

ADAMTS13 Activity and Antibody Profiles in TTP Arising in a Background of SLE

Elizabeth Biller, Shangbin Yang, and
Haifeng Wu

Department of Pathology, Ohio State
University, Columbus, OH

Background

- Acquired idiopathic TTP is an autoimmune disease, due to production of autoantibody against ADAMTS13
- TTP is known to coexist with other autoimmune diseases
- TTP occurs in up to 4% of systemic lupus erythematosus (SLE) patients
 - SLE usually precedes TTP

Case report

A longitudinal evaluation of a patient
with longstanding SLE who
developed TTP many years later

Case Summary

- 26 year old African-American female
- SLE diagnosed at age 9; no renal involvement
- Medications at the time of TTP presentation (no recent changes)
 - Immunosuppressive: prednisone, mycophenolate; previously on azathioprine until 2006
 - Others: hydroxychloroquine, clopidogrel, sulfamethoxazole/trimethoprim
- Presented to ED 1/1/2009 with cold symptoms, abdominal pain, petechiae, hematuria

Labs at the time of presentation

- Hemoglobin: 9.9 g/dL
- Platelets: 15 k/uL
- Direct bilirubin: 0.4 mg/dL
- LDH: 601 U/L
- Urinalysis: large blood
- Urine pregnancy test: negative
- Creatinine: 0.78 mg/dL
- C3: 38 mg/dL (80-178 mg/dL)
- Anti-dsDNA antibody: positive, titer 1:40
- Lupus anticoagulant: positive
- Peripheral blood smear: 1+ schistocytes

Clinical course

- Admitted into hospital
- Initial treatment: prednisone, IVIG
- Platelets continued to drop (8 k/uL)
- TTP suspected and begun on daily plasma exchange (PE)
- ADAMTS13 activity less than 2.5%
- Platelets and LDH normalized after 4 PE procedures
- Received two additional plasma exchanges, then discharged on prednisone and mycophenolate

Clinical course, continued

- Readmitted four days later with oozing from pheresis catheter site, platelets of 11 k/uL, LDH 446 U/L
- Resumed daily PE
- Mycophenolate discontinued, cyclophosphamide begun
- Platelets and LDH normalized after 19 PE procedures
- But unable to be weaned from PE afterwards—still required PE 2-3 times/week for 3 months, on cyclophosphamide and prednisone

Clinical course, continued

- Patient started on cyclosporine at end of April
- Was able to be weaned from plasma exchange on cyclosporine and cyclophosphamide
- Completed a one month course of cyclophosphamide, then restarted mycophenolate
- Completed six month course of cyclosporine
- Being maintained on prednisone, mycophenolate
- Has not required plasma exchange for >24 months

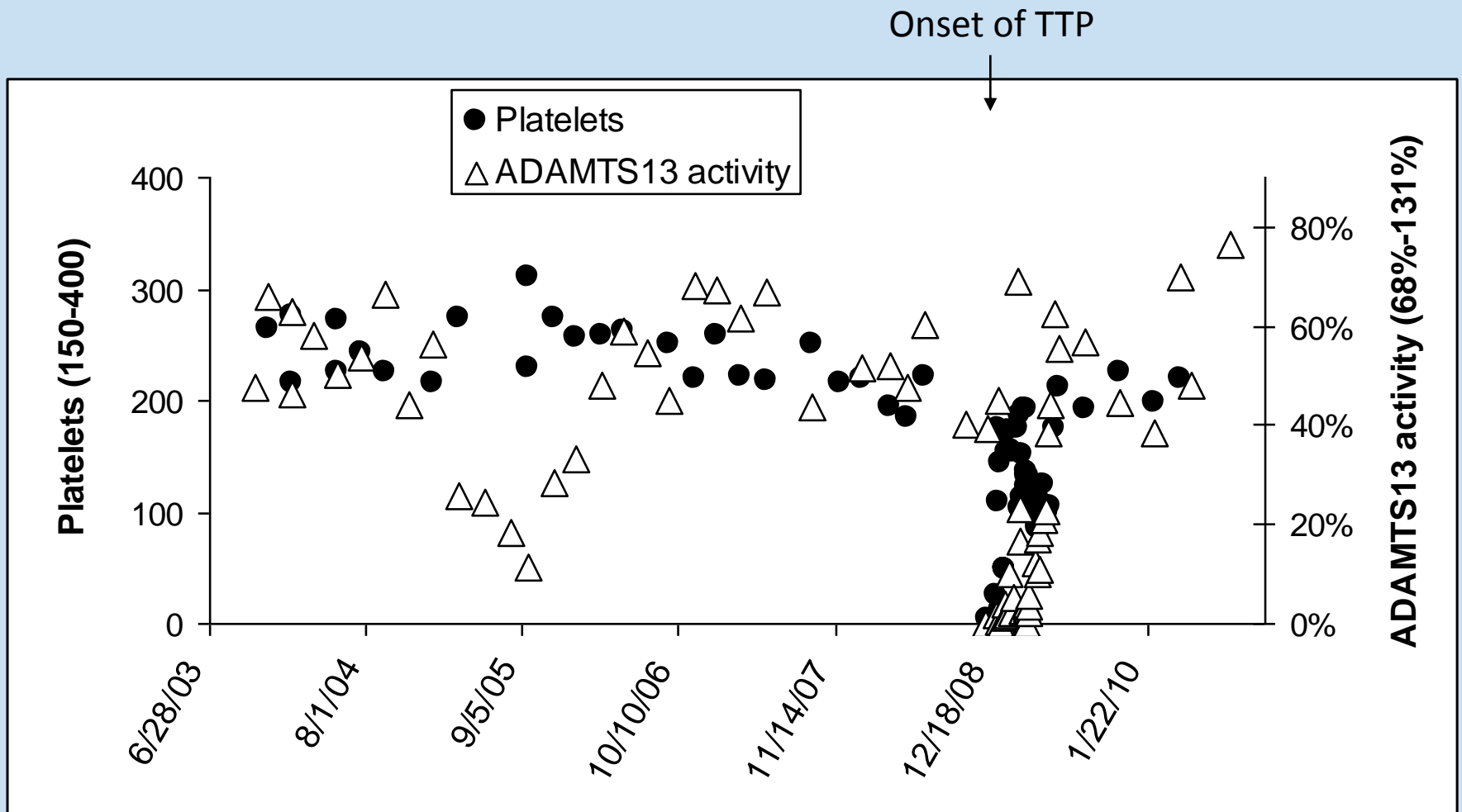
What can we learn from this intriguing case with 8 years of longitudinal followup and quarterly sample banking?

Examples of Questions

- What were the clinical factors that potentially triggered TTP in this patient?
 - Lupus flares?
- Were there any changes in ADAMTS13 activity levels or antibody titers over time, including before onset of TTP?
- Were there any alterations in ADAMTS13 antibody specificity or isotype during SLE, before onset of TTP, or during the course of TTP treatment/remission?
- What was the status of vWF multimer patterns and their changes over the course of SLE and TTP?
- More...

Methods

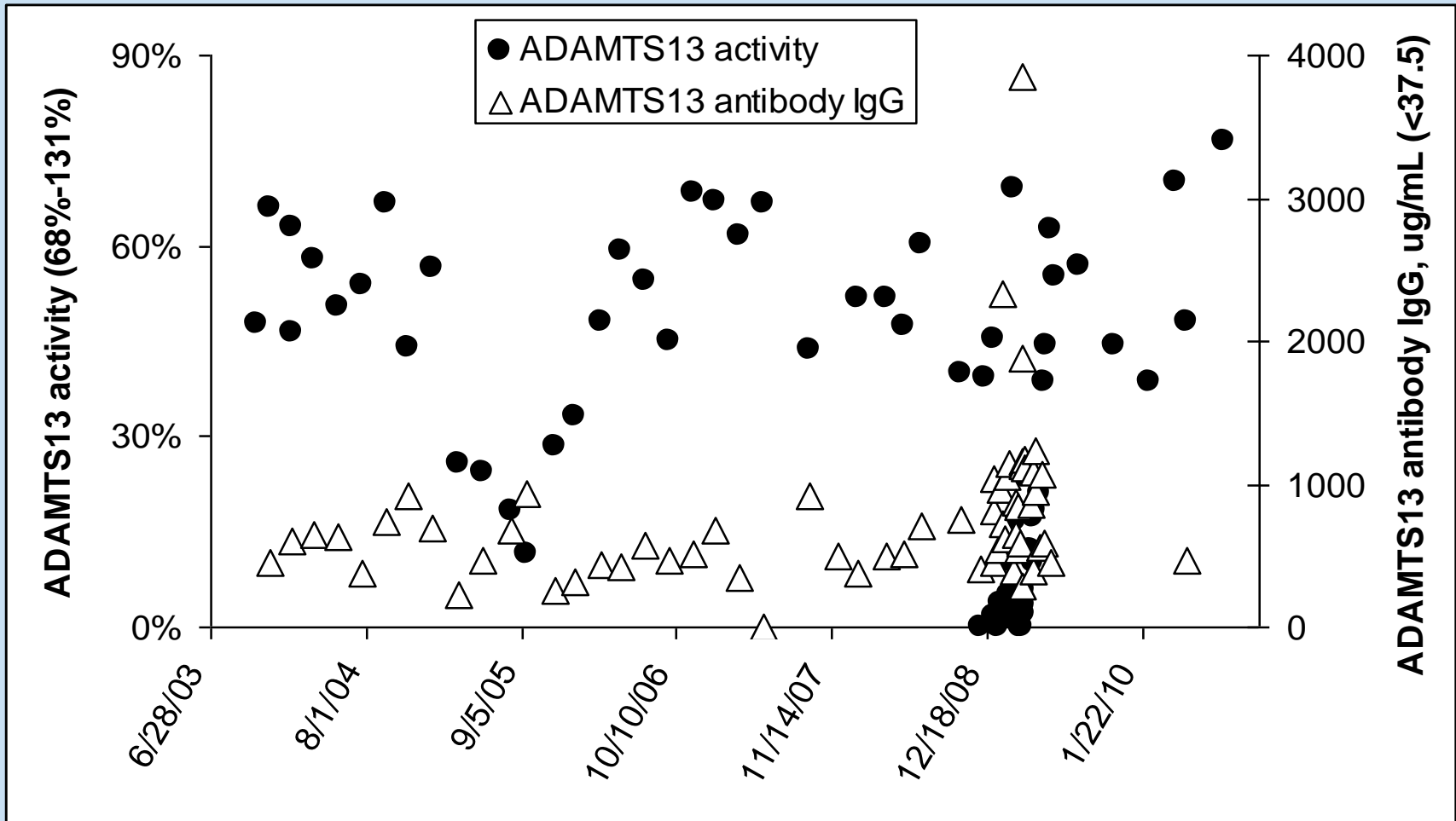
- Analyzed quarterly banked patient samples since 2003
- ADAMTS13 activity measured using SELDI-TOF mass spectrometry (PCS4000)
 - Samples with activity <5% retested using a protocol that measures activity to as low as 0.5%
- Determination of ADAMTS13 inhibitor titer
- Quantification of ADAMTS13 autoantibody (IgG) measured using commercially available ELISA kits



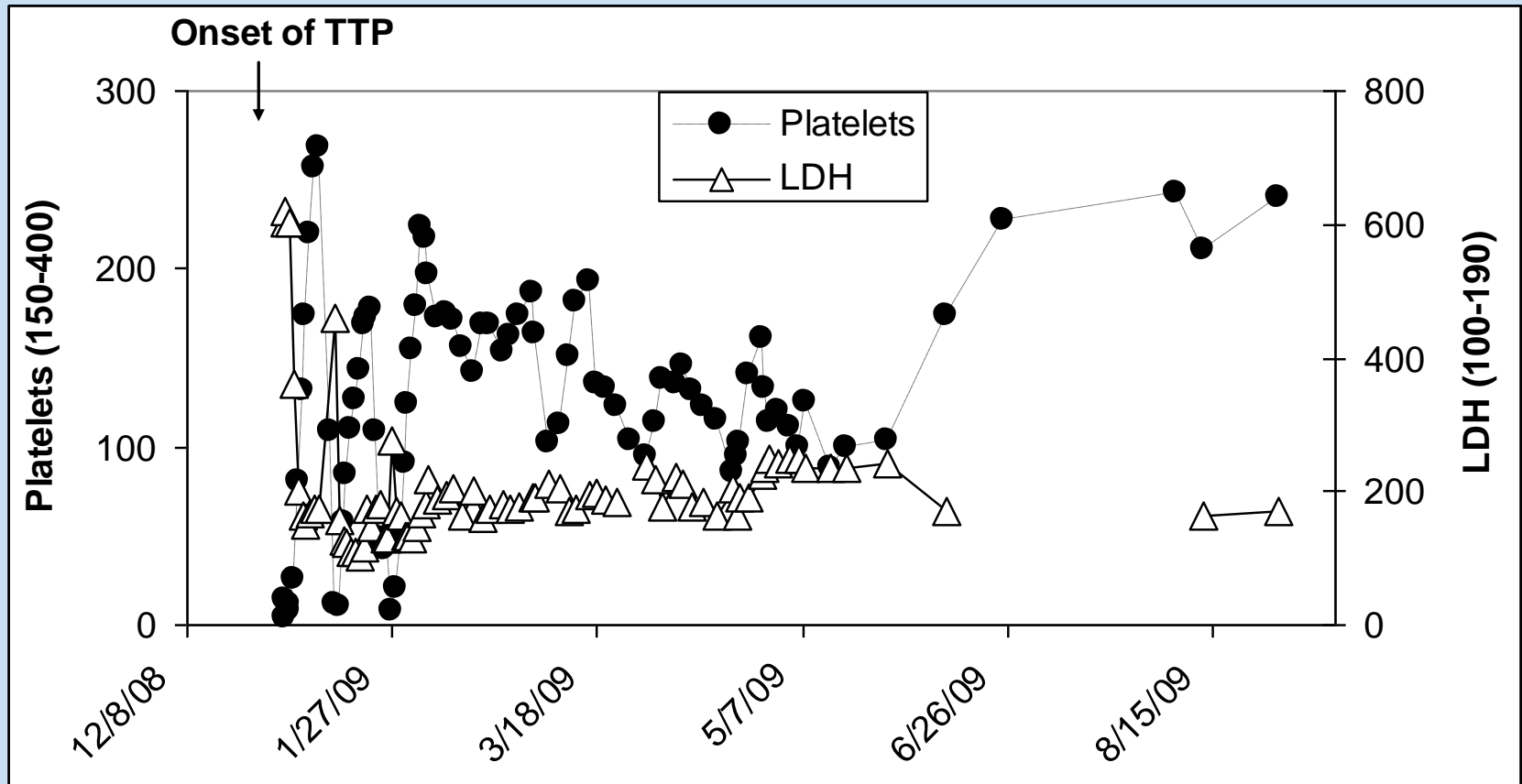
From 2003 to 2010

- Platelet counts had been normal until onset of TTP in 2009
- However, ADAMTS13 activity fluctuated, declined to 11% in 2005 without overt TTP, and eventually dropped to <2.5% in 2009 with a full blown TTP

Onset of TTP

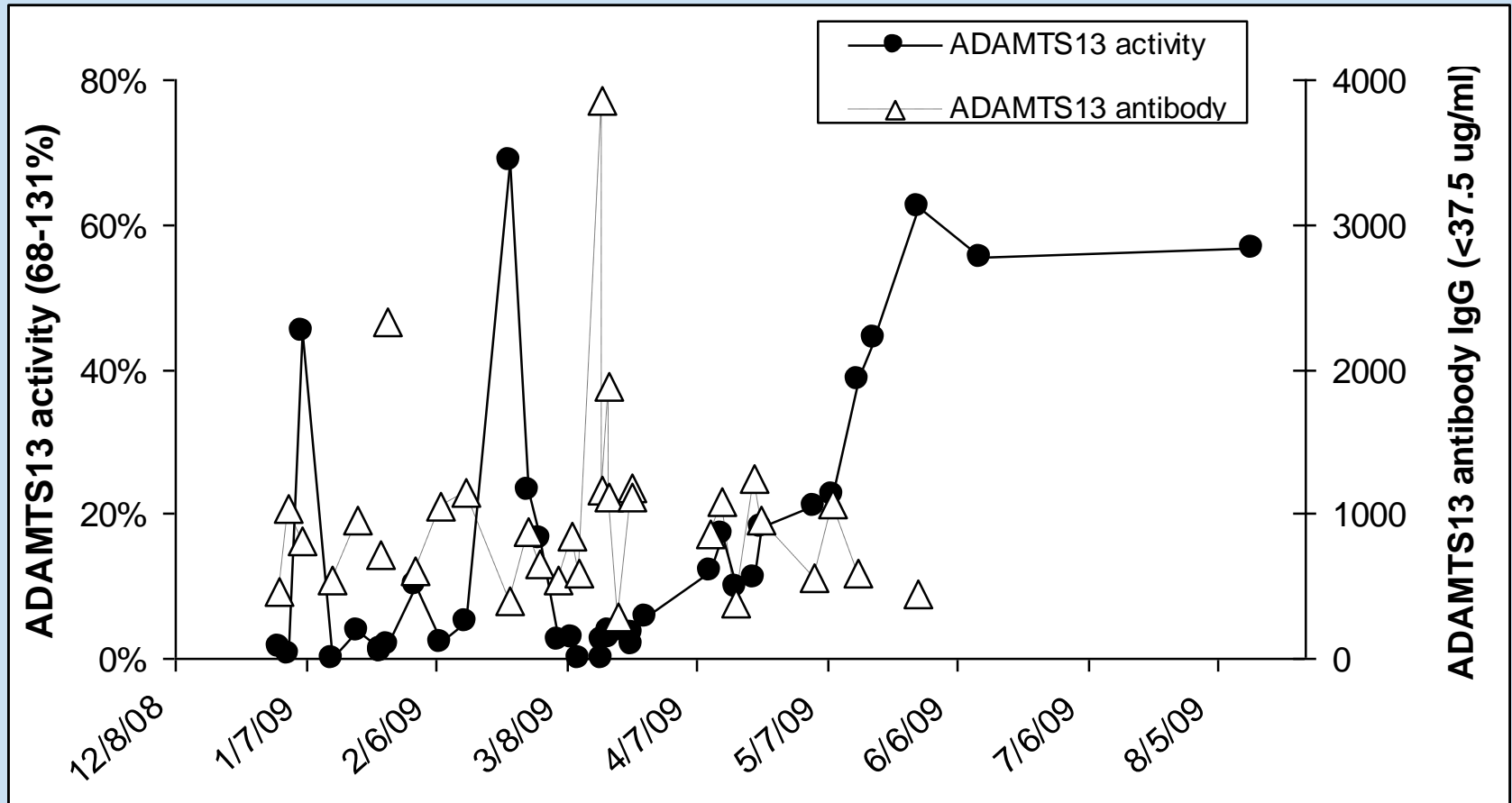


- In accordance with the changes of ADAMTS13 activity, ADAMTS13 IgG level increased to 1000 ug/mL in Sept. 2005 and at the onset of TTP, IgG level reached as high as >3000 ug/mL.
- The baseline level of ADAMTS13 IgG antibody in this patient is higher than the reference ranges, consistent with reports in the literature



- Patient responded well to PE therapy initially but shortly developed TTP exacerbation that required prolonged PE treatment to achieve a sustained remission

Onset of TTP



Western blotting to show different ADAMTS13 variants

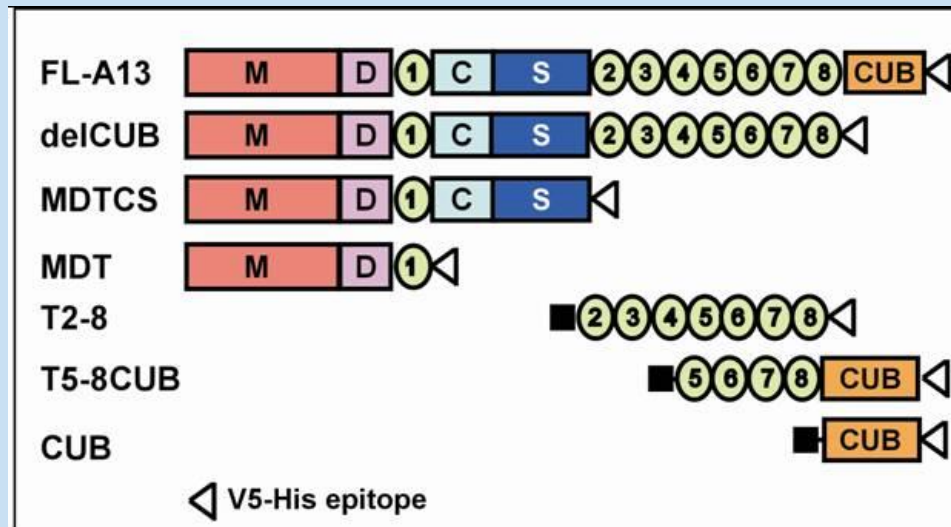
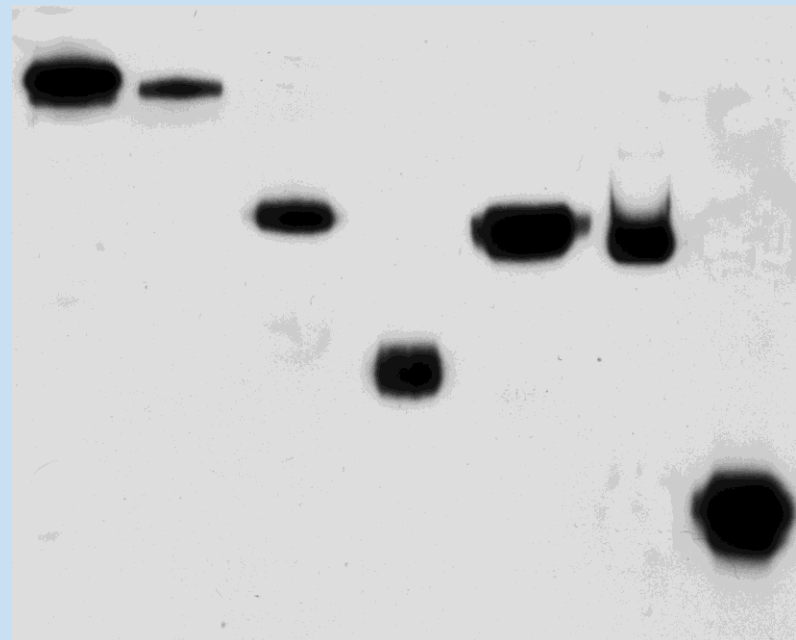


Figure courtesy Dr. Long Zheng, University of Pennsylvania

The proteins were fractionated on 10% SDS PAGE and transferred to the PVDF membrane. The membrane was incubated with monoclonal anti-V5 IgG (1:4000) in TBS containing 2.5% non-fat milk and 0.05% Tween-20, followed by a peroxidase-conjugated goat anti-mouse IgG (1:4000). The enhanced chemiluminescent reagents were used to determine the bound secondary antibody.

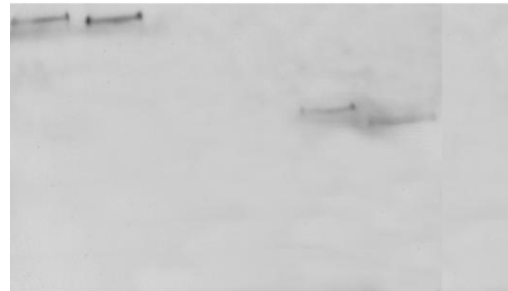
FL-A13 delCUB MDTCS MDT T2-8 T5-8CUB CUB



ADAMTS13 antibody specificity changes over the course of the disease

Sample 1109, 1/23/2004

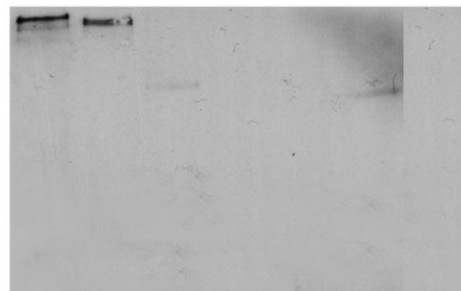
FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



SLE followup

Sample 1111, 5/19/2004

FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



SLE followup

Sample 1122, 3/27/2006

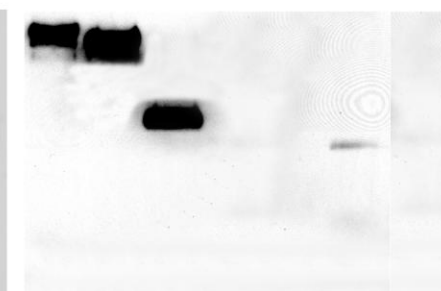
FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



SLE followup

Sample 1065, 1/24/2009

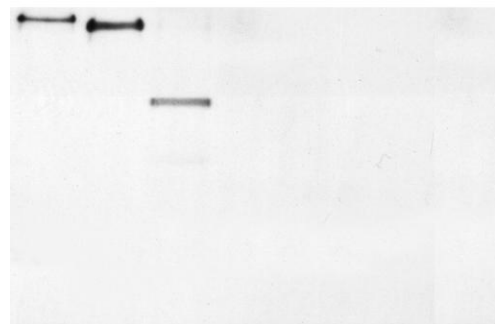
FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



TTP onset

Sample 1172, 3/9/2009

FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



During the treatment

Sample 1322, 5/14/2009

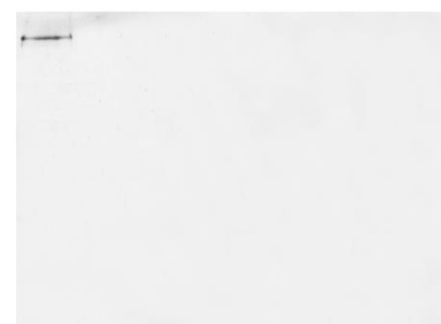
FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



Remission

Sample 1998, 5/13/2010

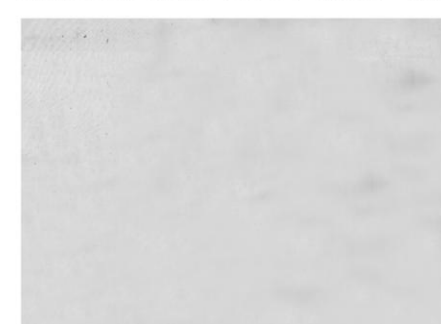
FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



Sustained remission with a full recovery of AD13 activity

Sample 2399, 11/18/2010

FL-A13 delICUB MDTCS MDT T2-8 T5-8CUB CUB



What we have learned so far from this case

- ADAMTS13 activity fluctuates over the course of SLE
- 11% ADAMTS13 activity appears to be hemostatic in this case
- The baseline ADAMTS13 IgG level is high and fluctuates longitudinally, and appears to be concordant with ADAMTS13 activity level
- ADAMTS13 antibody binding properties change over time
 - Without TTP: specificity largely against thrombospondin repeats and CUB domain at C terminus
 - During TTP: specificity mainly against cys rich/spacer domain with a high titer
 - A sustained recovery from TTP is associated with loss of antibody specificity against cys rich/spacer domains

Ongoing studies

- Completing antibody specificity studies
- Evaluating VWF multimer patterns over the course of disease
- Evaluating Ristocetin cofactor activity
- Evaluating ADAMTS13 antigen and inhibitor titer longitudinally

Acknowledgments

- Dr. Haifeng Wu
- Dr. Shangbin Yang
- Sara Haven
- April Horne
- Dr. Spero Cataland
- Dr. Lee Hebert