TTP and ADAMTS-13

Meredith Reyes, MD

October 17, 2005

Overview

- Thrombotic Microangiopathy
- TTP
  - Pathogenesis
  - Treatment
- HUS
- Laboratory assays of ADAMTS-13 activity

Thrombotic Microangiopathy

Thrombotic Microangiopathies (TMA)

- Thromboses in terminal arterioles and capillaries
- Organ ischemia
- Thrombocytopenia
- Erythrocyte fragmentation

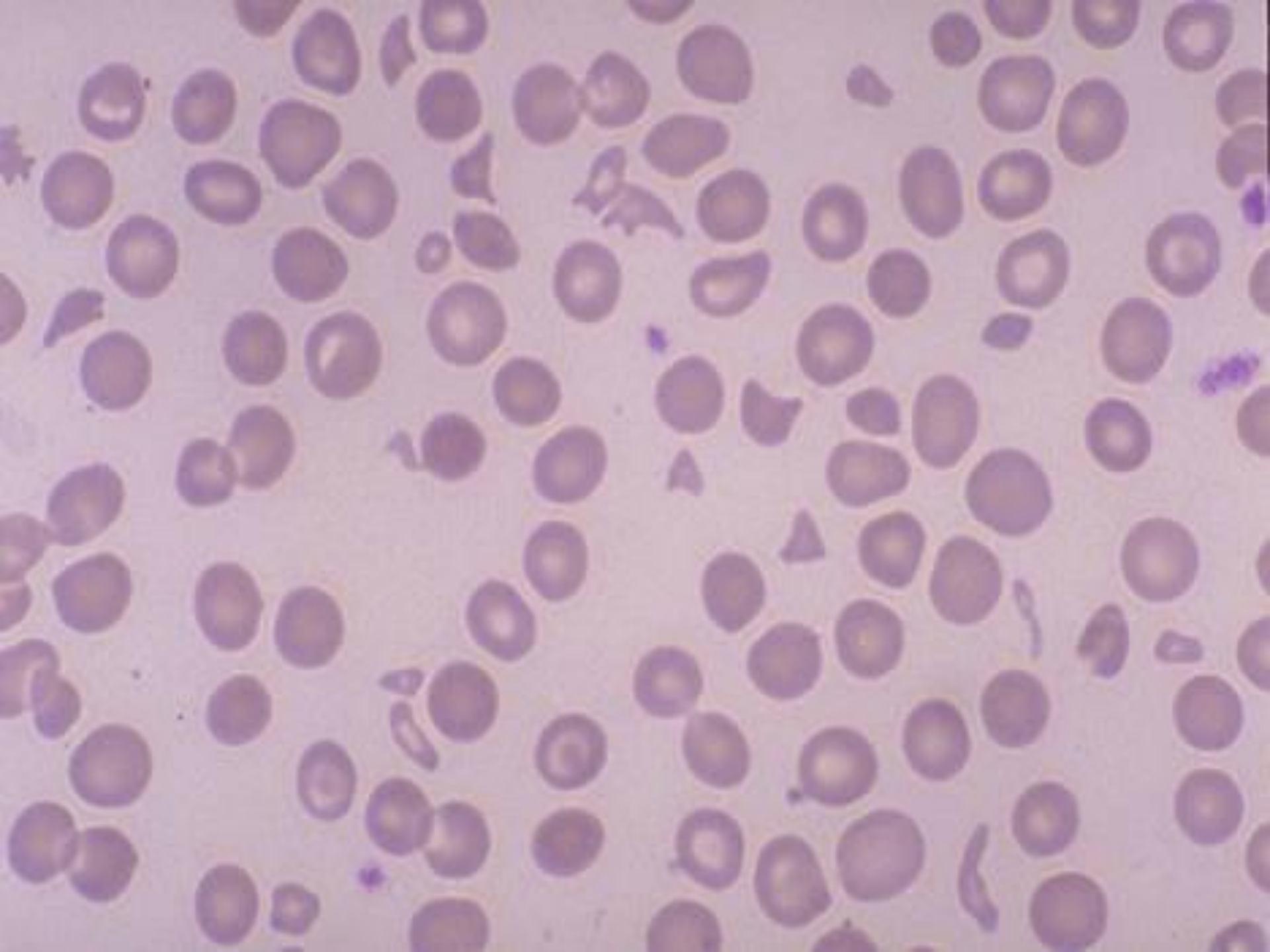
TMA Causes

- Medications
- Malignancies
- HIV
- Autoimmune Disorders
- Bone marrow transplantation
- Pregnancy
- Acquired / Idiopathic
  - Idiopathic TTP
  - Shiga-toxin producing *E. Coli*
- Familial

Thrombotic Thrombocytopenic Purpura

Clinical Features

- Fever
- Hemolytic Anemia with Schistocytes
  - At least 3/100 cells
  - Serum LDH increased
  - Serum haptoglobin decreased
- Thrombocytopenia (usually <10K)
  - Bone marrow with increased megakaryocytes
- Renal Dysfunction
- Neurological Deficits



von Willebrand Factor

- Central to TTP pathogenesis
- Multimers constructed w/in megakaryocytes and endothelial cells
- Stored in platelet α-granules & endothelial cell Weibel-Palade bodies
- Ultra-large multimers released & processed in plasma
  - 500-20,000 kd
  - Secretion stimulated by histamine, Shiga toxin, TNF-α, IL-8, IL-6

ULVWF Multimers

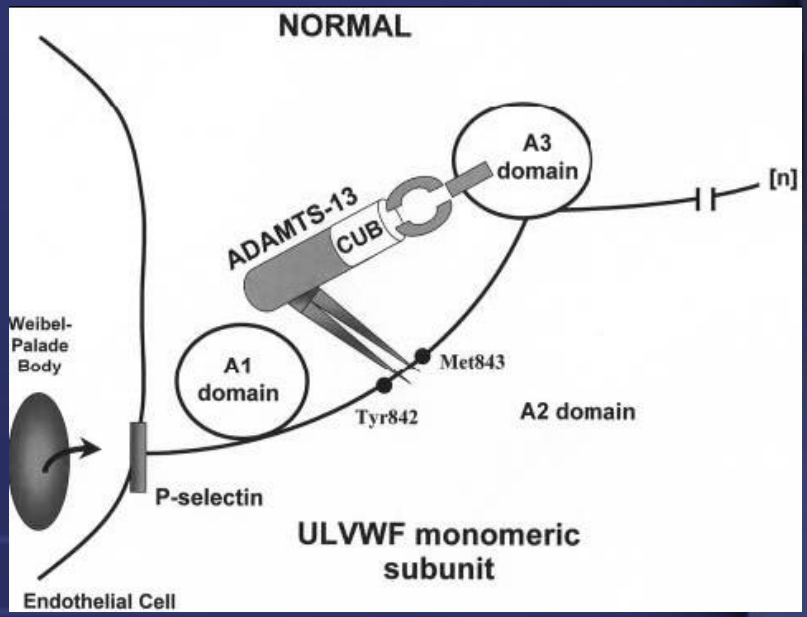
- Bind efficiently to platelet receptors
- More thrombi formation vs cleaved VWF
  - More binding sites
  - Closer proximity
- Thrombi embolize → organ ischemia
- Process controlled by ADAMTS-13

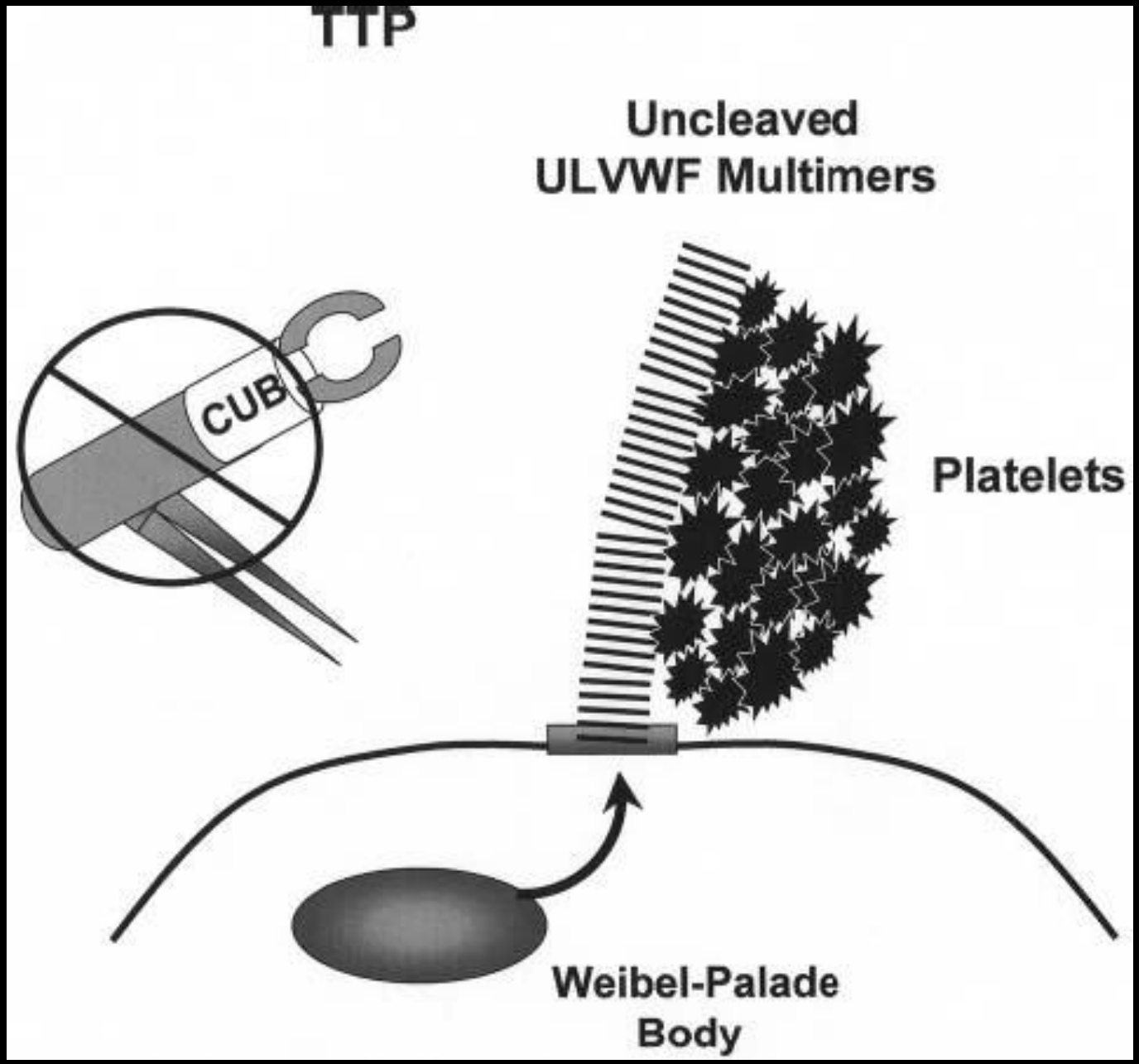
What is ADAMTS-13?

- "**A D**isintegrin **A**nd **M**etalloprotease with **T**hrombo**S**pondin domains" protease family
- Zn & Ca required for activity
- Synthesized in liver perisinusoidal cells
- Activity reduced in liver disease, malignancies, metabolic & inflammatory conditions, pregnancy, newborns

How Does ADAMTS-13 Work?

- Shear forces unfold ULVWF multimers
- ADAMTS-13 action
  - Binds A3 domain
  - Cleaves ULVWF
  - 140 kd & 176 kd fragments
- Multiple cleavages



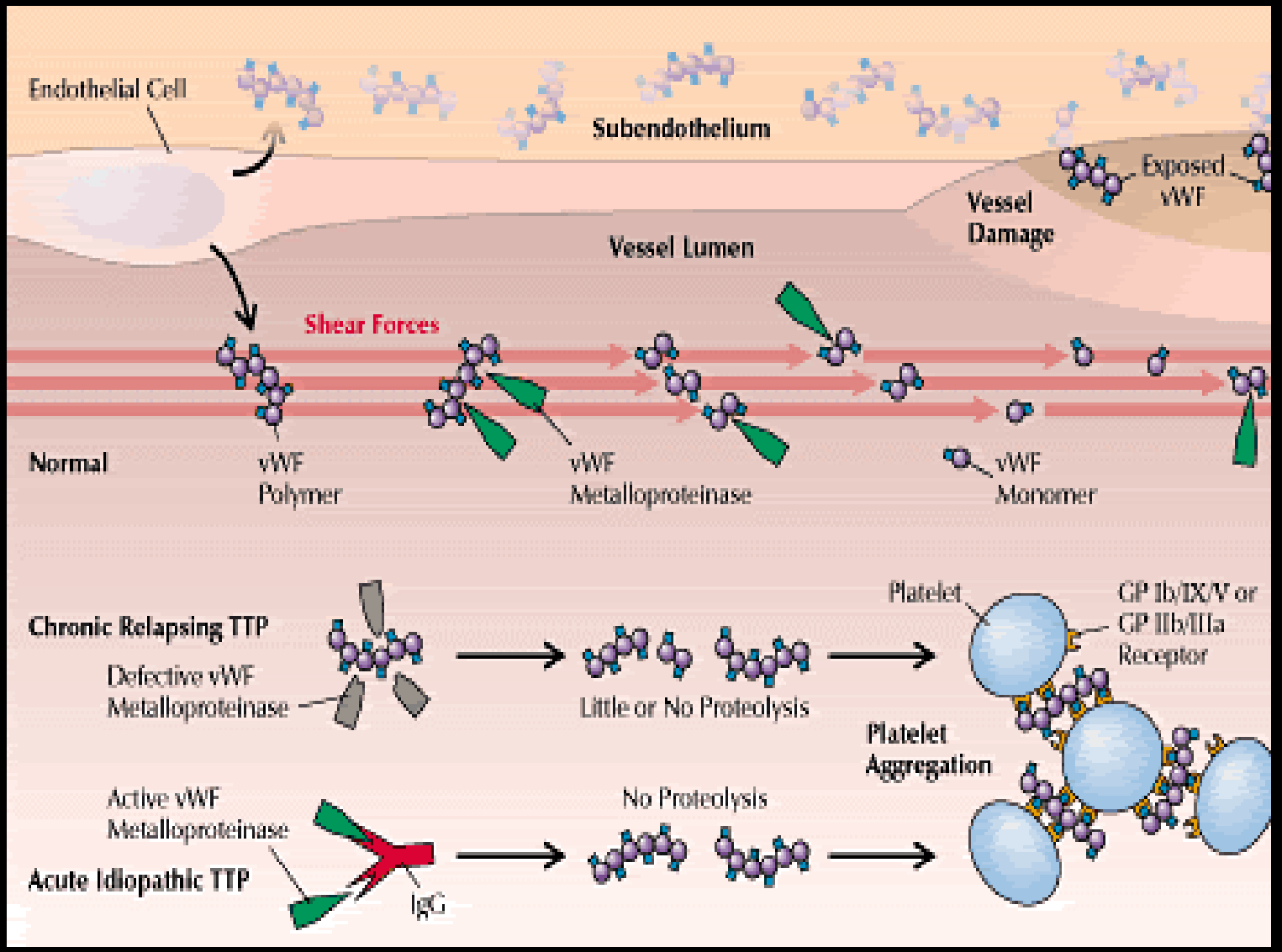

TTP
Uncleaved
ULVWF Multimers
CUB
Platelets
Weibel-Palade
Body

Acquired Idiopathic TTP

- Anti-ADAMTS-13 IgG
- Prohibits protease activity
- Associated with:
  - Autoimmune disorders
  - Ticlopidine
  - Clopidogrel (Plavix)

Familial TTP

- Upshaw-Schulman Syndrome
- Chronic relapsing disease
- 1st episode usually in childhood
- < 5% of normal plasma ADAMTS-13 levels
- Homozygous or compound heterozygous mutations in both ADAMTS-13 alleles
  - Chromosome 9q34
  - 70+ mutations described


Endothelial Cell
Subendothelium
Exposed vWF
Vessel Damage
Vessel Lumen
Shear Forces
Normal
vWF Polymer
vWF Metalloproteinase
vWF Monomer
Chronic Relapsing TTP
Defective vWF Metalloproteinase
Little or No Proteolysis
Platelet
GP Ib/IX/V or GP IIb/IIIa Receptor
Platelet Aggregation
Active vWF Metalloproteinase
Acute Idiopathic TTP
IgG
No Proteolysis

TTP Treatments

Classic TTP Treatments

- ADAMTS-13 Replacement!
- FFP
  - ADAMTS-13 + ULVWF polymers
  - Cryo-poor FFP: contains NO ULVWF polymers
    - Not making things worse!
  - Best for familial disease
  - Watch for hypervolemia
- Therapeutic Plasma Exchange
  - Giving FFP, plus REMOVING…
    - ULVWF-platelet aggregates
    - Stimulants of ULVWF secretion
    - Anti-ADAMTS-13 IgG

New TTP Treatments

- Glucocorticoids
- Rituximab
- Staphylococcal Protein A Immunoabsorption
- Truncated ADAMTS-13

TTP Look-Alikes

- Hemolytic Uremic Syndrome
- Disseminated Intravascular Coagulation
- Infections (Aspergillosis, RMSF, CMV)
- Pregnancy-induced thrombocytopenias
- Intravascular devices (heart valves)
- Malignant hypertension
- Vasculitis
- Antiphospholipid antibody syndrome

ADAMTS-13 activity level detectable & >5%

Hemolytic Uremic Syndrome

- Milder blood count abnormalities
- More severe renal failure
- Causes
  - E. coli O157:H7
  - Factor H deficiency
- Normal levels of ADAMTS-13 activity

Laboratory Assays

Assay Methods for ADAMTS-13

- Used to assess ADAMTS-13 activity levels (NOT protease itself)
- Substrate – VWF (purified or recombinant)
- VWF unfolding – urea or guanidine
- Activation – $BaCl_2$
- Detection – electrophoretic methods, decrease in related function
- ADAMTS-13 activity inhibited by EDTA
  - Must use citrate instead

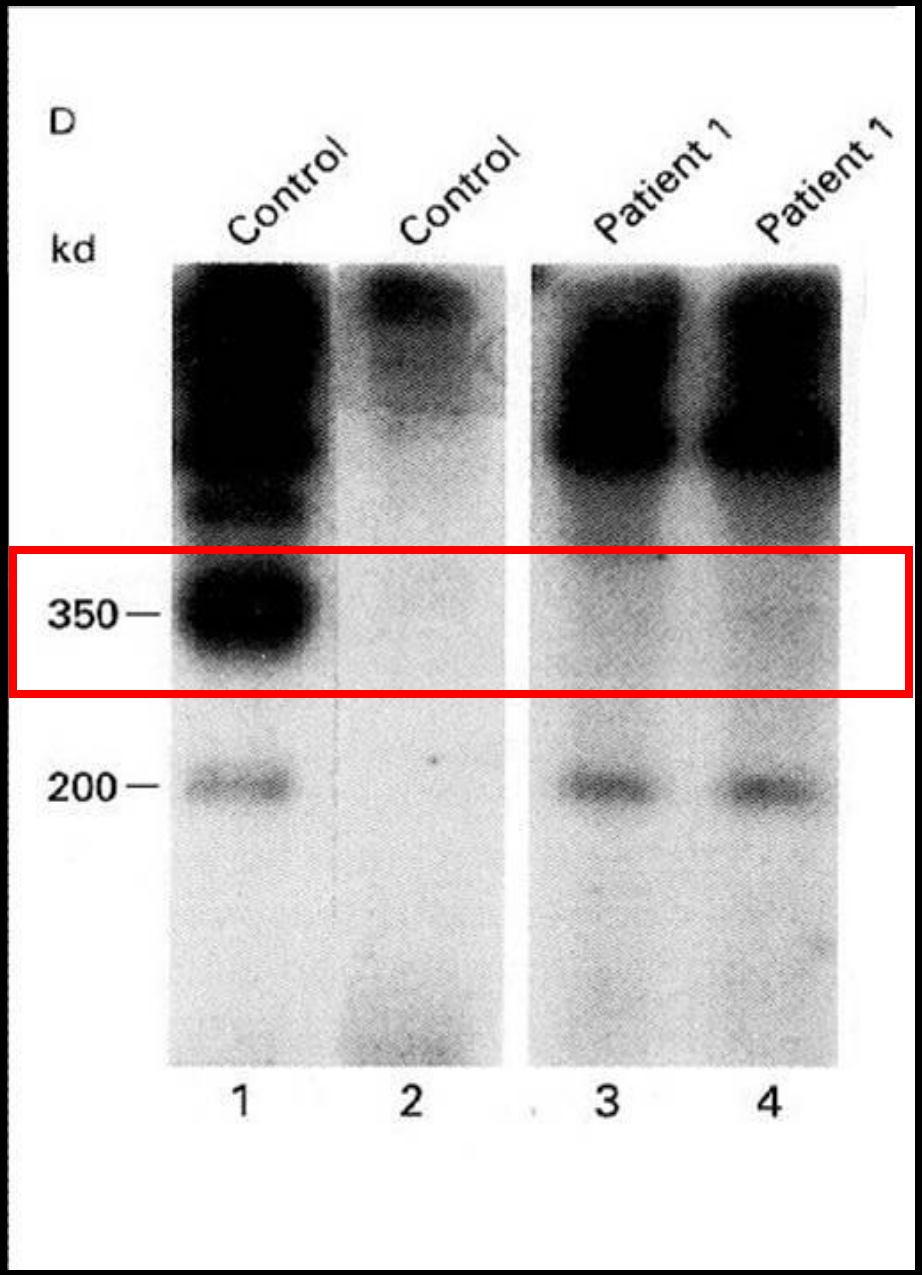
Loss of ULVWF Multimers

- Furlan, et. al.
- Looks for decreased multimer size
- Serially diluted plasma samples
- Purified VWF & urea added
- Overnight incubation
- SDS-agarose gel electrophoresis & immunoblotting with anti-VWF antibody
- Electrophoresis compared to serial dilutions of normal human plasma


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Analysis of Cleavage Products

- Tsai and Lian
- Purified VWF incubated with guanidine-HCL
- Plasma samples diluted & substrate added
- 1 hour incubation
- SDS-polyacrylamide gel electrophoresis & immunoblotting with anti-VWF antibody
- Dimers migrate as 200 kd and 350 kd bands


D
kd
Control
Control
Patient 1
Patient 1
350
200
1
2
3
4

Collagen-Binding Assay

- Gerritsen, et. al.
- Small VWF fragments do not bind collagen; large forms do
- Dilutions of plasma mixed with purified VWF
- Incubation – 2 hours
- ELISA – Microtiter plates coated with collagen type III
- Collagen-bound VWF quantified using labeled antibodies

Immunoradiometric Assay (IRMA)

- Obert, et. al.
- Plasma mixed with recombinant VWF
- Overnight incubation
- Residual activity estimated in microtiter plates via IRMA
  - Monoclonal antibody (epitope C-terminal to cleavage site)
  - 2nd monoclonal antibody labeled with I^{125} (epitope N-terminal to cleavage site)
- Cleavage of VWF detected by decreased binding of labeled antibodies

Ristocetin-Induced Aggregation

- Bohm, et. al.
- Ristocetin - *Norcadia lurida* glycopeptide antibiotic
  - Initiates binding of VWF to platelet glycoprotein Ib
  - Correlation between VWF size and ristocetin cofactor activity
- Purified VWF mixed with plasma
- Overnight incubation
- Residual VWF:ristocetin cofactor activity assayed
- Turbidity compared to serial dilutions of normal human plasma

Fluorogenic Assay for VWF Cleavage

- Substrate is FRET-VWF73
  - C-terminal 2/5 of A2 domain of VWF
  - Cleaved in absence of denaturants & shear forces
  - Cleavage causes fluorescence
- Plasma added & fluorescence counted over time
  - Normal plasma: fluorescence increases with time
  - ADAMTS-13 deficient plasma: fluorescence fails to increase or increases by smaller amounts

Bethesda Inhibitor Assay

- Mixing studies
  - Normal human plasma mixed with patient's plasma
- Residual activity measured via ANY assay
- One Bethesda Unit = quantity of inhibitor that neutralizes 50% of the ADAMTS-13 activity in normal plasma
    - Increase in Bethesda units is exponential
    - Normal is ≤ 0.3 Bethesda Units

Comparing the Assays

- 30 plasmas tested with various assays
  - ADAMTS-13 levels from <3% to 100%
- Severe ADAMTS-13 deficiency
  - Good interassay & interlaboratory agreement
- Normal or moderately reduced ADAMTS-13
  - Less concordant results
- Few errors with collagen-binding assay

When ADAMTS-13 assay is ordered here…

- The Blood Center of Southeastern Wisconsin Reference Laboratory
- Gerritson method and Bethesda Inhibitor Assay
- Sample collected in citrate and sent frozen
- Assay run 2x per week
- Turnaround time 7-10 days
- Cost $105

Test Utility

- Patient presentations vary greatly
- Can help to refine treatment course
- May help to anticipate clinical course of patients with TTP

Test Drawbacks

- Clinical course and ADAMTS-13 levels don’t always correlate
- Transfusion of RBC’s and platelets can increase ADAMTS-13 activity
- Assays are time consuming and must be sent to reference labs

Resources

- Kokame, K, et. al; “FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay”, British Journal of Haematology, 129, 93-100.
- Kremmer Hovinga, J, et. al; “The von Willebrand Factor-Cleaving Protease (ADAMTS-13) and the Diagnosis of Thrombotic Thrombocytopenic Purpura (TTP), Pathophysiol Haemost Thromb 2003/2004, 33, 417-421.
- Mayer, SA, et. Al; “Thrombotic Microangiopathy: Differential diagnosis, pathophysiology and therapeutic strategies”, The Mount Sinai Journal of Medicine, May 2005, 72 (3), 166-175.
- Sadler, JE, et. al; “Recent advances in Thrombotic Thrombocytopenic Purpura”, Hematology 2004, 407-423.
- Tripodi, A, et. al; “Measurement of von Willebrand factor cleaving protease (ADAMTS-13): results of an international collaborative study involving 11 methods testing the same set of coded plasmas” J of Thrombosis and Hemostasis, Sep 2004, 2 (9), 1601-1609.
- Tsai, Han-Mou; “Advances in the Pathogenesis, Diagnosis, and Treatment of Thrombotic Thrombocytopenic Purpura”, J Am Soc Nephrol. 2003 Apr;14(4):1072-81.
- Veyradier, A, et. al; “Assays of ADAMTS-13 Activity”, Sem in Hematology, Jan 2004, 41 (1), 41-47.