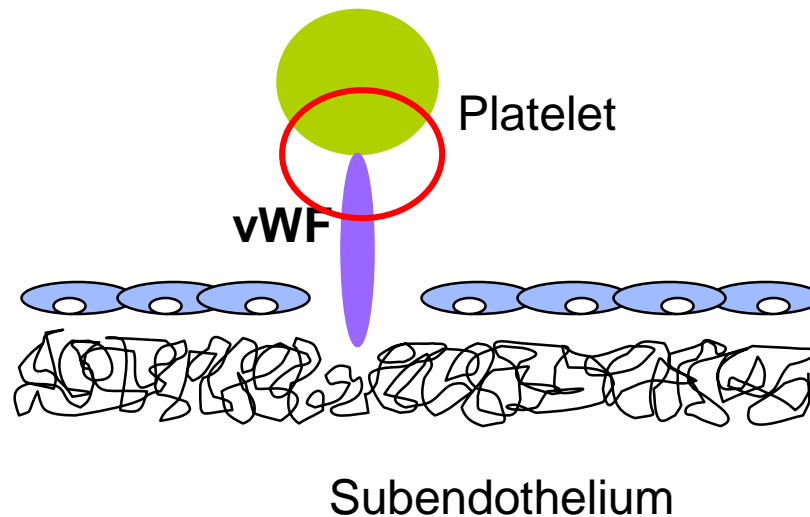
- Measures the ability of patient plasma to agglutinate lyophilized and reconstituted platelets.
- Can be automated
- Can be performed on a platelet aggregometer manually

# Ristocetin Cofactor Assay

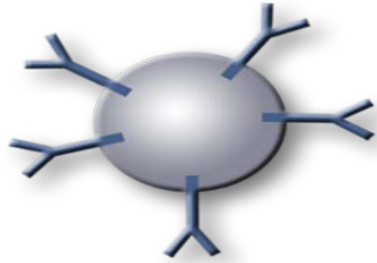
- Ristocetin – used first as an antibiotic, was withdrawn from the market as it agglutinates platelets
- Ristocetin is added to fixed platelets in the presence of the patient plasma. Binding of vWF to platelets results in agglutination
- The assay is performed on a platelet aggregometer or can be automated
- The Ristocetin Cofactor assays that are currently cleared by the Food and Drug Administration (FDA) are cumbersome, time consuming and give poor precision.
  - CAP survey CGS3-A2014 Sample 01 (mean 11.8) = 31.2% CV  
Sample 02 (mean 205) = 23.5% CV

# vWF Activity Assay – LIA Assay

- Detects the vWF binding site where it binds to platelets – GPIb



# vWF Activity Assay – LIA Assay

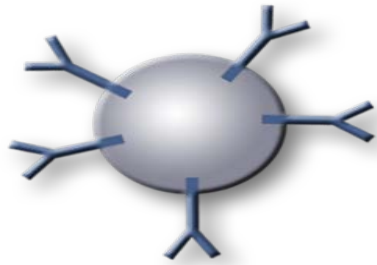


Latex Particle



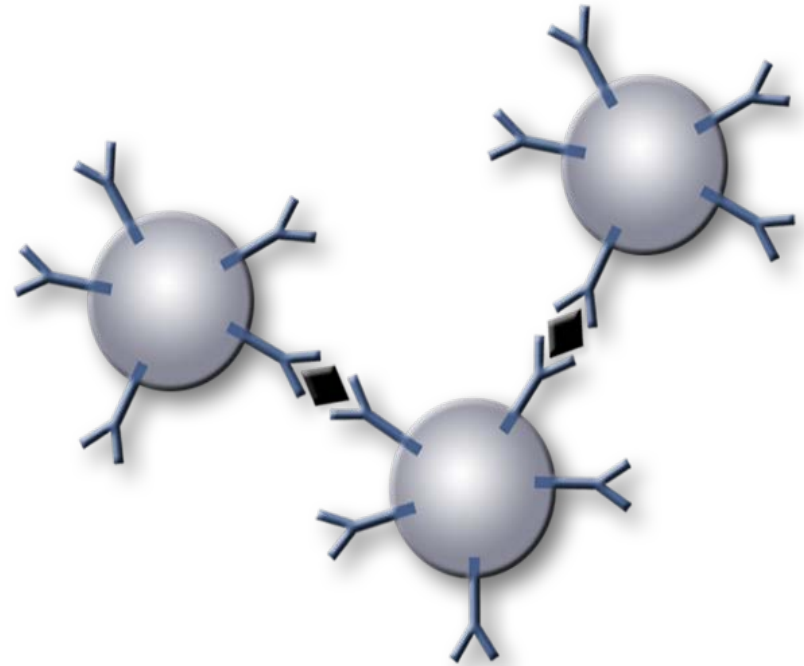
vWF Molecule

# vWF Activity – LIA Assay



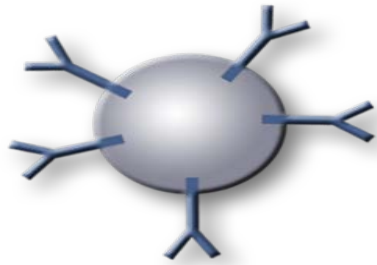
Latex Particle

vWF Molecule

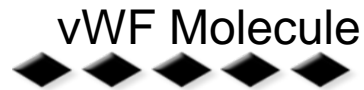


Specific anti-vWF  
monoclonal antibody  
directed against the  
platelet binding site of  
vWF

# vWF Activity – LIA Assay

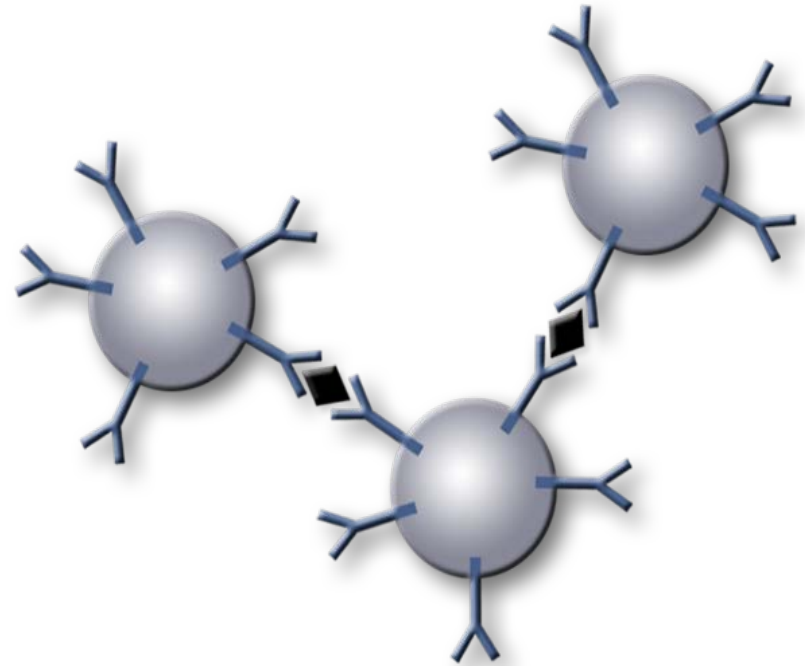


Latex Particle



Agglutination of the latex occurs in proportion to the vWF's GPIIb binding site

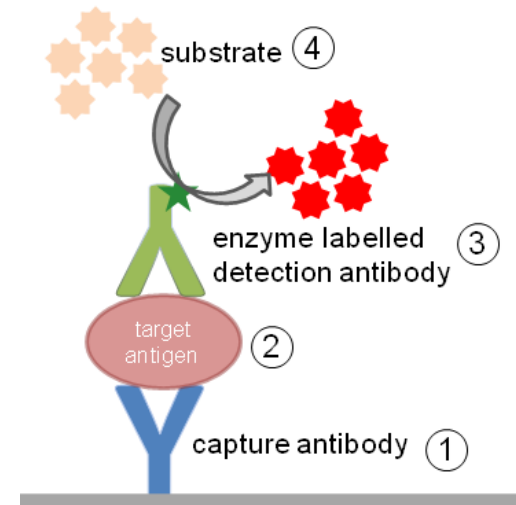
Automated and precise (3.4 – 8.0% CV on TOP)



Specific anti-vWF monoclonal antibody directed against the platelet binding site of vWF

# vWF Antigen: vWF:Ag

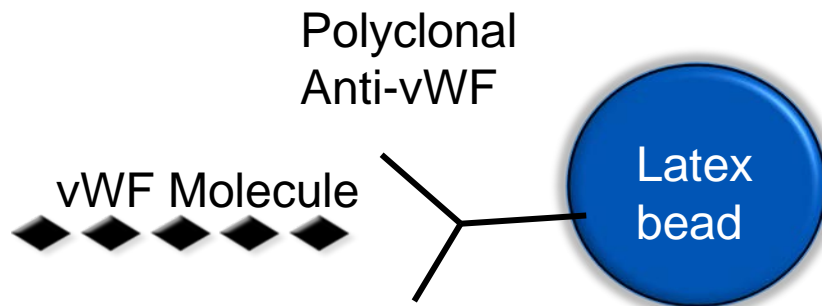
- vWF Antigen quantitative assays
  - test for the presence of the protein
- ELISA
- Latex immunoassay





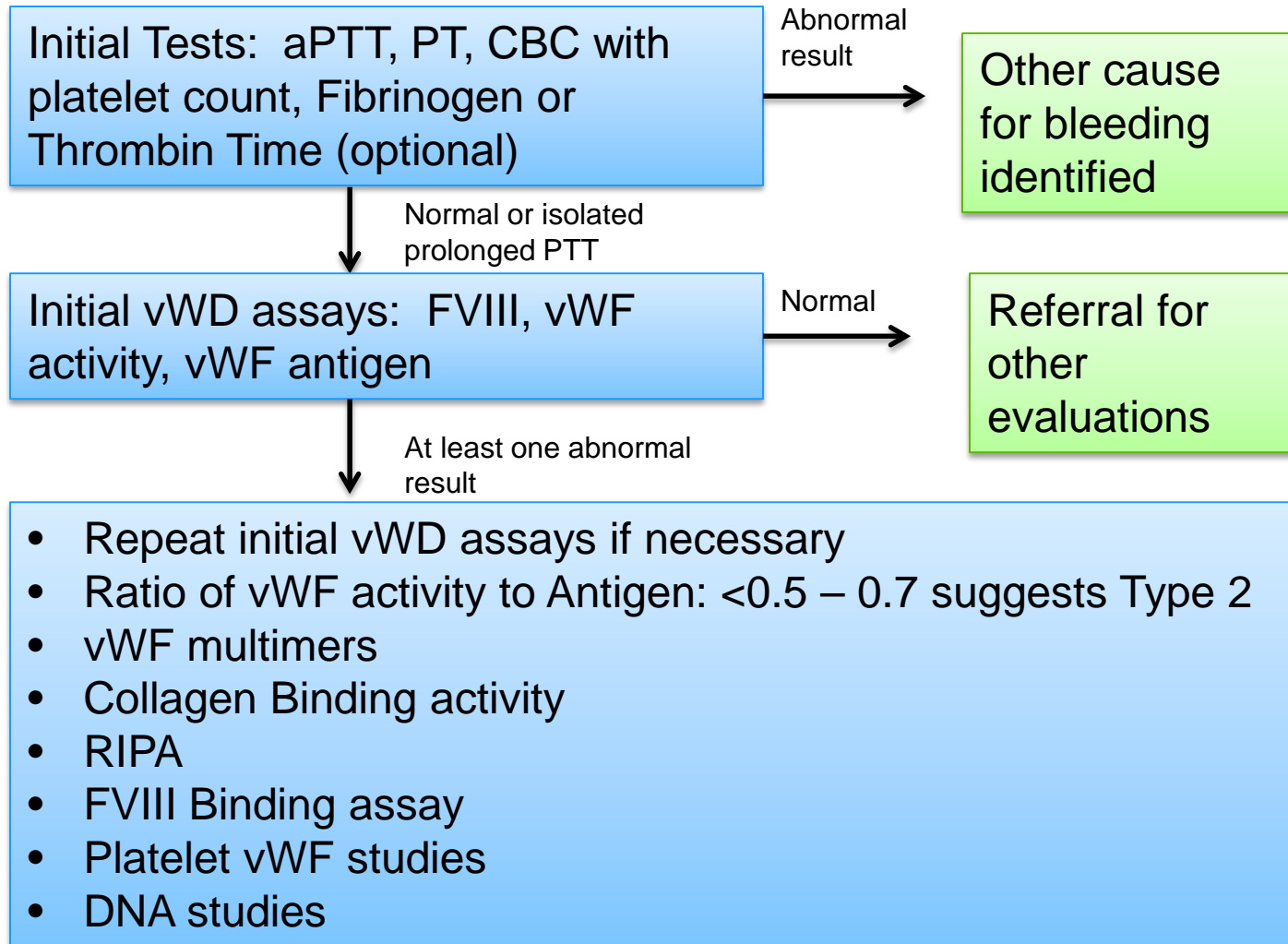
# vWF Antigen: vWF:Ag

- Latex immunoassay



Rate of aggregation is proportional to the vWF concentration in the sample

# vWD Panel: Test Algorithm With Positive History



# vWF Reference Interval

- *Remember: vWF is an acute phase reactant so may increase with stress*
- Few labs define by blood group, with Type O lower than A,B,AB
- Most labs use one reference range
- Generally 40 – 170%

# vWD NHLBI Guidelines

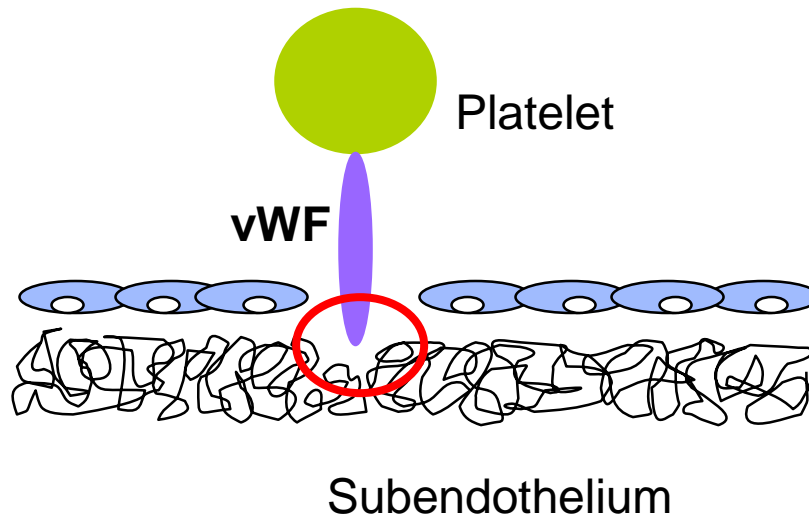
Assay	vWD	Possible vWD
vWF:RCo	< 30 IU/dL**	30 – 50 IU/dL
vWF:Ag	<30 IU/dL or normal	30 – 50 IU/dL
Factor VIII	↓ or normal	↓ or normal

\*\*30 IU/dl recommended for definitive “cut-off” for vWD  
Does not preclude vWD @ 30–50 IU/dL vWF if supporting clinical history is present, nor therapy to elevate vWF if there is a clinical bleeding risk

Nichols W et al., The Diagnosis, Evaluation and Management of von Willebrand Disease, NHLBI guideline, Dec 2007.

# Collagen Binding Activity: vWF:CB

- Measures the ability of vWF to bind to collagen



ELISA-based assays available (None are FDA cleared)

Chemiluminescent assays are in development

# vWF Multimer Analysis

## vWF Multimers

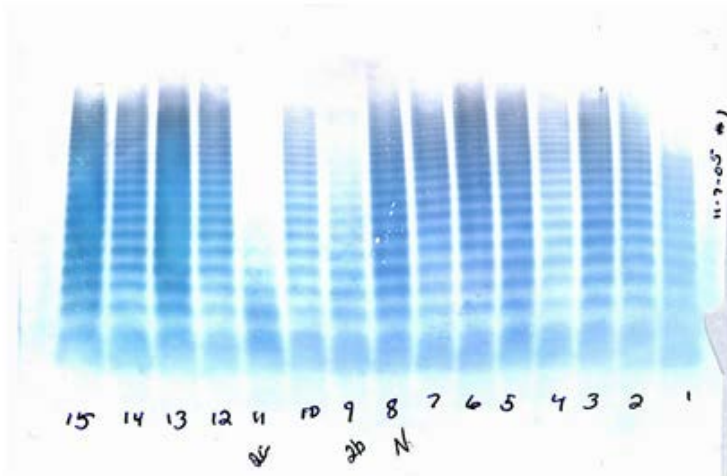


Photo used with permission of Dr. D Adcock

- Plasma digestion by sodium dodecyl sulfate
- Electrophoresis in agarose gel
- Western blotting to nitrocellulose
- Tagging with antibodies to vWF, labeled with horseradish peroxidase (HRP)
- Development with HRP substrate

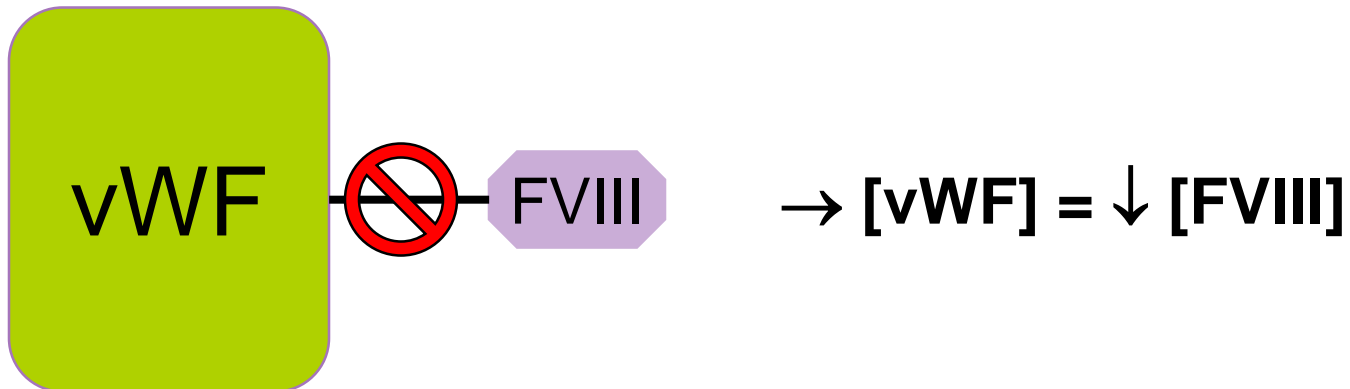
# vWF 2N Binding Assay

- Measures the ability of a patient's vWF to bind to FVIII in a microtiter plate
- Lab defined assay – not FDA cleared
- Performed in a few select laboratories

[http://www.practical-haemostasis.com/Factor%20Assays/vWF/factor\\_viii\\_binding\\_assay.html](http://www.practical-haemostasis.com/Factor%20Assays/vWF/factor_viii_binding_assay.html)

## vWD 2N

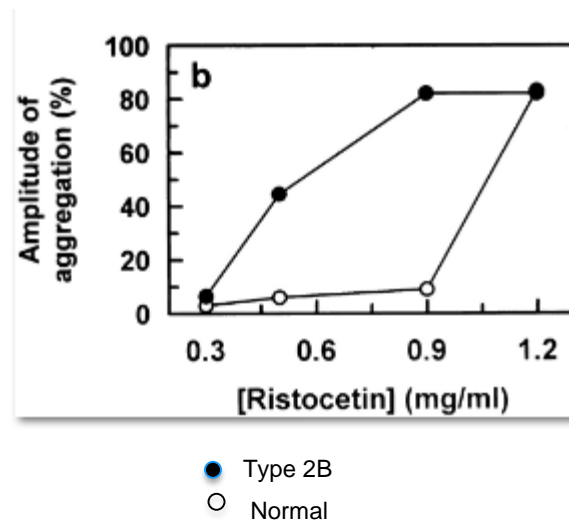
- Normandy variant “autosomal hemophilia”
- Abnormal vWF protein that cannot bind and stabilize FVIII
  - Diagnose using vWF 2N Binding Assay
- Low FVIII activity with normal vWF levels and function





# Ristocetin Induced Platelet Aggregation (RIPA)

- Measures the aggregation response of the patient's platelets to ristocetin
- Type 2B vWD patients will show an exaggerated aggregation response to low dose ristocetin (0.5mg/mL)



Hoylaerts MF, et al., Recurrent Arterial Thrombosis Linked to Autoimmune Antibodies Enhancing von Willebrand Factor Binding to Platelets and Inducing FcγRII Receptor-Mediated Platelet Activation., Blood Apr 1998,91(8)2810-2817;

# Acquired vWD

- New onset bleeding, no family history
- Associated with clonal hematoproliferative disorders, autoimmune disease, malignancies, cardiac defects
  - Antibody, adsorption, loss of high molecular weight multimers (HMWM)
- Similar clinically to hereditary vWD
- vWF Antigen, vWF Activity, FVIII Activity
  - Type 1 or Type 2 pattern
  - Limited laboratory studies to aid in distinction between hereditary and acquired vWD
    - Mixing studies positive for inhibitor ~ 15%

Tiede A, Diagnosis and Treatment of Acquired von Willebrand Syndrome, Throm Res. 2012 Dec;130 Suppl 2:S2-6

# Case #1

- 56-year-old woman referred due to recent onset of easy bruising and nosebleeds, weight gain, fatigue, forgetfulness
- No medications
- TSH = 7.4 mIU/mL ( 0.5 – 4.0 reference range)
- PTT = 44 seconds (26 – 36 sec reference range)
- PT, fibrinogen, CBC parameters including platelet count all normal

# Case #1

- FVIII = 15%
- vWF activity = 20%
- vWF antigen = 18%

# Case #1

- FVIII = 15%
- vWF activity = 20%
- vWF antigen = 18%
  
- **Diagnosis – acquired vonWillebrand Disease**  
secondary to hypothyroidism (Hashimotos thyroiditis)
- Treated with Synthroid®
- After 2 months, bleeding symptoms resolved

## Case #2

- 28-year-old woman with menorrhagia since initial menses
- Adopted, no family history available
- Normal physical exam
- No medications
  
- Lab results:
- PT, PTT, fibrinogen all normal
- CBC – decreased hematocrit and hemoglobin

## Case #2

- FVIII = 30%
  - FIX = 90%
  - FXI = 105%
  - vWF R:Co activity = 34%
  - vWF antigen = 60%
  - Activity/Antigen ratio = 0.6
- 
- Multimers show a normal pattern with slightly decreased intensity

## Case #2

- FVIII = 30%
  - FIX = 90%
  - FXI = 105%
  - vWF R:Co activity = 34%
  - vWF antigen = 60%
  - Activity/Antigen ratio = 0.6
- 
- Multimers show a normal pattern with slightly decreased intensity

## Diagnosis – Type 2M vWD



# vWD Type 2M

- Type 2M – stands for “multimer”
- Rare disorder
- vWF antigen is normal
- vWF R:Co is decreased
- Multimer analysis shows a normal pattern with all multimers present

# vWD: Treatment

# Treatment of vWD

- DDAVP = desmopressin (Stimate<sup>®</sup> 1.5 mg/mL nasal spray, or IV for serious bleeds)
  - Causes release of vWF from endothelium
  - Most useful in Type 1 (quantitative defect)
  - May be useful in Type 2A and 2M
    - Cautious about administering to Type 2B
  - Can increase vWF approx. 2-5 fold
  - Increase tapers off with additional dosing due to depletion
  - DDAVP Challenge: measure FVIII and vWF activity pre-dose, 1 hr post dose, and 2-4 hrs post-dose to determine functional longevity

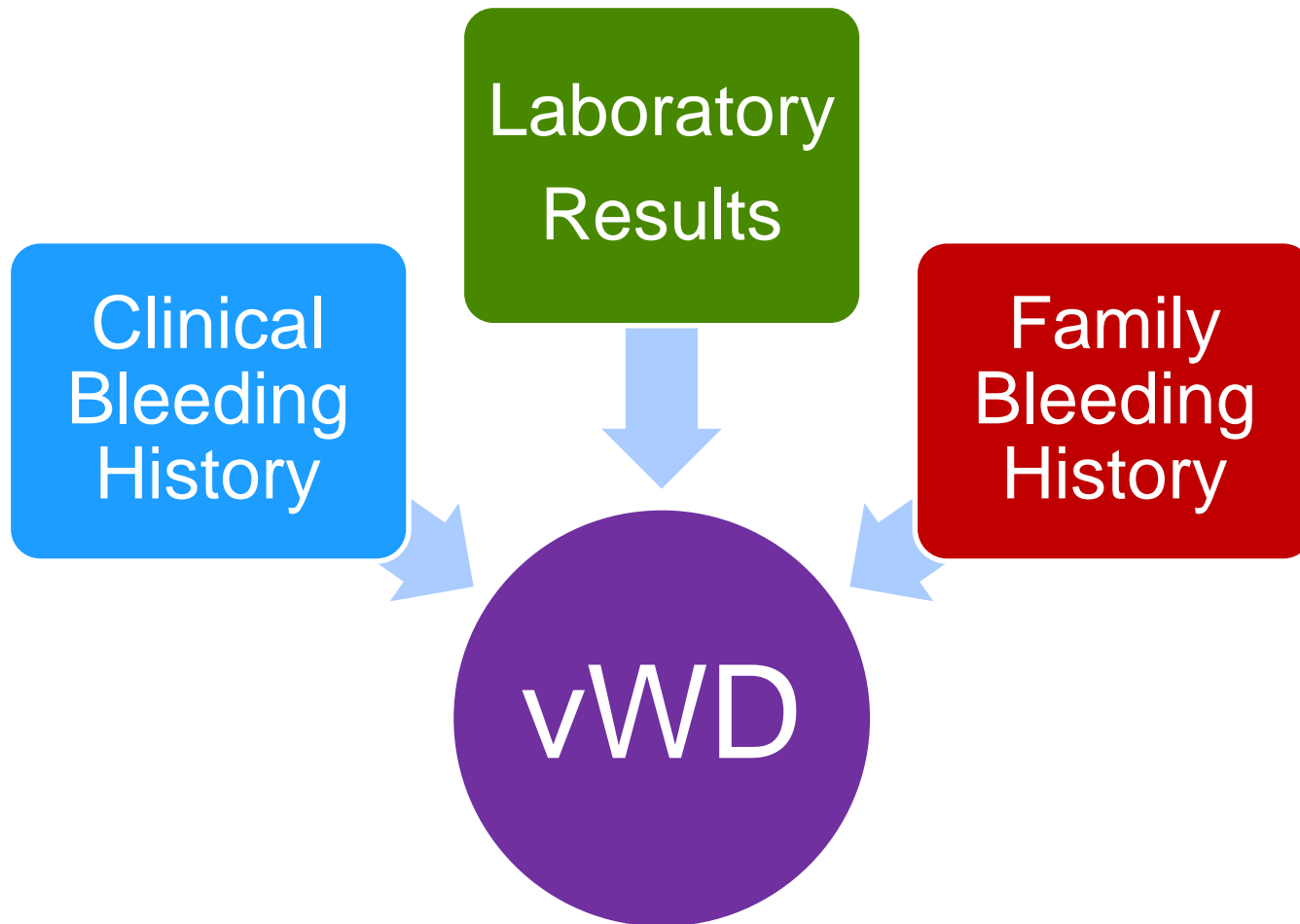
Mannuci PM, Desmopressin (DDAVP) in the Treatment of Bleeding Disorders, Blood, Vol. 90, No. 7, 1997. Revised 2012..

# Treatment of vWD

- Replacement products
  - Humate P<sup>®</sup>
  - Alphanate SD/HT<sup>®</sup>
  - Wilate<sup>®</sup> – 1:1 ratio of FVIII/vWF
  - Cryoprecipitate – rarely used except in life and limb threatening bleeds with no other product available

<http://www.nhlbi.nih.gov/health-pro/guidelines/current/von-willebrand-guidelines/full-report/4-management-of-vwd.htm>

# Conclusion



# Conclusion

- If patients with vWD are diagnosed correctly and treated appropriately, fewer bleeding episodes will lead to an improved quality of life
- Routine coagulation assays (PT, aPTT, fibrinogen) will not always detect vWD
- Testing for aPTT, FVIII, vWF activity and vWF antigen should be performed in patients with a strong clinical suspicion of the disease
- Testing may need to be repeated several times in order to exclude vWD