ADAMTS13: Its Impact on the Diagnosis and Management of MAHA

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What is TTP?

- **A clinicopathological entity (Moschcowitz, 1924):**
  - **MAHA, thrombocytopenia, neurological deficits**
  - **Widespread arteriolar and capillary thrombi at autopsy**
- **Efforts to define TTP as a clinical syndrome has led to confusion**
  - **Pentad: MAHA, thrombocytopenia, neurological deficits, renal abnormalities & fever**
  - **Triad: MAHA, thrombocytopenia & neurological deficits**
  - **Diad: MAHA, thrombocytopenia**
- **Definition differs in**
  - **Exclusion of patients with co-morbidity**
    - **Stx-HUS**
    - **HSCT, lupus, LA, drugs, HIV infection, cancer, etc.**
  - **Exclusion of patients with renal failure**
- **There has been no basis to determine which definition of TTP is valid.**

TTP without ADAMTS13 Deficiency? TTP is Defined Differently

Authors, year	No.	Severe def.	Excl. renal failure	Exclude co-morbidity
Furlan et al, 1998	24	83%	Yes (HUS by referral)	No
Veyradier et al, 2001	111	89%	Yes (HUS by referral)	Yes (by referral)
Bohm et al, 2002	22	91%	Not indicated	No
Rick et al, 2002	50	78%	Not indicated	No
Hovinga et al, 2004	396	57%	Yes (HUS by referral)	Yes (by referral)
Matsumoto et al, 2004	108	52%	Yes (HUS by referral)	Yes (by referral)
Coppo et al, 2004	46	67%	No	Yes (by referral)
Peyvandi et al, 2004	100	48%	No	No
Terrel et al, 2005	70	31%	No	Yes (by referral)
Kokame et al, 2005	41	80%	Not indicated	Not indicated
Tsai et al, 1998	39	100%	Yes (Cr_{max} > 2.5)	Yes (overall)
Zhou et al, 2004	34	100%	Yes (Cr_{max} > 2.5)	Yes (overall)

ADAMTS13 Deficiency in the CAG Studies

	No.	*Severe def.*	*Exclude co-morbidity*	*Exclude renal failure*
CAG (2013)	**61**	**25%***	**No apparent alternative causes**	**No**
CAG-1 (1991)	**41**	**(90%) (Inh 76%)**	**DIC, cancer, preeclampsia**	**Yes (anuria)**

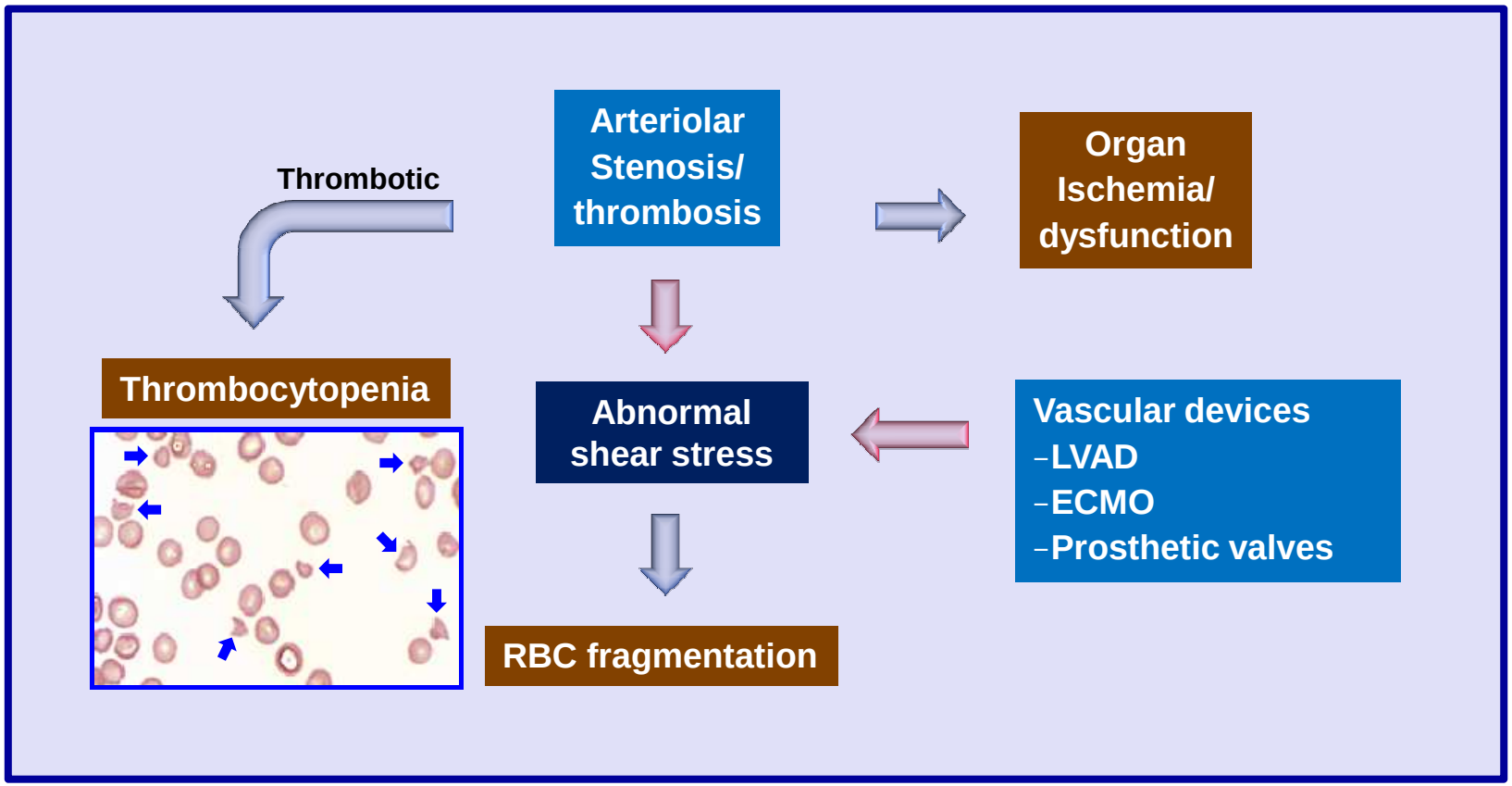
***P<0.0001**

- **The difference in the prevalence of severe ADAMTS13 between the two series likely result from inclusion of patients with renal failure in the 2013 series.**
- **Variation in the ADAMTS13 assays may also contribute to the difference.**

Rock GA et al, Thromb Research 2013 (ePub)

Tsai HM et al, Clin Lab 2001;47:387-392

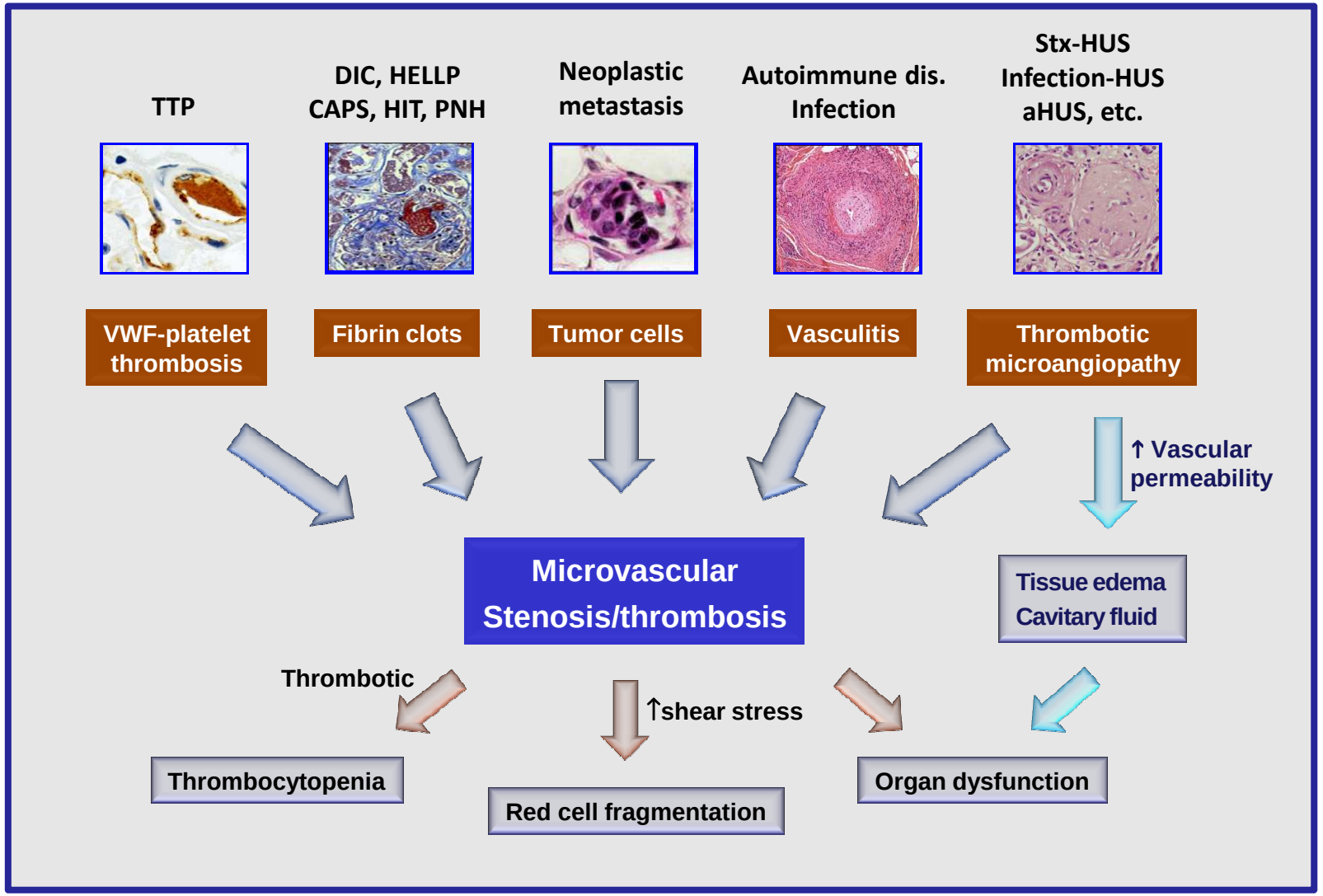
MAHA: Mechanical Injury of the Red Cells



- **RBC fragmentation may result from mechanical devices or arteriolar stenosis/thrombosis**
- **In patients without mechanical devices, arteriolar stenosis/thrombosis is the cause.**

Based on Tsai HM, Am J Med 2013;126:200-209

Five Different Types of Pathology May Cause MAHA



Adapted from Tsai HM, in Wintrobe's Clinical Hematology 13/e (in press)

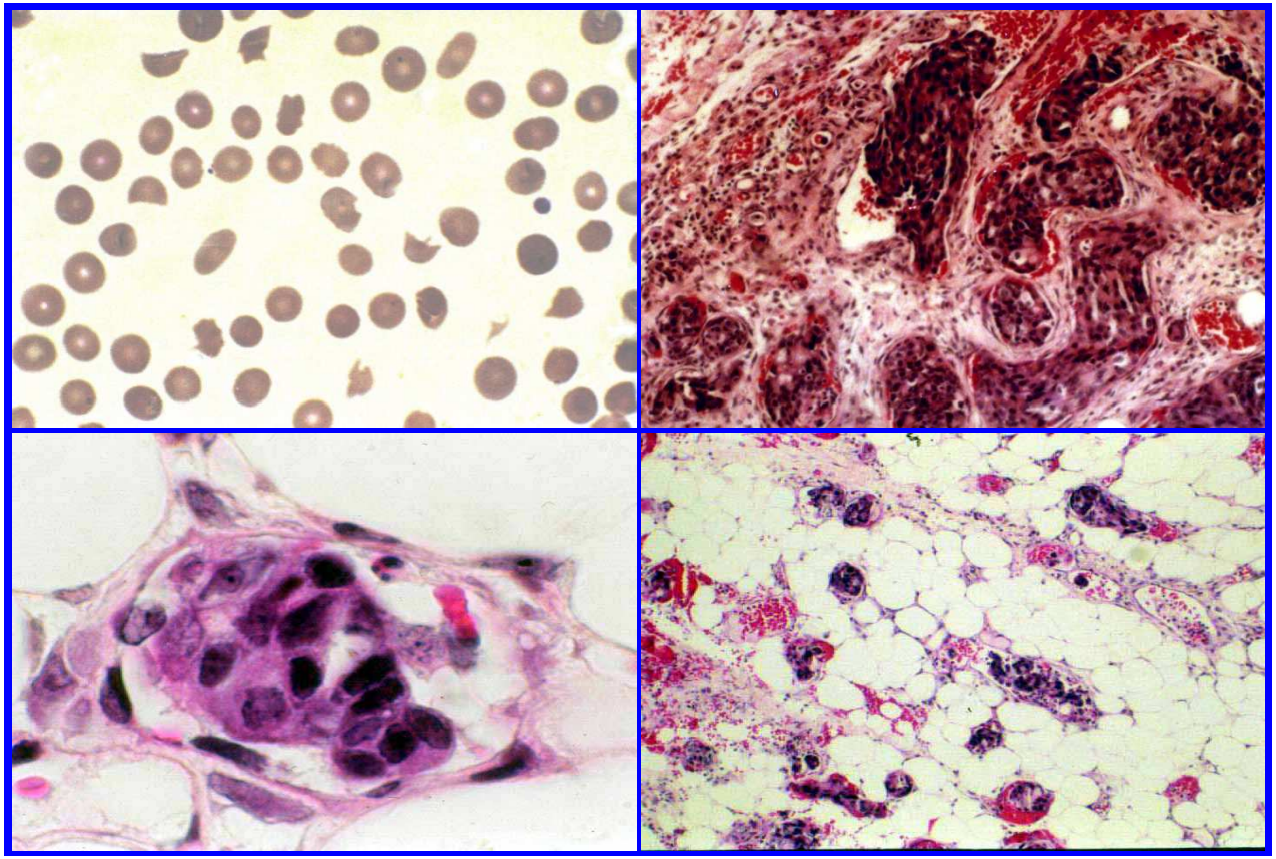
Case 1: TTP without ADAMTS13 Deficiency?

•Age/Sex	56 y / Female
•Presentation	MAHA ↓ Platelet count
•Clinical dx	TTP
•Treatment	Plasma Ex
•Problem	Normal ADAMTS13
•Final dx	?

Test	Day 1	Day 7
Platelets/L (x10^{-9})	76	24
Hb (g/L)	62	90
LDH (U/L)	4,900	>5,000
vWF:Ag (%)	221	111
HMW fraction (%)	85	83
ADAMTS13 (%)	112	92

Personal unpublished data

Case 1: Microvascular Invasion by Tumor Cells



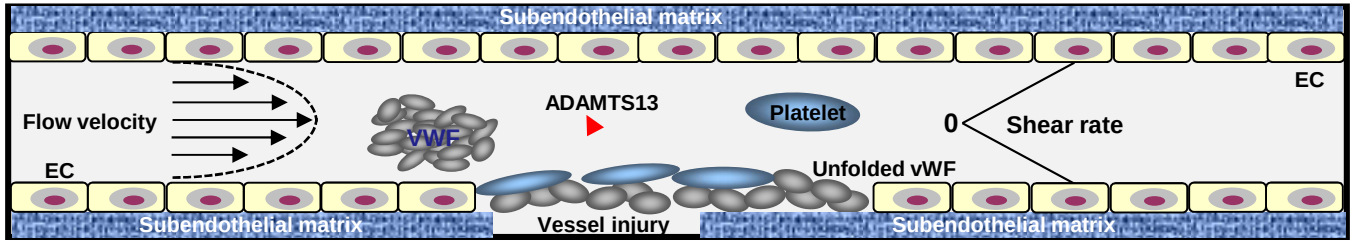
Personal unpublished data

A Molecular Definition of TTP, the Disease

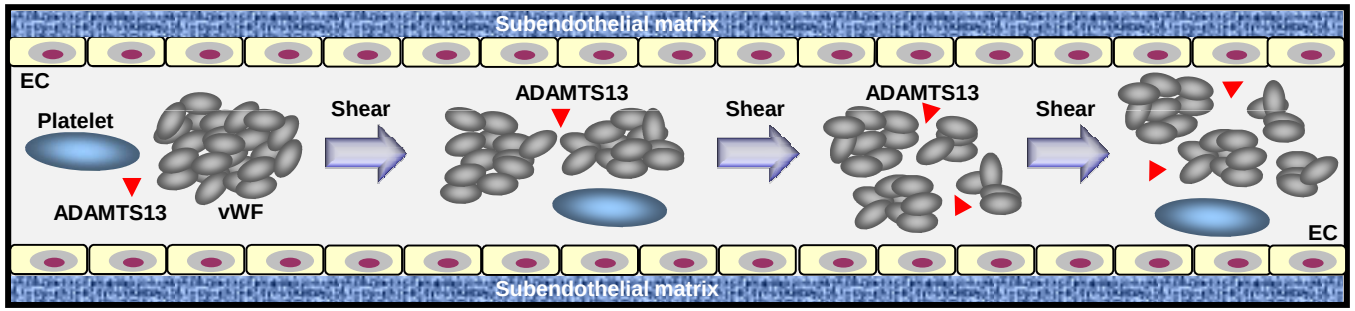
- **Propensity to VWF-platelet thrombosis resulting from severe ADAMTS13 deficiency:**
  - **Causes of ADAMTS13 deficiency**
    - **Autoimmune inhibitors**
    - **Genetic mutations**
- **Phenotyes**
  - **Active thrombosis: ADAMTS13 <10%**
    - **Conventional TTP**
      - ❑**Thrombocytopenia, MAHA, neurologic complications, and other organ dysfunction**
    - **Chronic thrombosis**
      - ❑**MAHA with mild thrombocytopenia or even thrombocytosis**
    - **Incomplete TTP phenotype**
      - ❑**Thrombocytopenia only (often mistaken to be ITP)**
      - ❑**Neuro defects without thrombocytopenia or MAHA (often mistaken to be CVA)**
      - ❑**Neuro defects with thrombocytopenia but no MAHA (often mistaken to be CVA)**
  - **No active thrombosis (remission): ADAMTS13 is normal, decreased or <10%**
    - **Patients still have the disease during clinical remission**

How Does ADAMTS13 Deficiency Predisposes the Patient to Microvascular Thrombosis

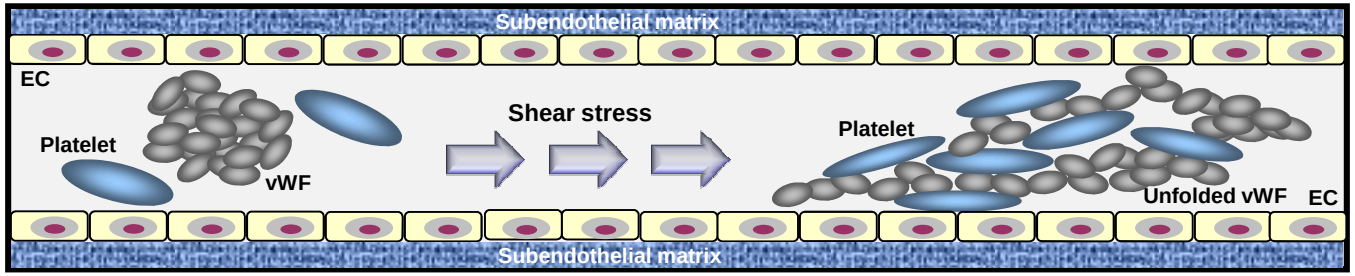
A: At sites of microvascular injury



B: Normal circulation

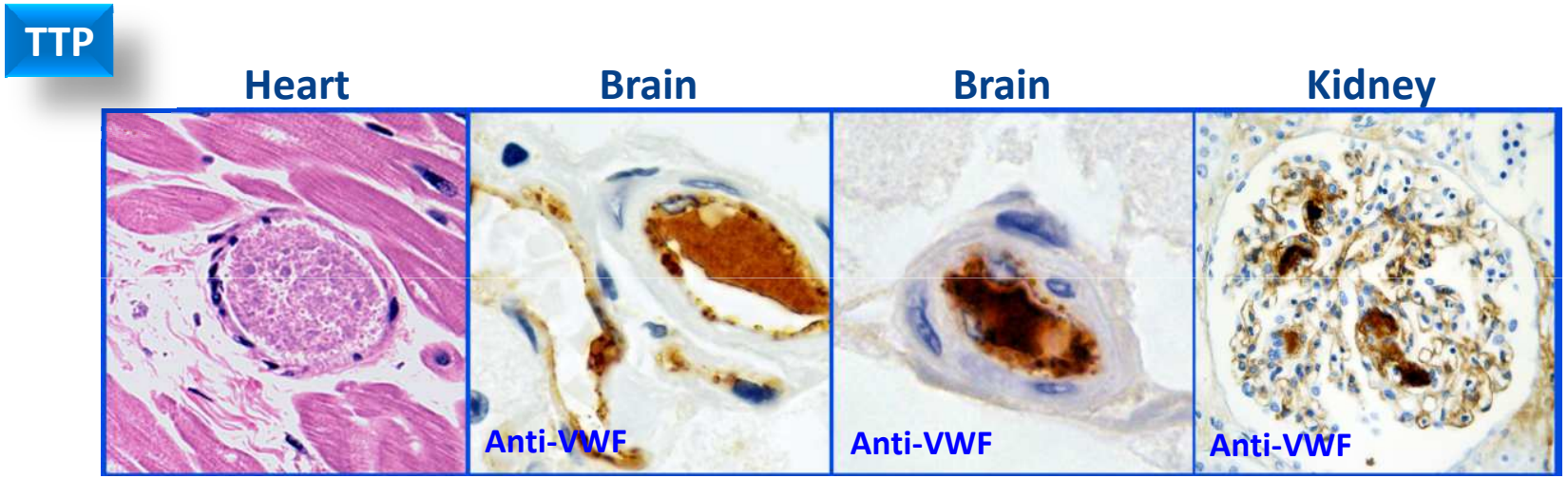


C: ADAMTS13 deficiency



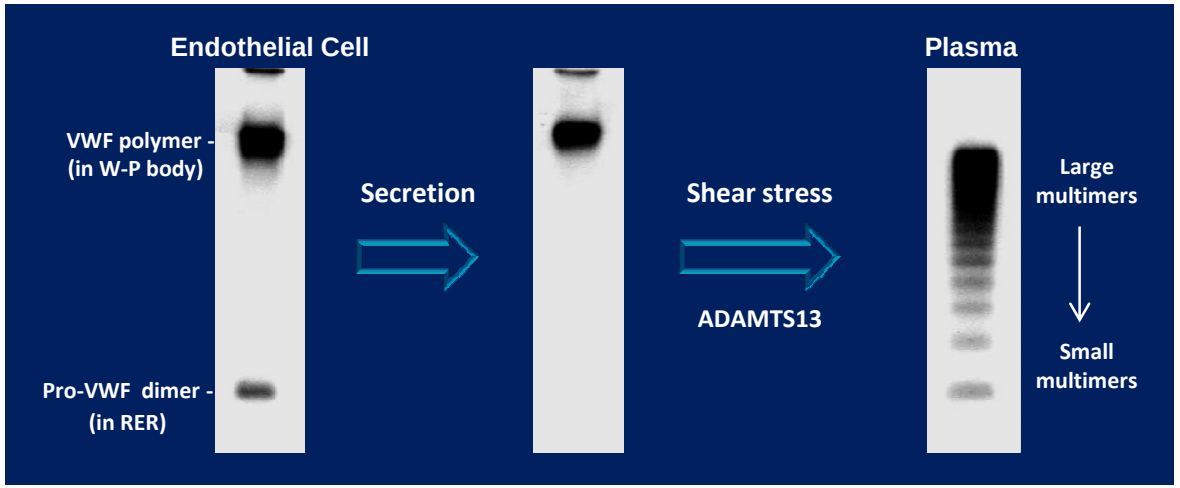
Tsai HM, Am J Med 2013;126:200-209

TTP: Microvascular Thrombosis, No Microangiopathy



Tsai HM, Am J Med 2013;126:200-209

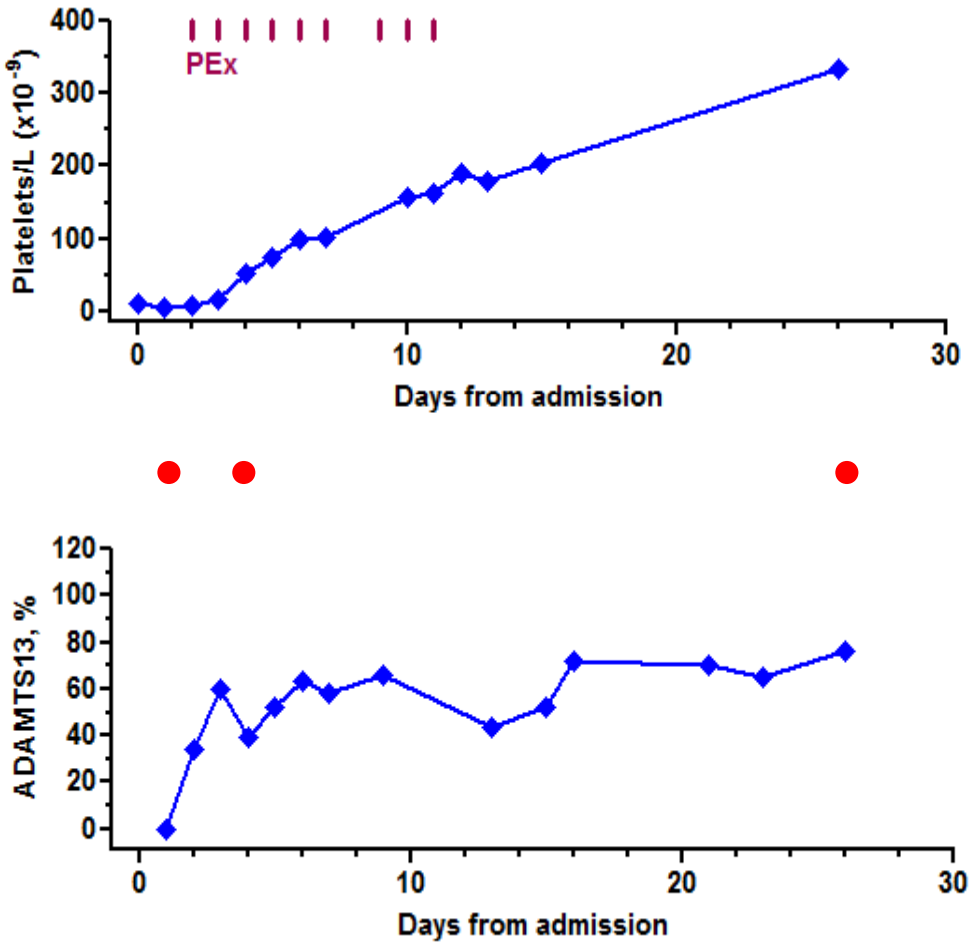
Factors Affecting the Size Distribution of VWF Multimers

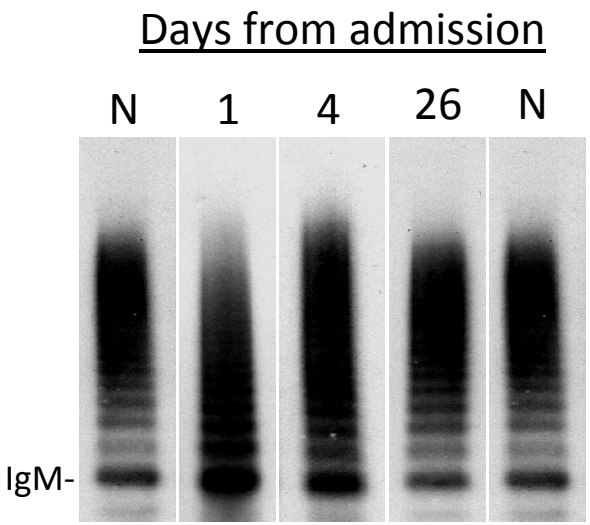


Altered VWF size distribution	Example
Upward:	
Decreased ADAMTS13	TTP
Augmented secretion	dDAVP
VWF mutant with ↑ clearance	VWD- Vicenza
Downward:	
Consumption in platelet aggregation	TTP, VWD 2B
Abnormal shear stress	MAHA without ADAMTS13 def.
VWF mutants with ↑ susceptability	VWD 2A

Based on Tsai HM. Microangiopathic hemolytic anemia. In Encyclopedia of Human Biology (in press)

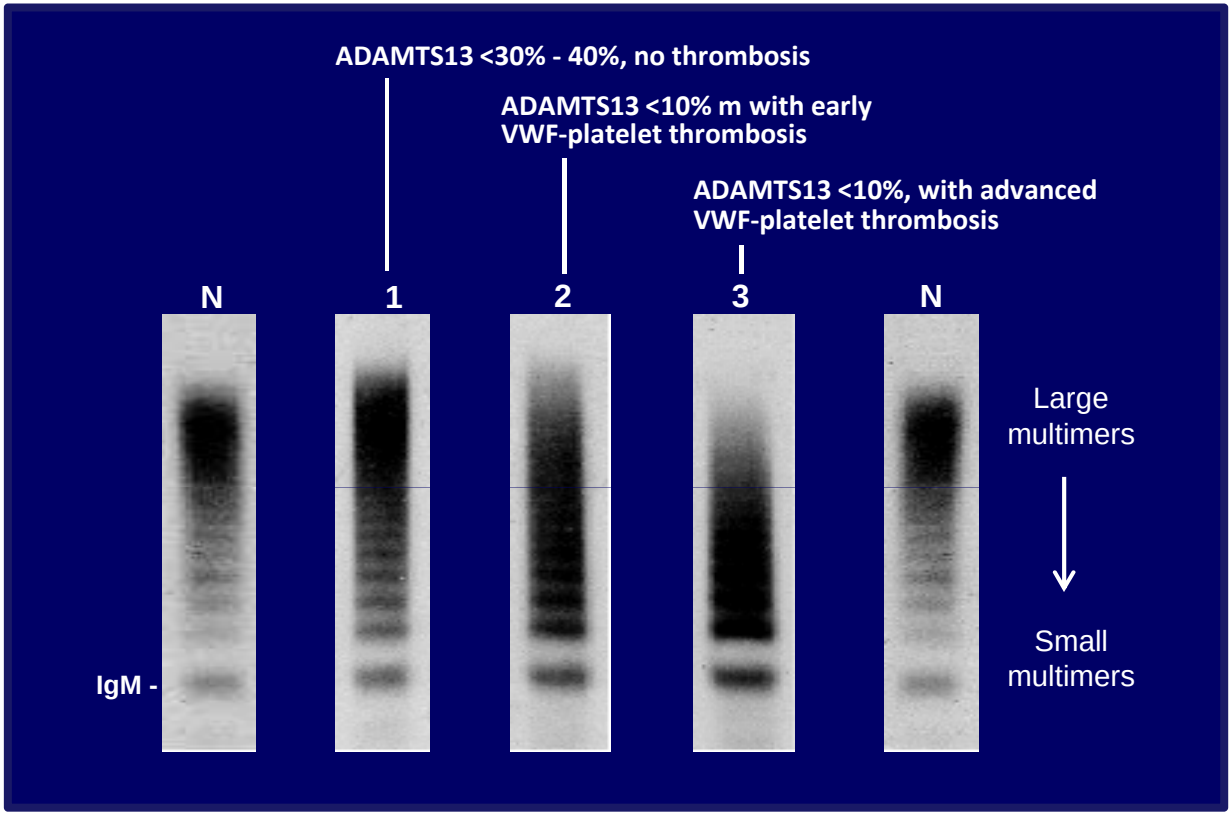
Case 2: Multimer Changes in TTP





Based on personal unpublished data

VWF Multimers in TTP

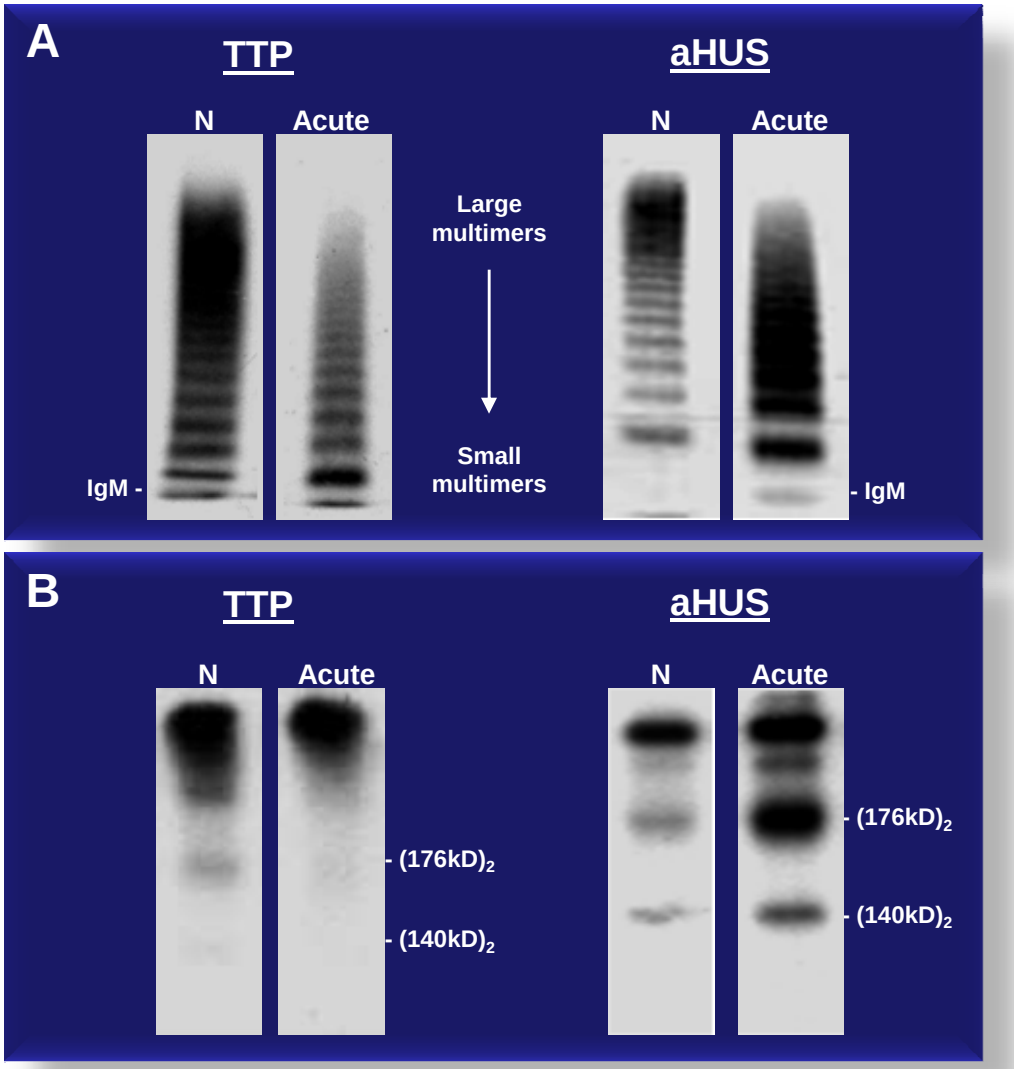


Depletion of VWF is not limited to extra large multimers

Depletion from the top: Responsiveness to shear stress depends on the size

Adapted from Tsai HM, in Wintrobe's Clinical Hematology 13/e (in press)

Decrease of Large VWF Multimers Are Mediated by Different Mechanisms in TTP and aHUS



SDS agarose electrophoresis

SDS PAGE (non-reducing)

Based on:

Tsai HM, Microangiopathic hemolytic anemia. In Encyclopedia of Human Biology (in press)
Also Pediatric Res 2001;49:653-659 (ADAMTS13 and VWF multimers in stx-HUS)

Modifiers of Thrombosis Predisposition in TTP

- **ADAMTS13**
  - **>10%, no thrombosis**
  - **Threshold of thrombosis is not fixed**

- **Modifiers of VWF-platelet aggregation**
  - **Shear stress profile**
  - **Blood components**
    - **Thrombospondin: protective of VWF from proteolysis**
    - **B2GP1: decreasing VWF-platelet binding**
    - **Other proteins and factors**

ADAMTS13 – Impacts on Clinical Practice

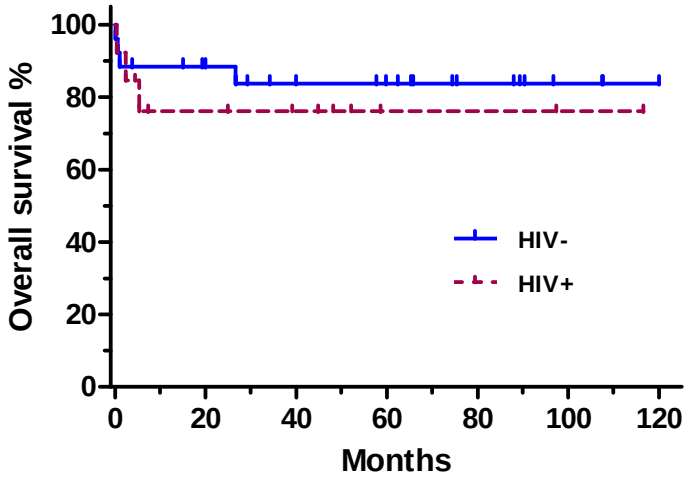
- **A molecular basis distinguishing TTP from other causes of MAHA**
- **Rationale for plasma exchange and plasma infusion**
  - **Replenishes ADAMTS13**
  - **Removal of inhibitors during plasma exchange**
- **Rationale for rituximab and other immunotherapy**
  - **Persistent disease activity**
  - **Intermittent relapses**
  - **Possibly acute cases**
- **Therapeutic development**
  - **Recombinant ADAMTS13**
  - **Non-suppressible ADAMTS13 variants**
  - **Blockers of VWF-platelet aggregation**
- **Precise definition of TMA**

Problems and Solutions

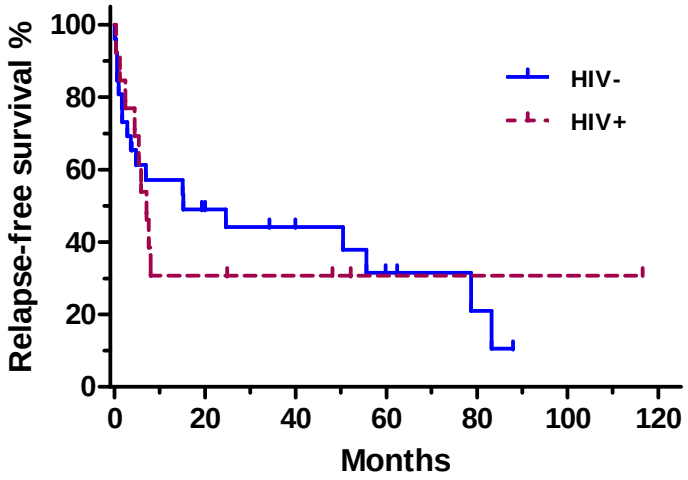
Problem	Potential solution
• Death	• Rituximab (late death >2 weeks) • Belimumab (anti-BAFF) • Bortezomab (plasma cells) • Recombinant ADAMTS13 • Blockers of VWF-platelet binding – Aptamer ARC1799 – Anti-VWF nanobodies (e.g. ALX-0681)
• Early relapse or • Persistent disease activity	• Rituximab • Belimumab (anti-BAFF) • Bortezomab (plasma cells) • CSA
• Late relapse	• ADAMTS13-guided – Rituximab – Belimumab (anti-BAFF) – Bortezomib (plasma cells) • CSA

Long-Term Outcome of TTP

Overall survival



Relapse free survival

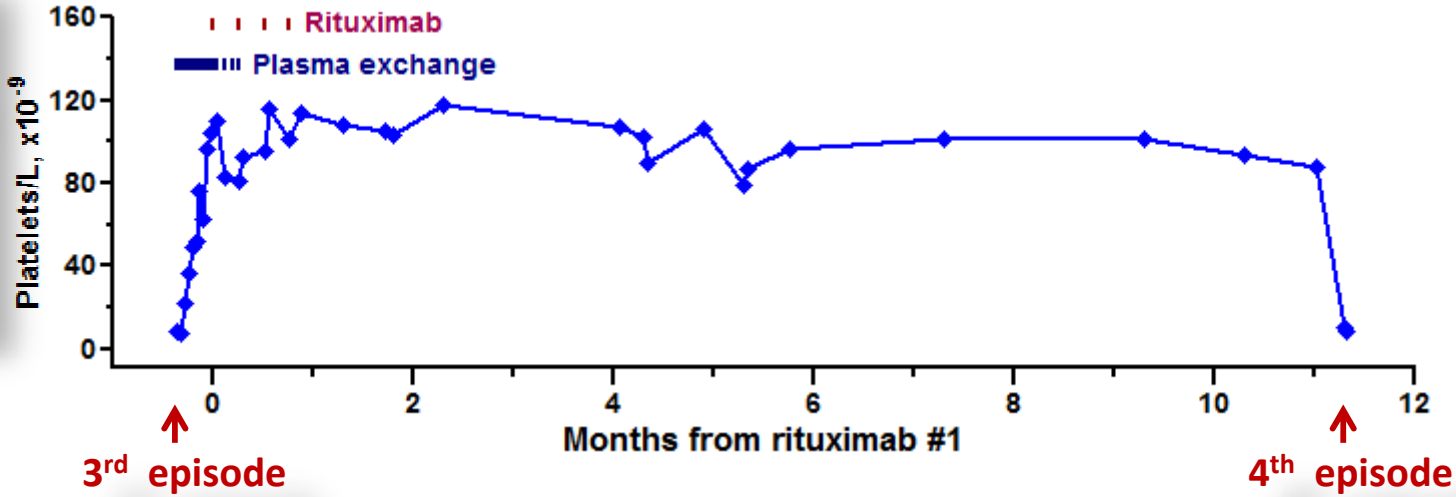


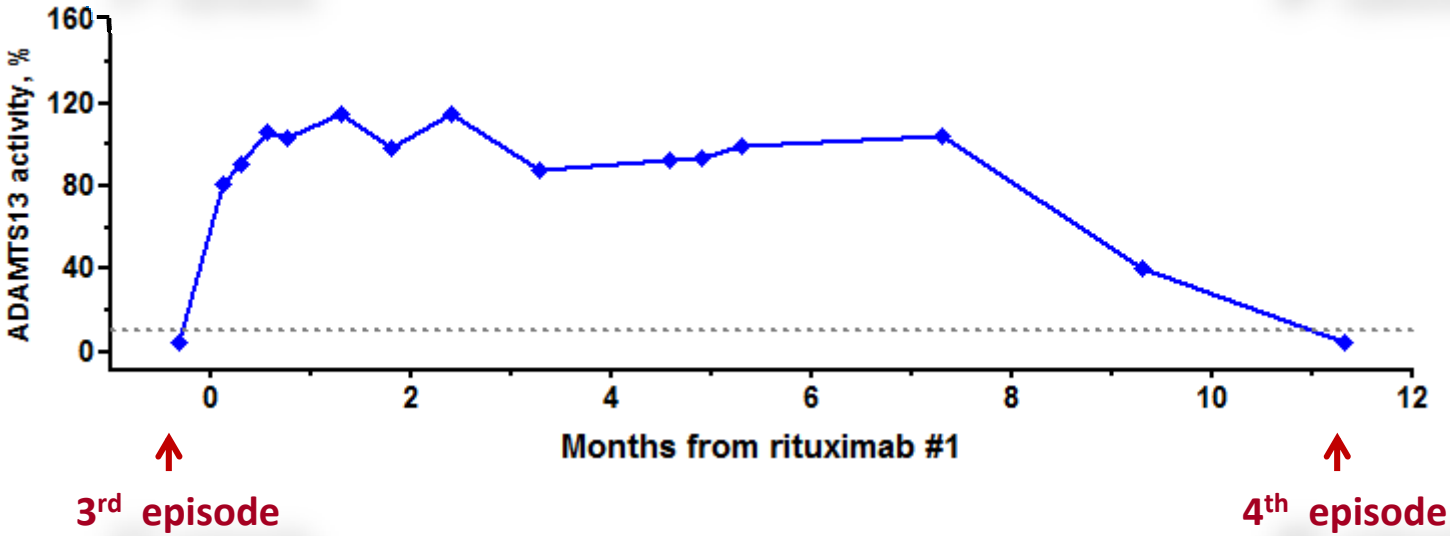
- **TTP continues to relapse without reaching a plateau in patients without HIV infection.**
- **In HIV infected patients, anti-retroviral therapy prevents relapse after one year.**

Tsai HM. TTP and aHUS: an update. Hem Onc Clin NA (in press)

Case 3: Decrease of ADAMTS13 Precedes TTP Relapse

45 y/o female
History:
- Cutaneous lupus
- Hb CC
- HCV
- Splenomegaly
- Recurrent TTP





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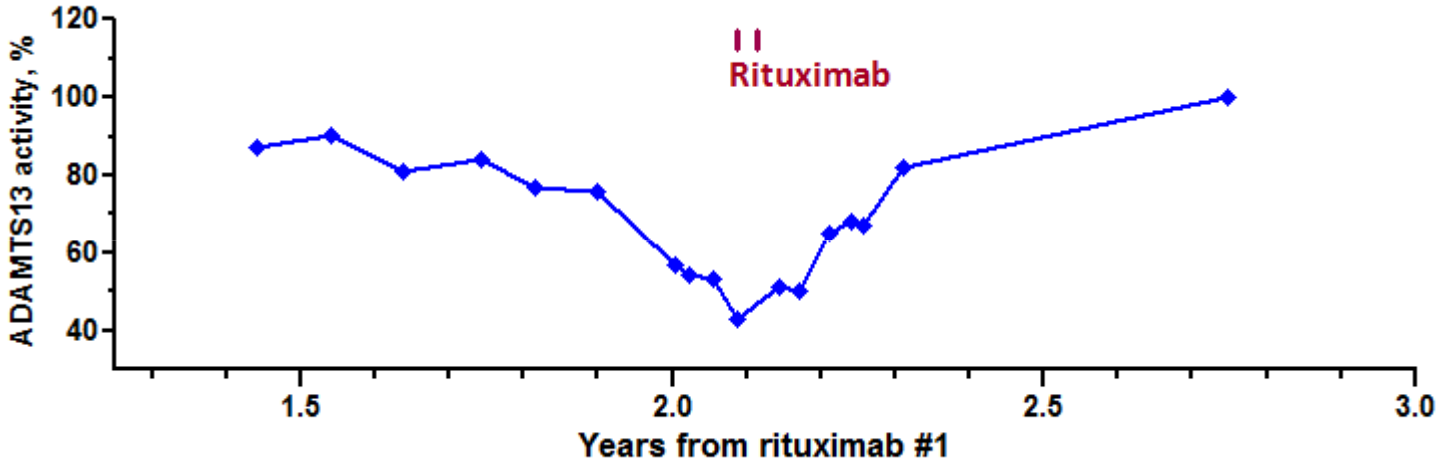
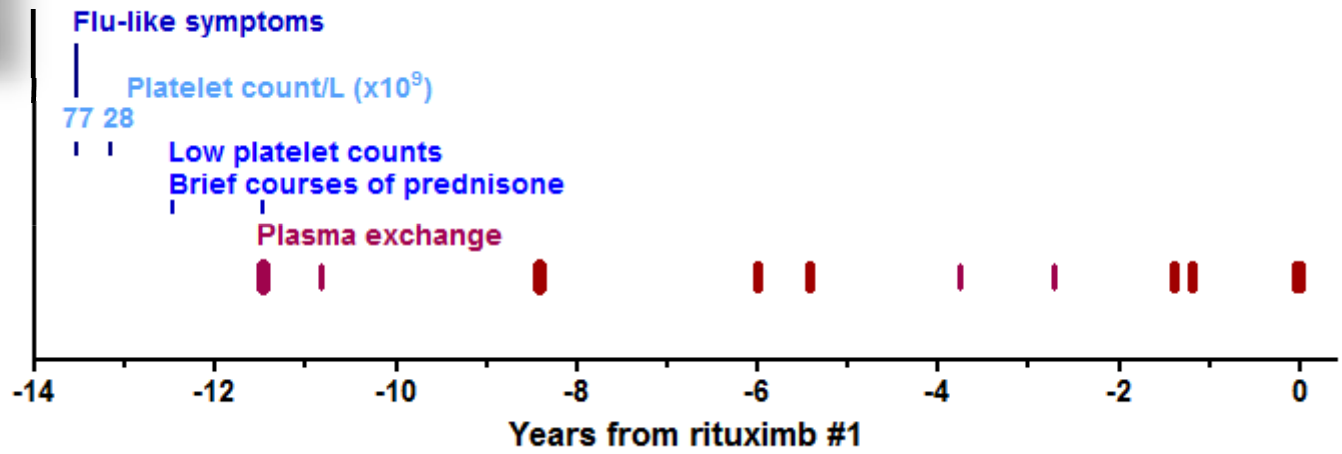
Case 3: ADAMTS13-Guided Rituximab to Prevent TTP Relapse



Tsai HM, Hematol Oncol Clin North Am (in press)

Case 4: TTP with Multiple Relapses

51 y/o female
Recurrent TTP



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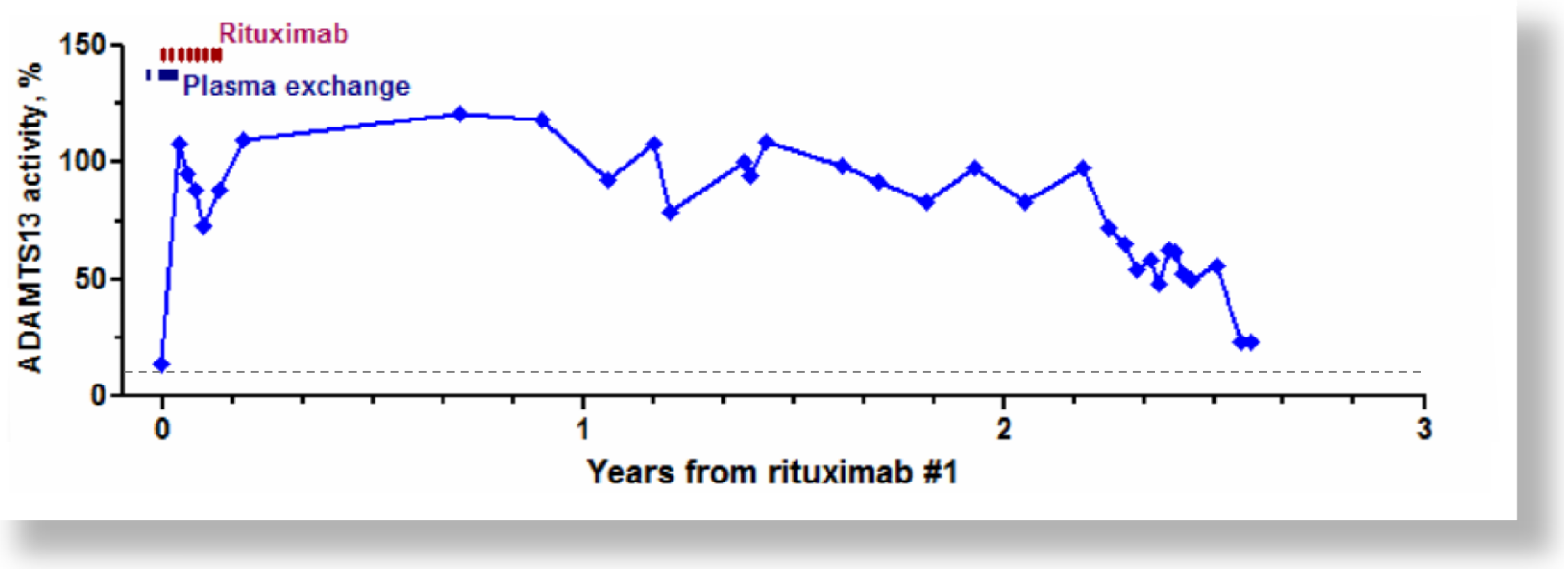
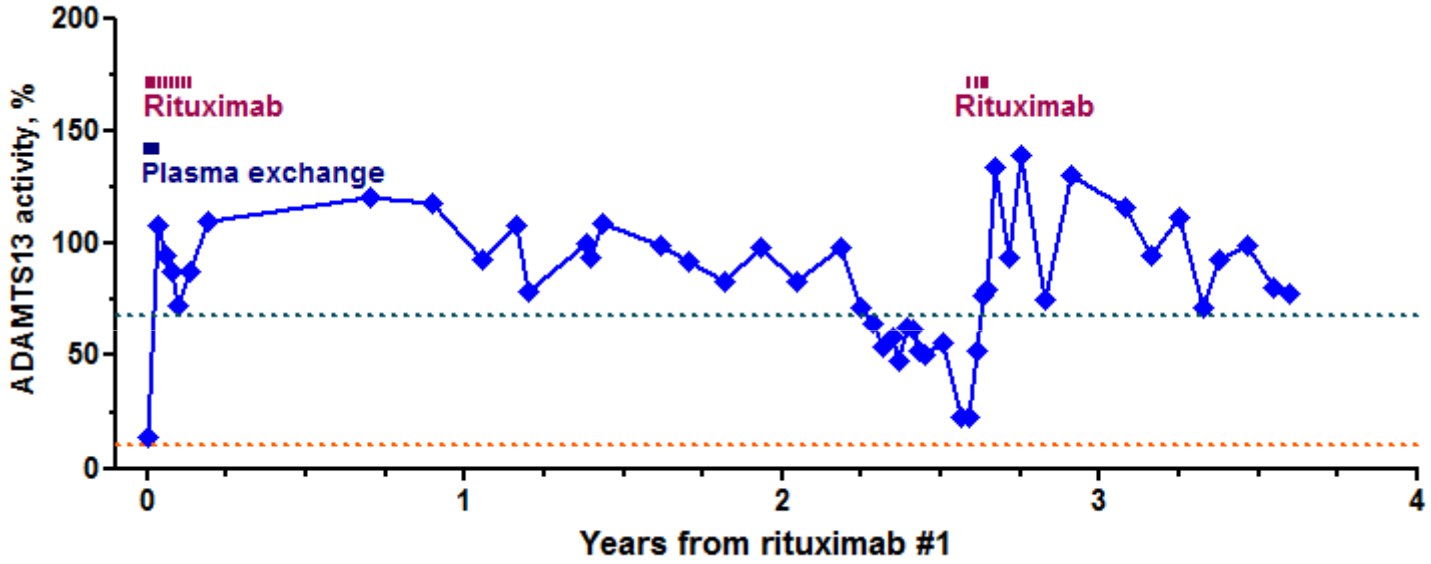
Case 4: TTP with Declining ADAMTS13 after Rituximab



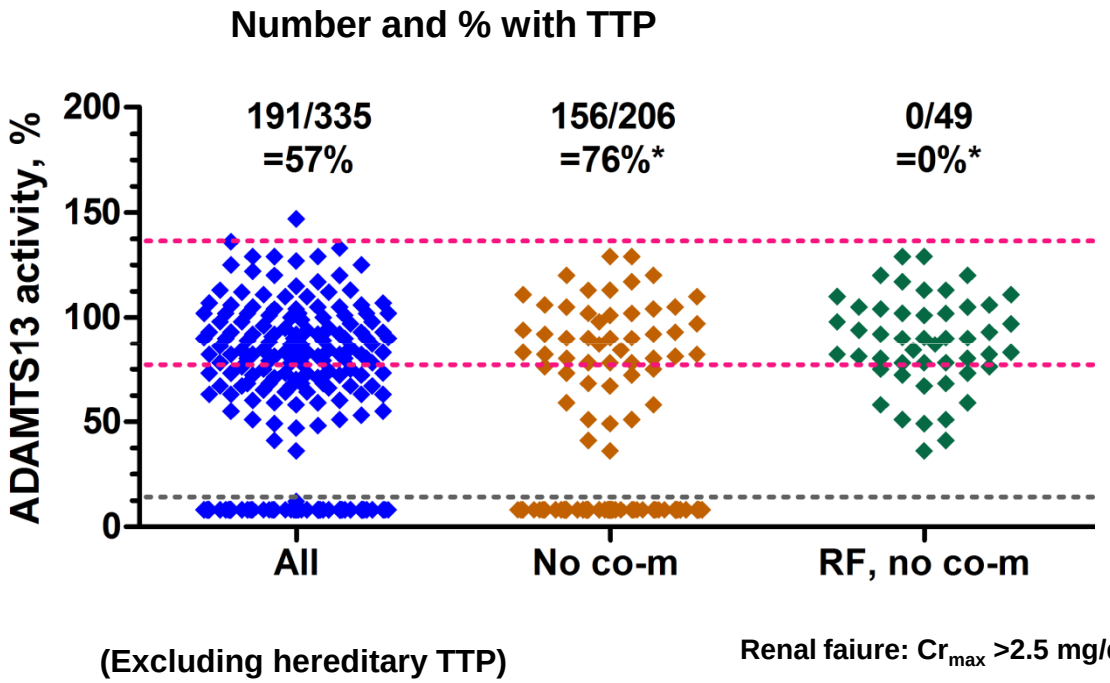
Tsai HM. TTP and aHUS, an update. Hematol Oncol Clin North Am (in press)

Case 4: ADAMTS13-Guided Rituximab to Avert a TTP Relapse



Based on
Tsai HM. TTP and aHUS, an update. Hematol Oncol Clin North Am (in press)

ADAMTS13 Activity Segregates TTP from Other types of MAHA



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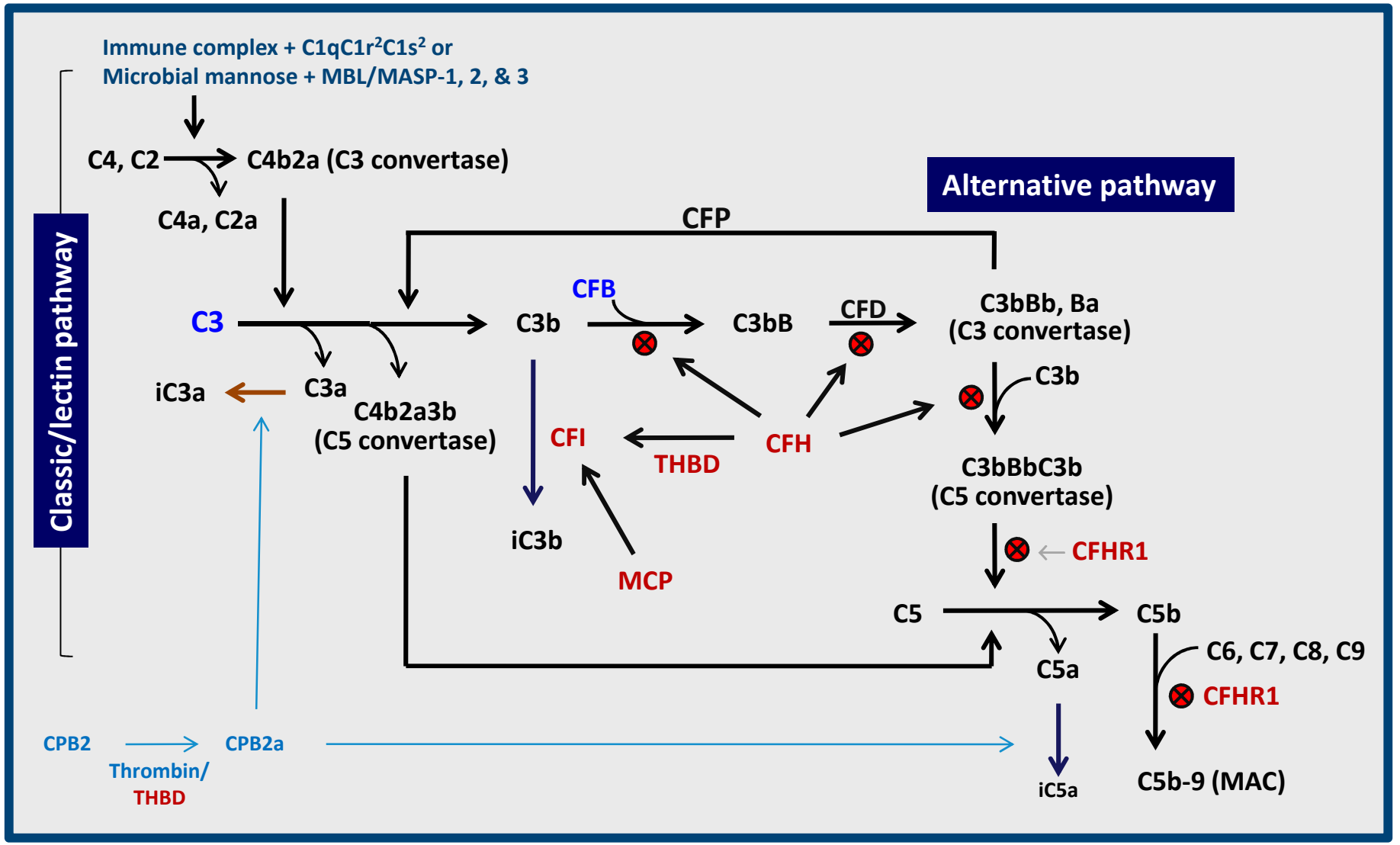
Regulation of Complement Activation



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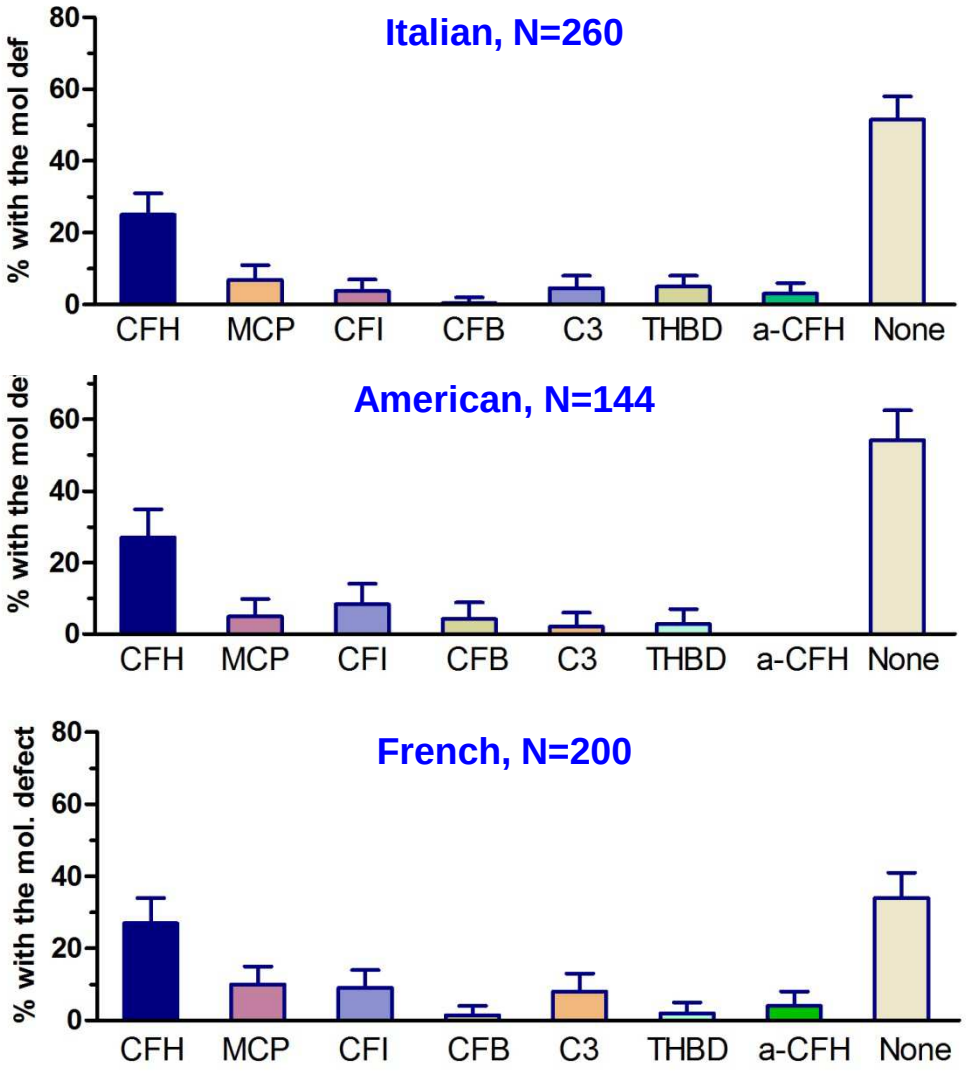
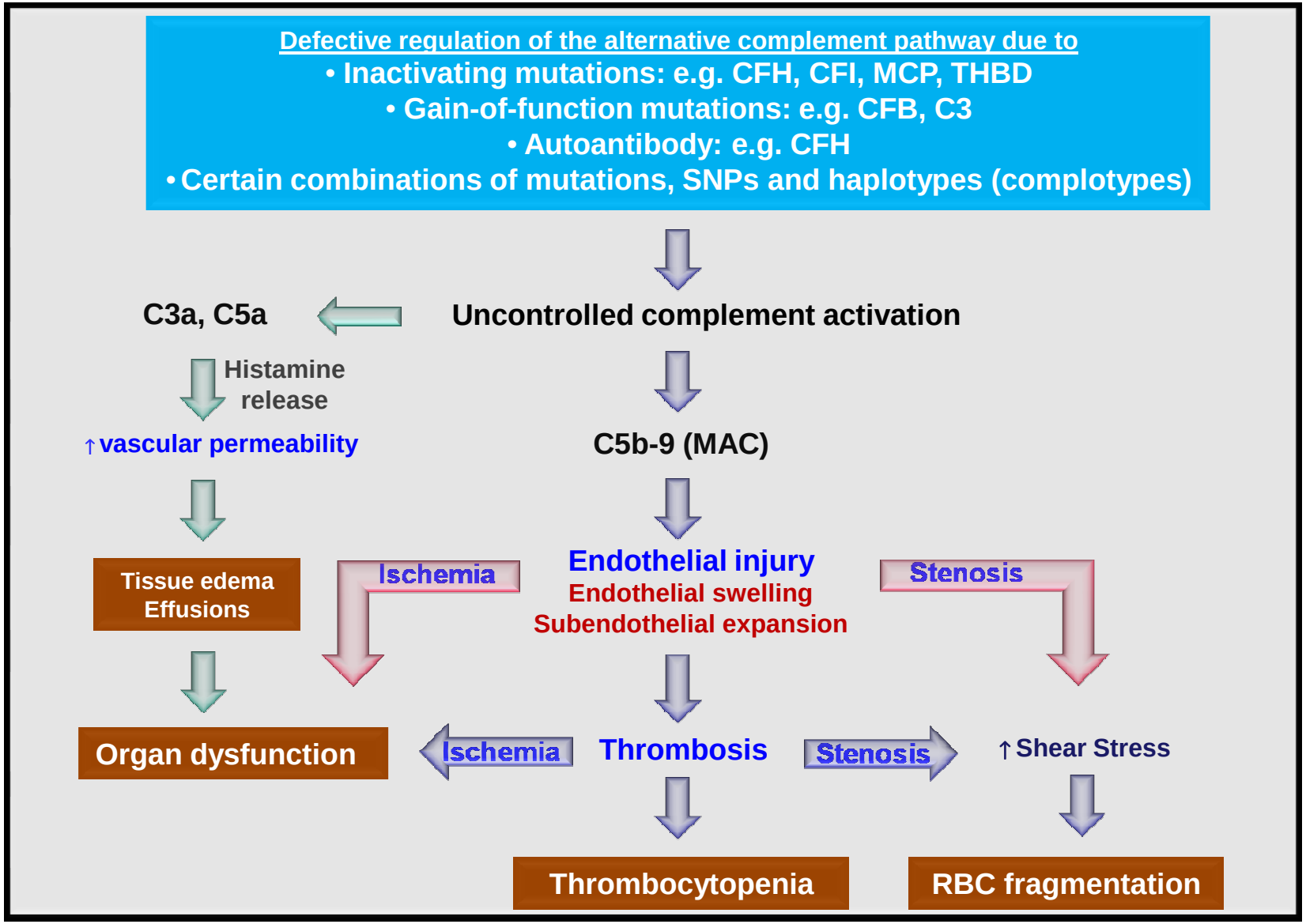
Genetics of aHUS

Vertical bar = 95% CI



Based on
Noris et al, CJASN 5:1844, 2010
Maga CH et al, Hum Mutat 2010;31:E1445
Fremeaux-Bacchi et al, CJASN 2013;8:554

Pathophysiology of aHUS



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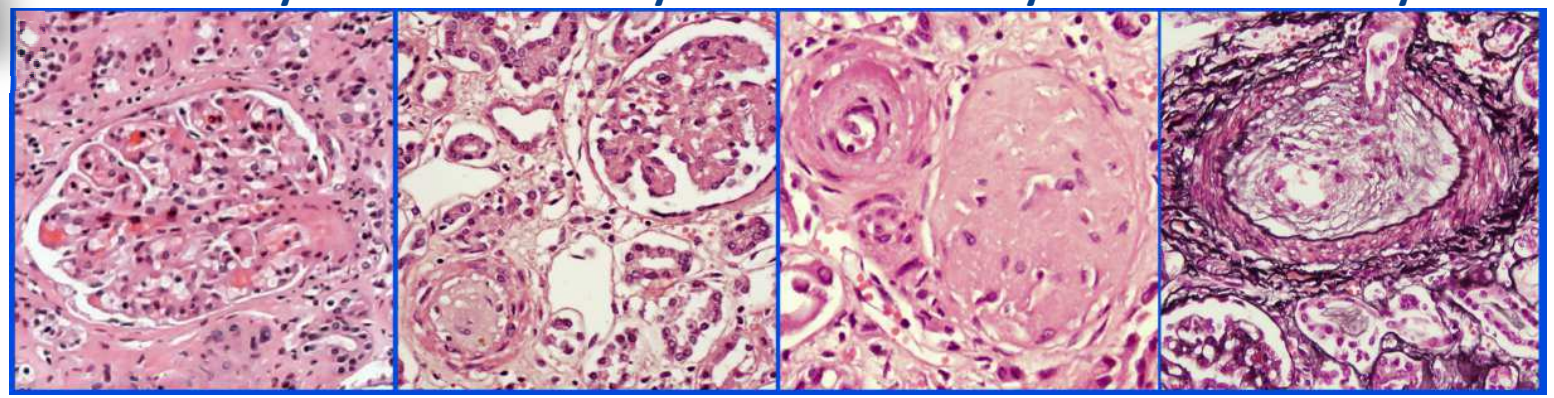
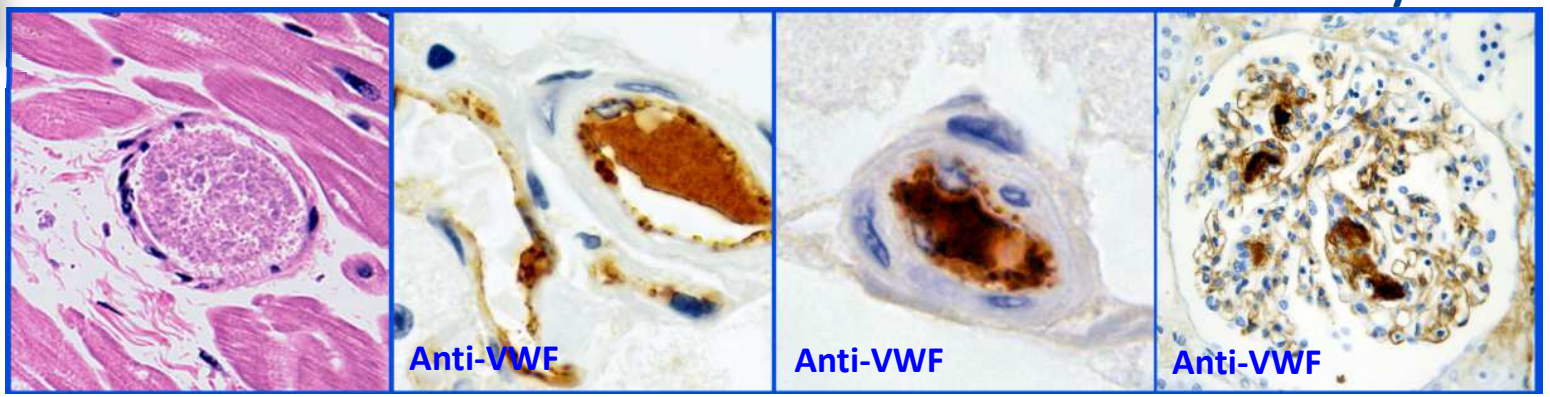
TTP and aHUS: Different Pathology

aHUS



1. **Interstitial edema of soft tissue, brain and other organs is common in aHUS**
2. **Organ dysfunction does not always correlate with thrombocytopenia or MAHA**
3. **Hypertension, often severe but brittle, may result from involvement of the juxtaglomerular arterioles**

TTP



Based on Tsai HM, Am J Med 2013;126:200-209

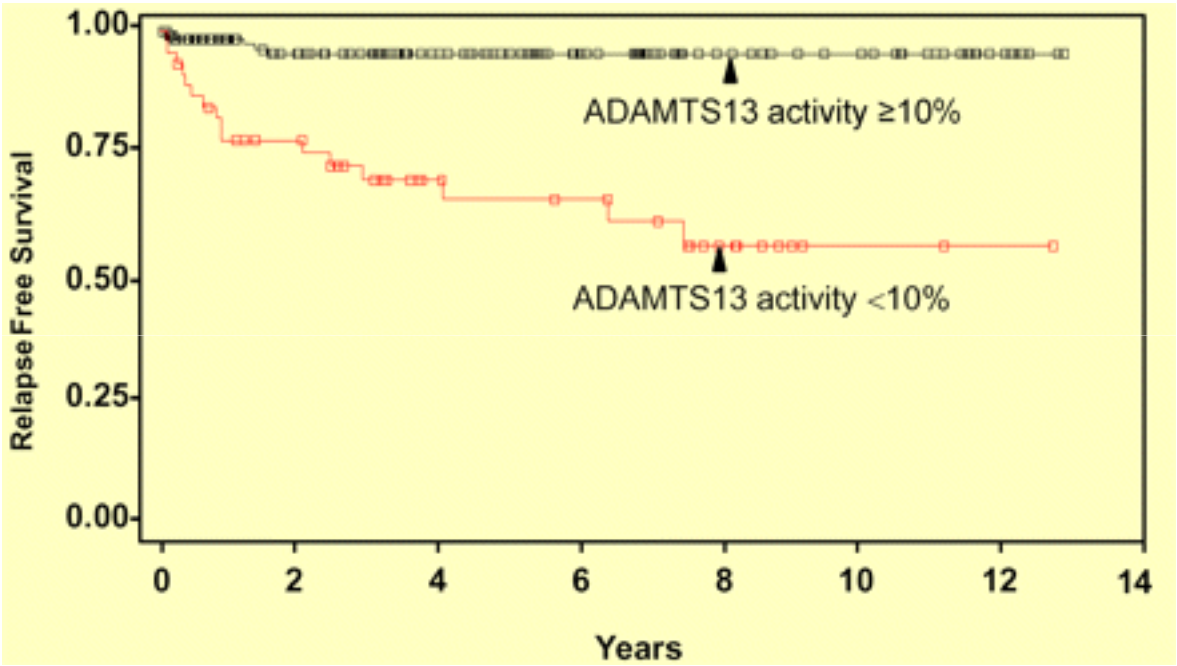
Laboratory Diagnosis of aHUS

- **Conventional complement tests: C3, C4, CH50, AH50**
  - **Abnormal in 30%**
  - **Not specific for aHUS**
- **Plasma CFH and CFI protein concentrations**
  - **Decreased in 30% of patients with CFH or CFI mutations**
- **Mutation analysis (CFH, MCP, CFI, CFB, C3, THBD)**
  - **Abnormal in 40% (sporadic) - 70% (familial)**
- **CFH antibody**
  - **5% - 10%**

Clinical Differentiation between aHUS and TTP

- **Under-appreciated in aHUS**
  - **CNS: Mental changes, seizures, focal deficits**
- **More common in aHUS**
  - **GI: abdominal pain, vomiting, pancreatitis, diarreha**
- **Rare in TTP**
  - **Advanced renal failure (Cr_{max} >2.5 mg/dL), hypertension**
  - **Abnormal vascular permeability**
    - **Pleural/pericardial effusions, ascites, anasarca**
    - **Non-cardiogenic pulmonary edema (ARDS)**
    - **Cerebral edema (MRI: posterior reversible encephalopathy syndrome, PRES)**
    - **Exudative retinopathy**
- **Correlation between CBC and disease severity**
  - **TTP: the platelet count reflects the severity of the disease**
  - **aHUS: Organ dysfunction may occur without worsening thrombocytopenia**

Does the Presence of ADAMTS13 Signify a Better Prognosis?

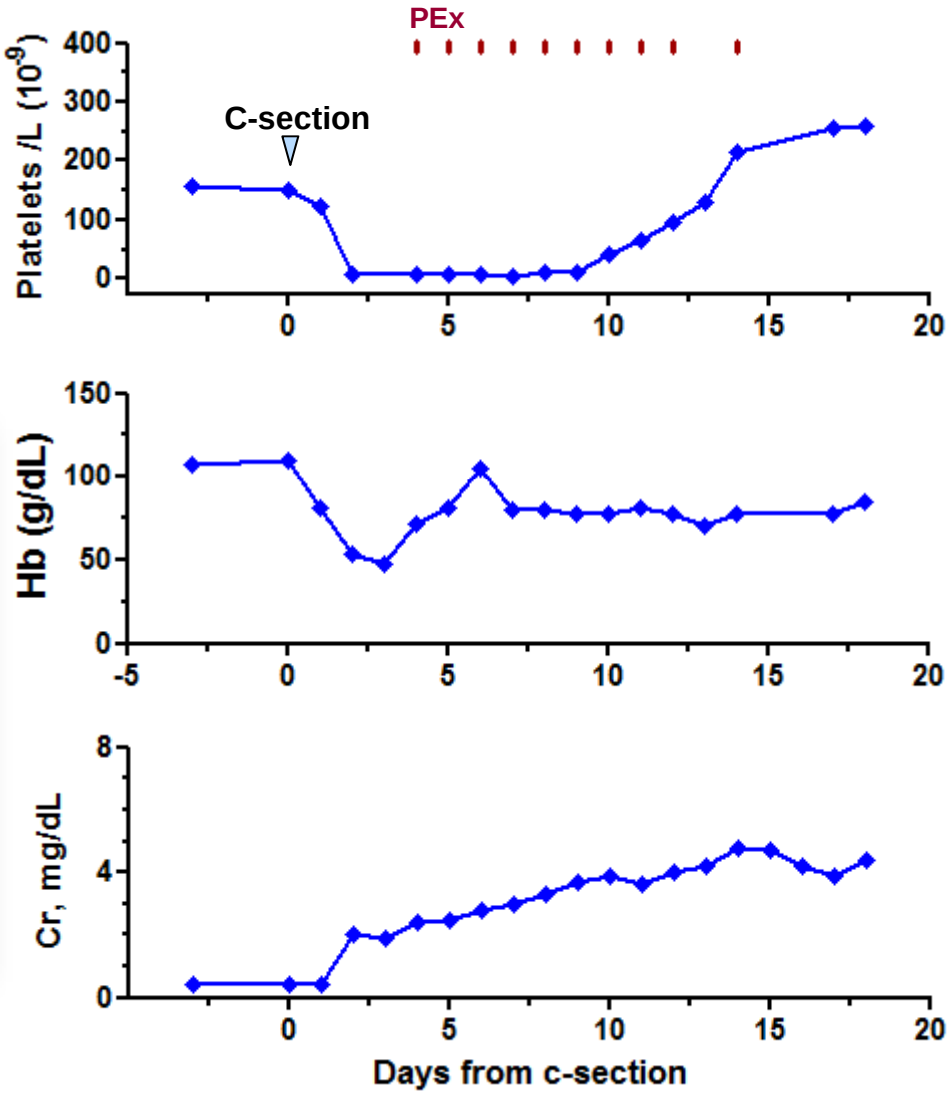


Long XL, Blood 2010;115:1475

Case 5: Relapse of aHUS May Not Be Accompanied with Thrombocytopenia

26 y/o
- **D2 after C/S, headache, listless, abdominal pain**
- **Thrombocytopenia MAHA, LFT normal**
- **ADAMTS13=70%**

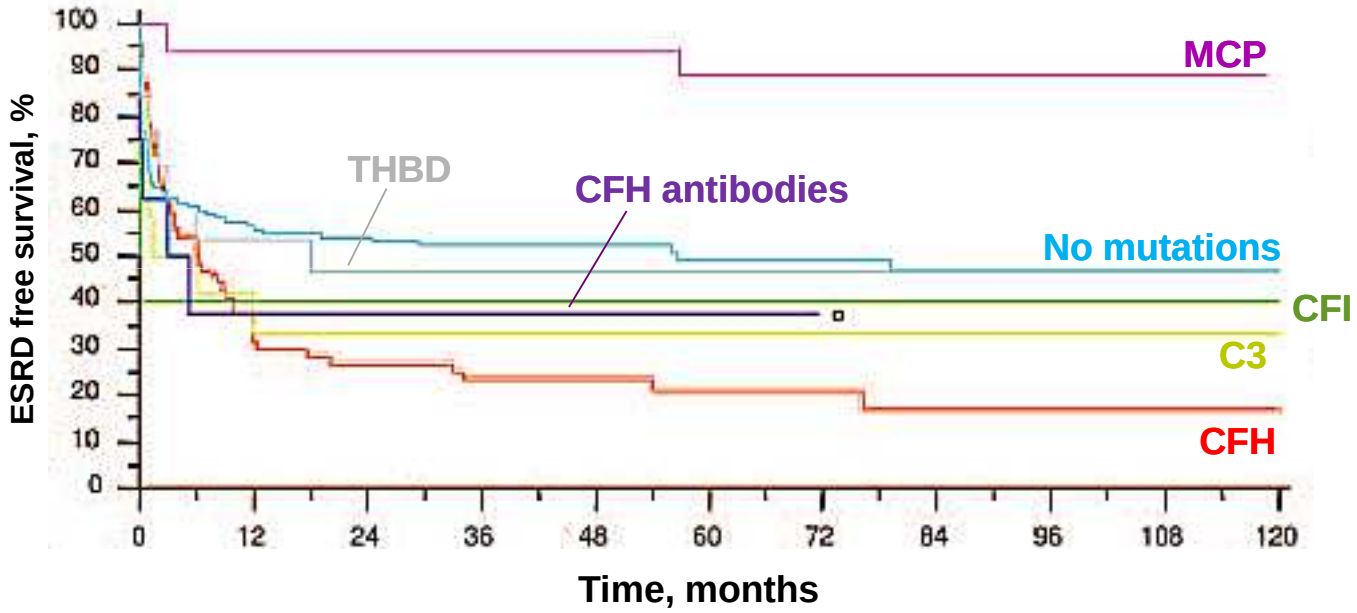
Day 55:
- **Headache**
- **A brief episode of syncope**
- **Respiratory distress**
- **Bilateral chest infiltrates**
- **Intubated**
- **Hb 94 g/L, MAHA**
- **Platelet 180x10^9/L**
- **Cr 7.1**
- **ADAMTS13=72%**



(Personal unpublished data)

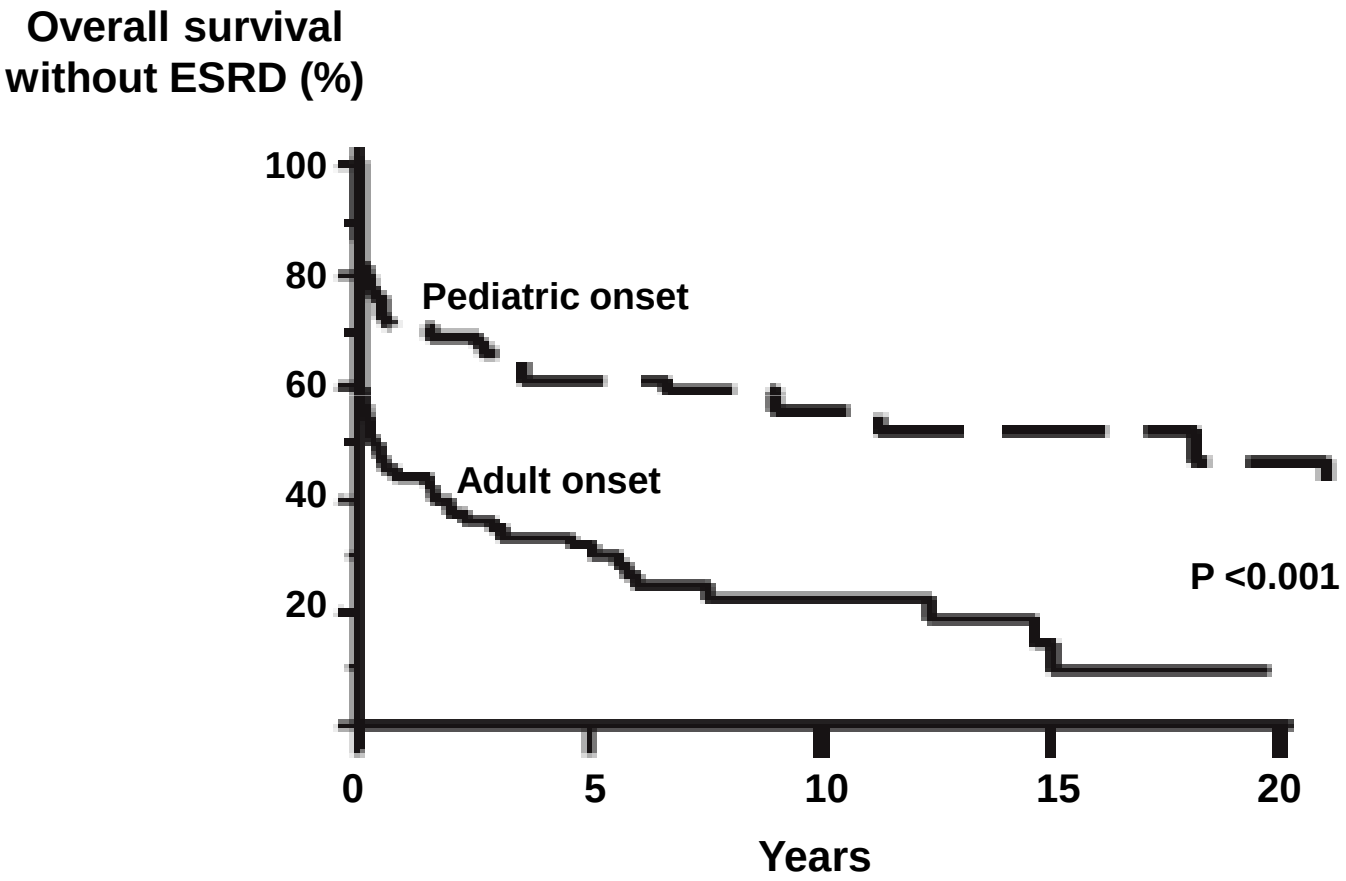
aHUS: Molecular Defects and Prognosis

Event: relapsing aHUS, death or ESRD



Based on
Noris M et al, CJASN 2010;5:1844

ESRD-Free Survival of aHUS: Worse in Adults

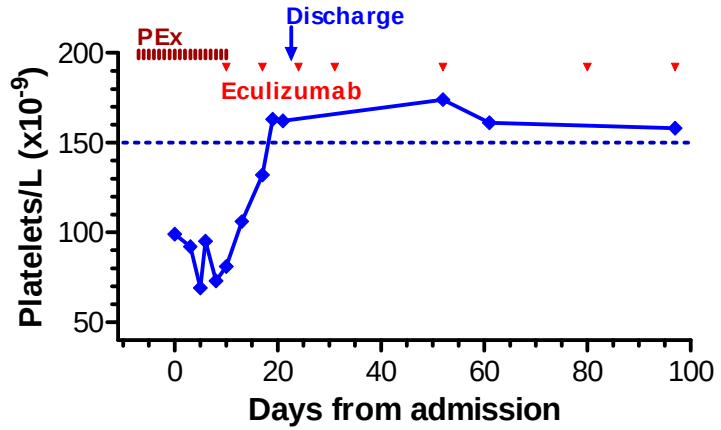


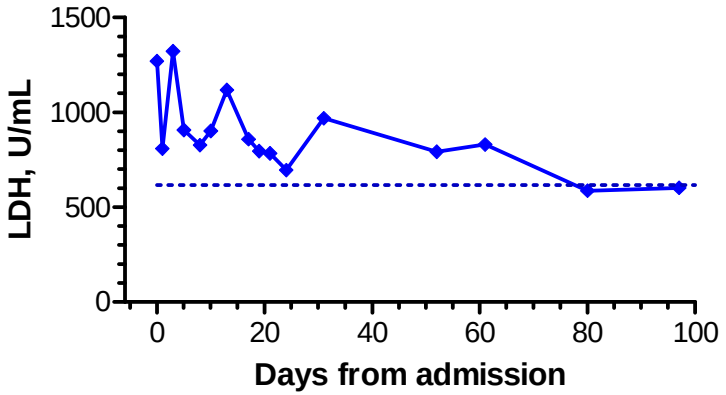
Based on
Fremeaux-Bacchi et al, CJASN 2013;8:554

Case 6: aHUS after Autologous HSCT

54 year-old female

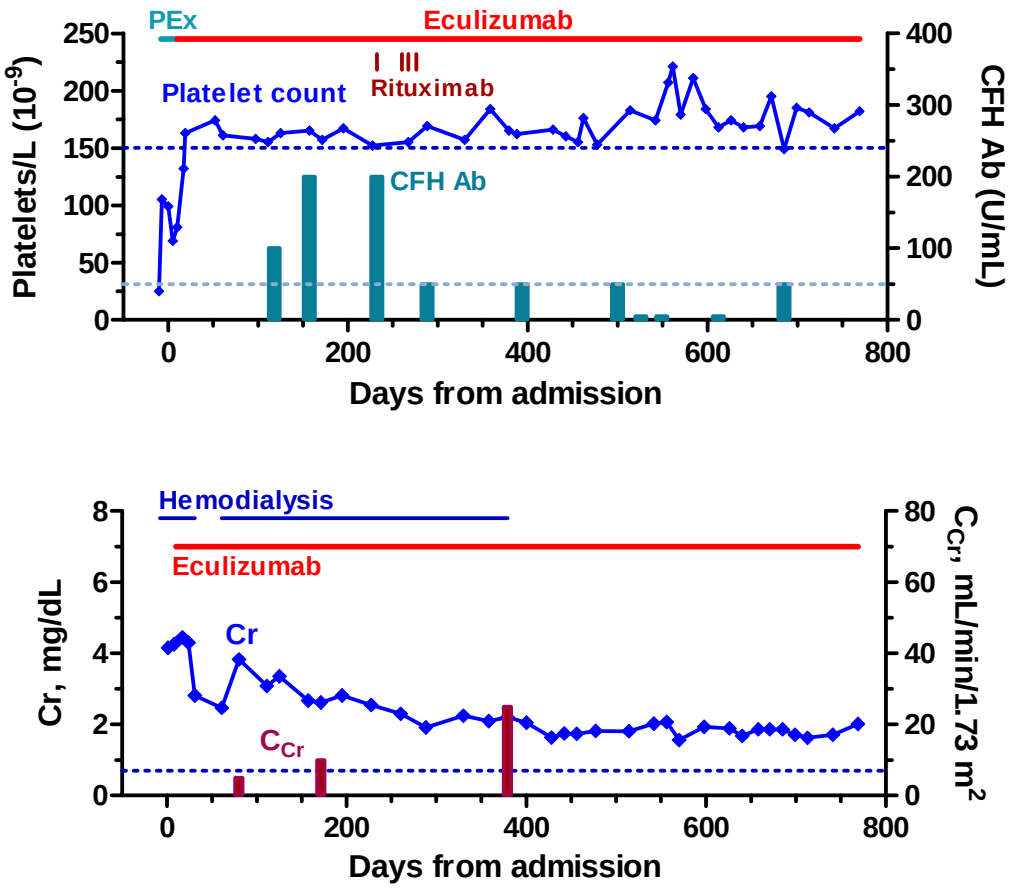
- **Three months PTA: auto-HSCT for myeloma**
- **Day -9:**
  - **Abdominal pain, vomiting, diarrhea**
  - **Mild thromobocytopenia, anemia & azotemia**
  - **Stool: *C. difficile* +**
  - **Tx: metronidazole**
- **Day -7:**
  - **Seizures, anuric**
  - **Platelet 25x10^9/L, MAHA**
  - **Thalidomide, acyclovir were discontinued**
  - **PEx for 'TTP'; HD for ARF**
- **Day -1: ΔMS, vomiting, intubated**
- **Labs at transfer:**
  - **Hb 85 g/L, platelet 99x10^9/L, , LDH 1,271 U/L**
  - **Cr 4.0 mg/dL**
  - **Smear: schistocytes**
  - **ANA, shiga toxin assay, viral cultures: negative**
  - **(ADAMTS13: 60%)**





Tsai HM. TTP and aHUS, an update. Hematol Oncol Clin North Am (in press)

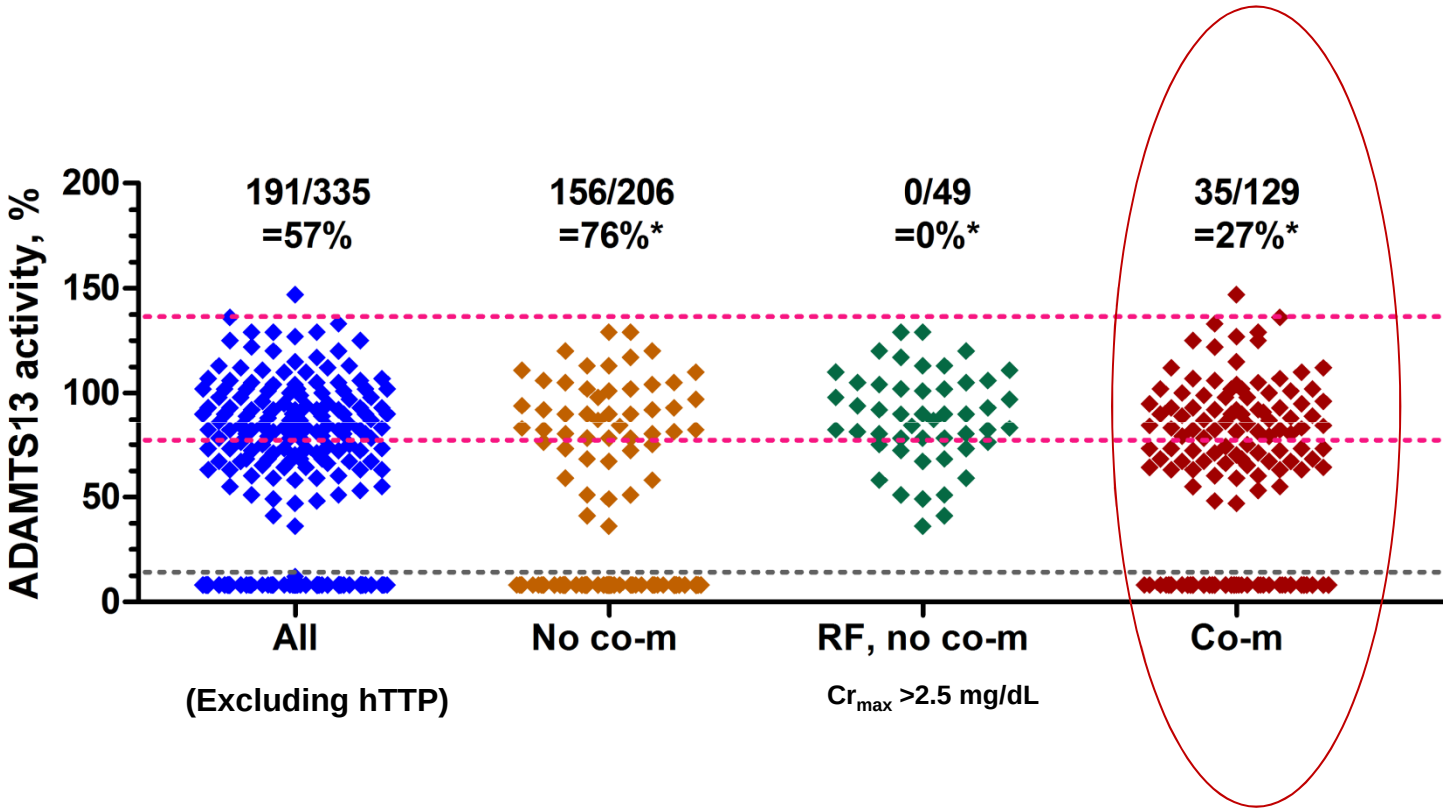
Case 6: Atypical HUS after Autologous HSCT



Progressive renal failure is not inevitable nor irreversible in aHUS.

Tsai HM. TTP and aHUS, an update. Hematol Oncol Clin North Am, in press

MAHA with Comorbidity



Tsai HM. TTP and aHUS, an update. Hematol Oncol Clin North Am (in press)

Classification of Comorbidity in Patients with MAHA

I. Triggers of TTP or aHUS presentation in patients with the disease
- **Pregnancy, IV contrasts, pancreatitis, infection, inflammation, surgery, trauma, etc.**

II. Inducers of ADAMTS13 inhibitor or defective complement regulation (e.g. CFH autoantibodies)
- **Ticlopidine, HIV (ADAMTS13 inhibitors)**
- **Hematopoietic stem cell therapy**

III. TMA via other mechanisms
- **Shiga toxin associated HUS**
- **Microbial neuraminidases (e.g. Streptococcus pneumonia or influenza virus)**
- **Angiogenesis inhibitor: e.g. bevacizumab**

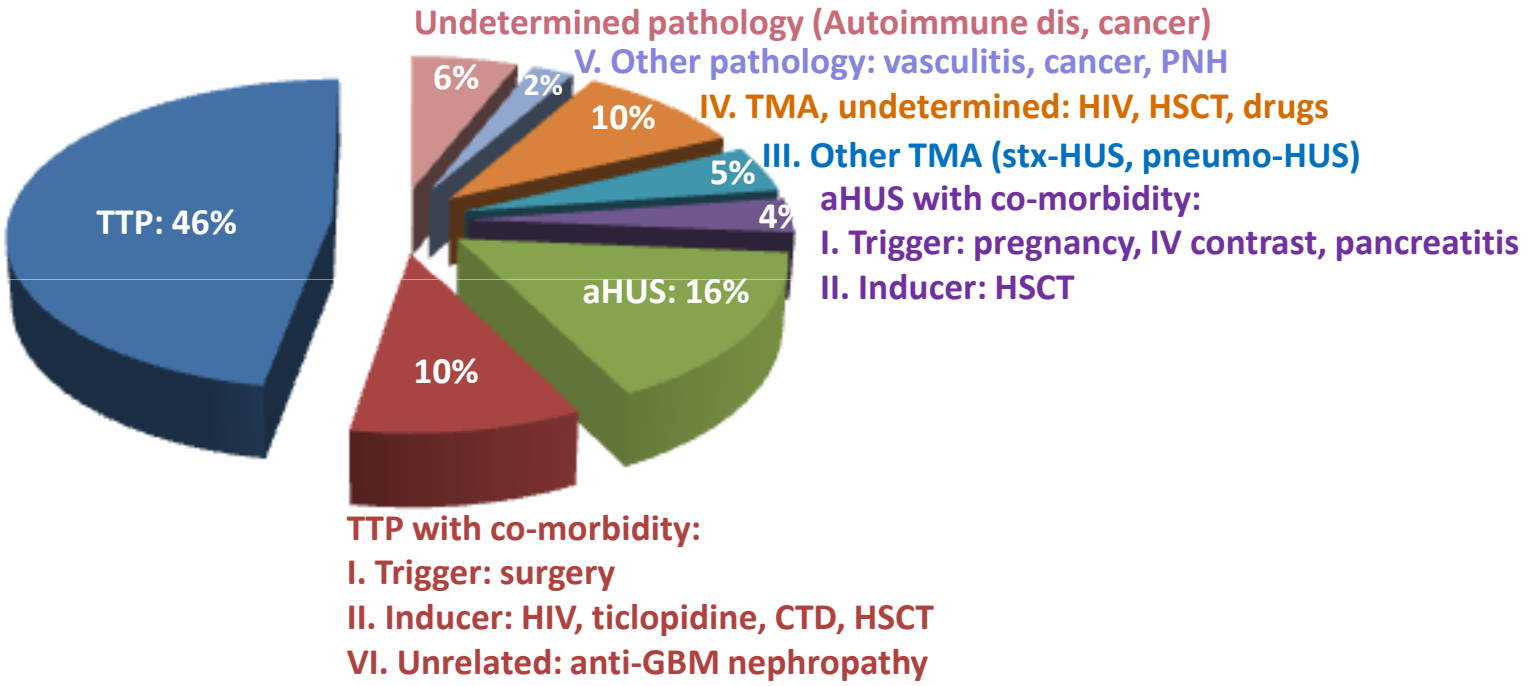
IV. TMA, undetermined mechanisms
- **Hematopoietic stem cell therapy, HIV infection**
- **Systemic autoimmune diseases, drugs (e.g. gemcitabine, mitomycin, quinine, cocaine, etc.)**
- **Calcineurin inhibitors/post-renal transplants: may represent aHUS triggered by surgery**
- **Severe hypertension: may be a consequence of aHUS**

V. Other types of pathology
- **Fibrin thrombosis: DIC, CAPS, HELLP syndrome, HIT, PNH, etc.**
- **Vasculitis: Autoimmune diseases (e.g. lupus); infections (e.g. Rocky Mountain spotted fever)**
- **Intravascular clusters of neoplastic cells**
- **Intravascular devices: e.g. VAD, ECMO, prosthetic heart valves**

VI: Not directly related to MAHA

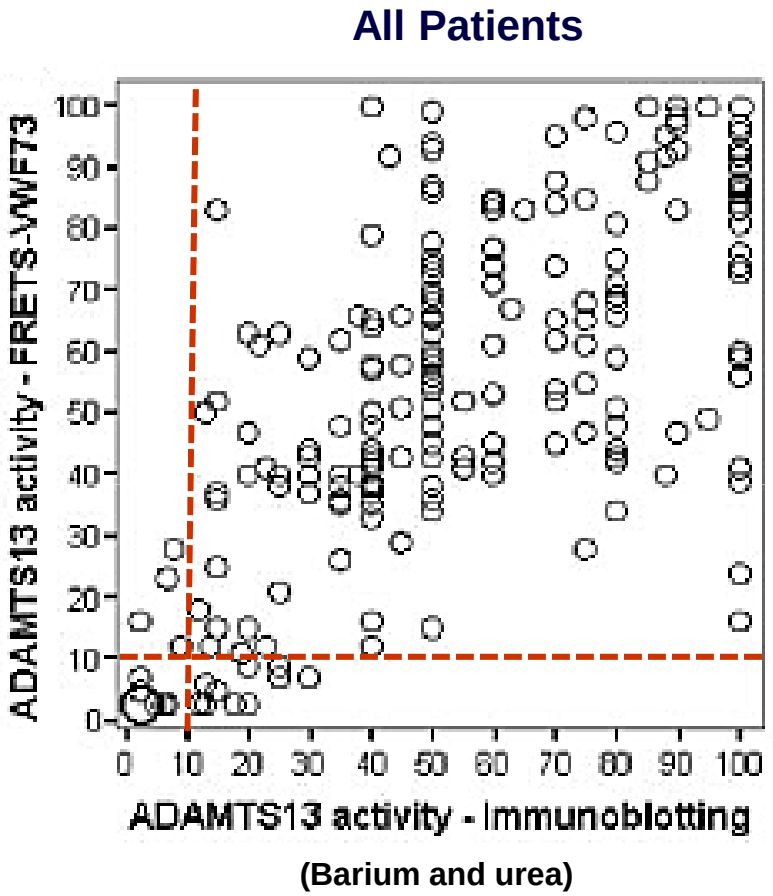
Causes of MAHA

N = 337 (excluding 27 cases of hereditary TTP)

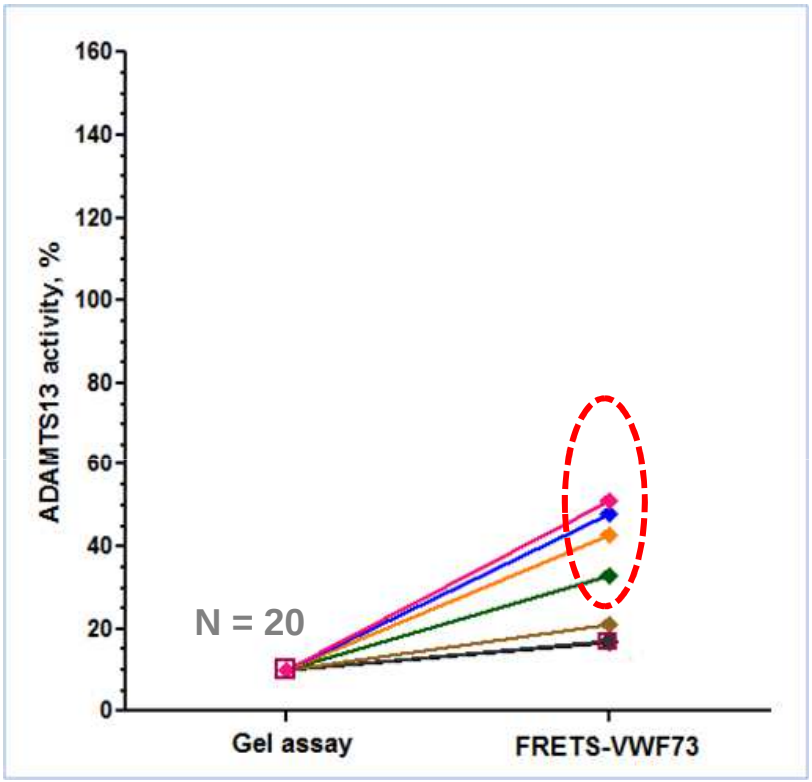


Tsai HM, Microangiopathic hemolytic anemia. In Encyclopedia of Human Biology (in press)

ADAMTS13 Assay Variability Remains a Critical Issue



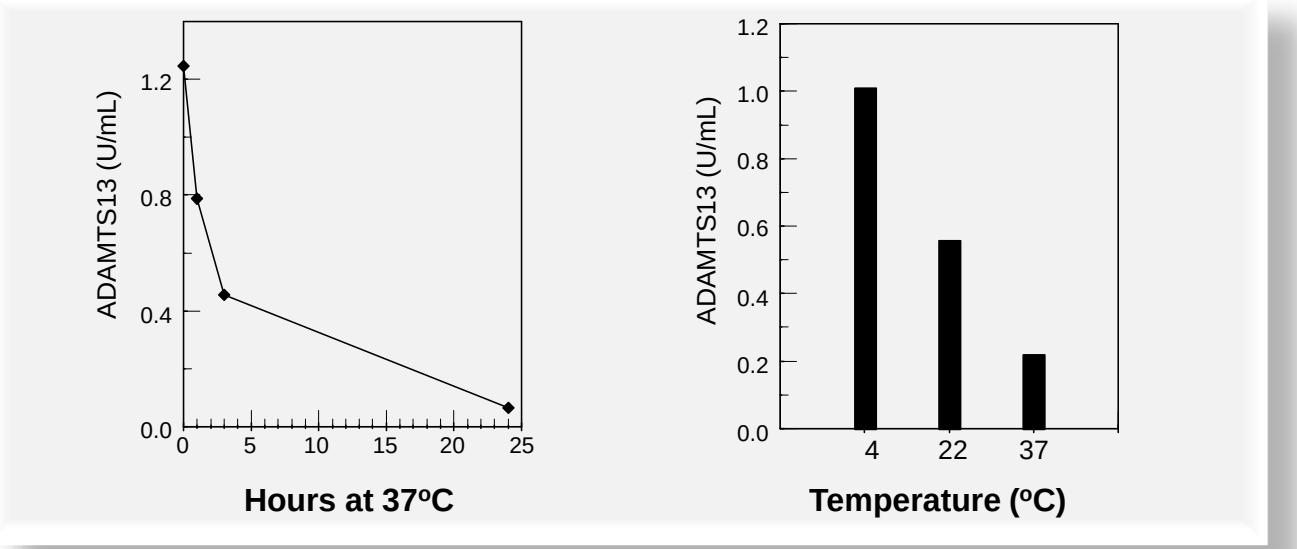
Kremer Hovinga JA et al, Blood 2010;115:1500

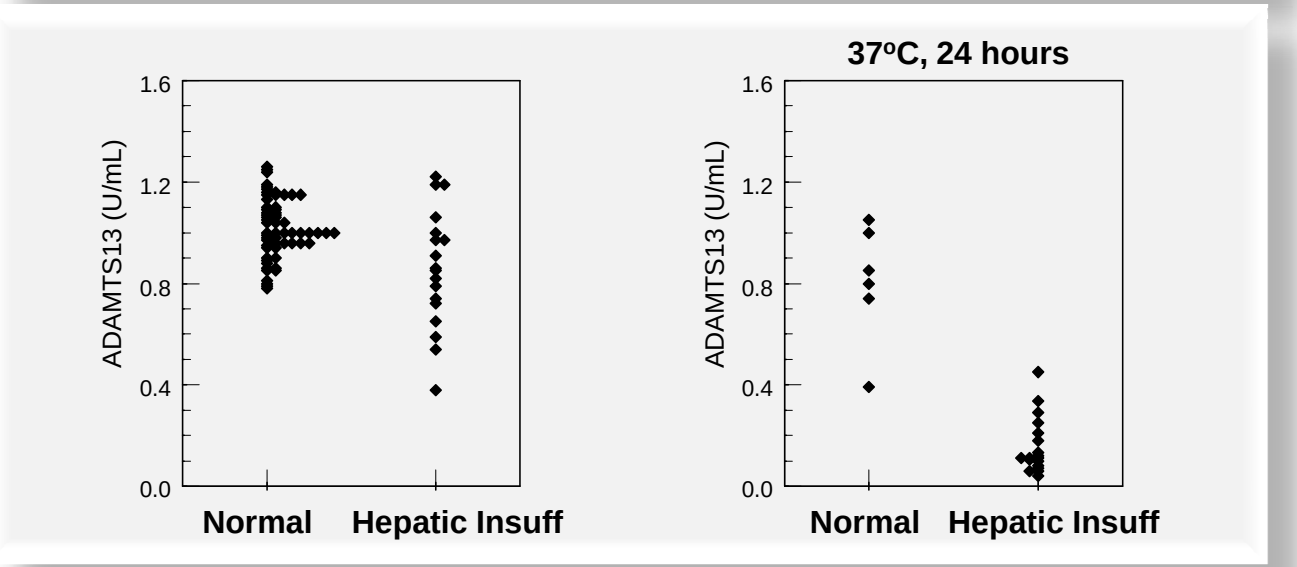


Tsai HM, Hematol Oncol Clin North Am, in press

With assays that do not completely segregate TTP from other causes, the diagnosis of TTP is problematic.

Stability of Plasma ADAMTS13 Activity





Personal unpublished data

Reliability of ADAMTS13 Assays

VWF cleavage in the presence of Ba^{++} and urea

- Certain ADAMTS13 polymorphisms are more sensitive to urea
- Ba^{++} activation may be affected by plasma protein conc. and other factors
- High plasma VWF levels cause spuriously low levels

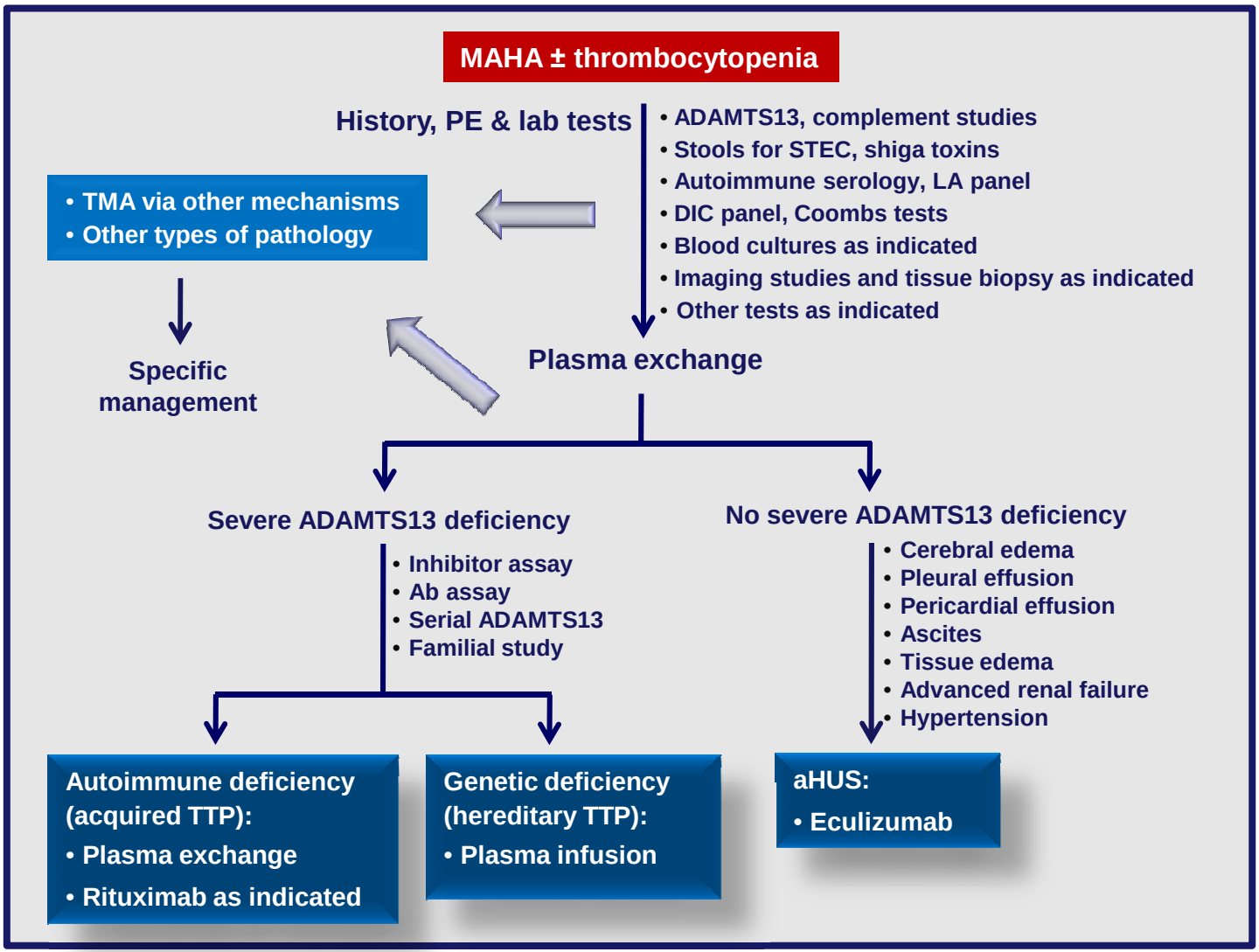
VWF73-FRETS

- ***Spuriously high levels***:
  - Non-specific cleavage of the peptide substrate
  - Dissociation of ADAMTS13 from inhibitors at low pH (6.1)
- ***Spuriously low levels***
  - Plasma Hb
  - Plasma bilirubin
  - Other unknown factors of fluorescence quenching

Stability of ADAMTS13 in plasma

- Stable in normal plasma
- Unstable in pathological conditions: DIC, liver diseases, sepsis, etc.

Approaches to the Management of MAHA



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