ADAMTS13 and the pathogenesis of thrombotic thrombocytopenic purpura (TTP)

9th ECAT Participants' Meeting
Leiden, 13.-14.11.2014

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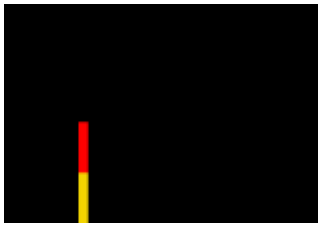


New affiliation

Bernhard Lämmle

Guest Professor/Senior Consultant

(since November 2013)

Disclosure

- Employed by CTH, University Medical Center, Mainz
- Research grant from Baxter Bioscience (Hereditary TTP Registry, Investigator-initiated Research)
- Chairman Data Safety Monitoring Board, BAX 930 study (started in 2014)

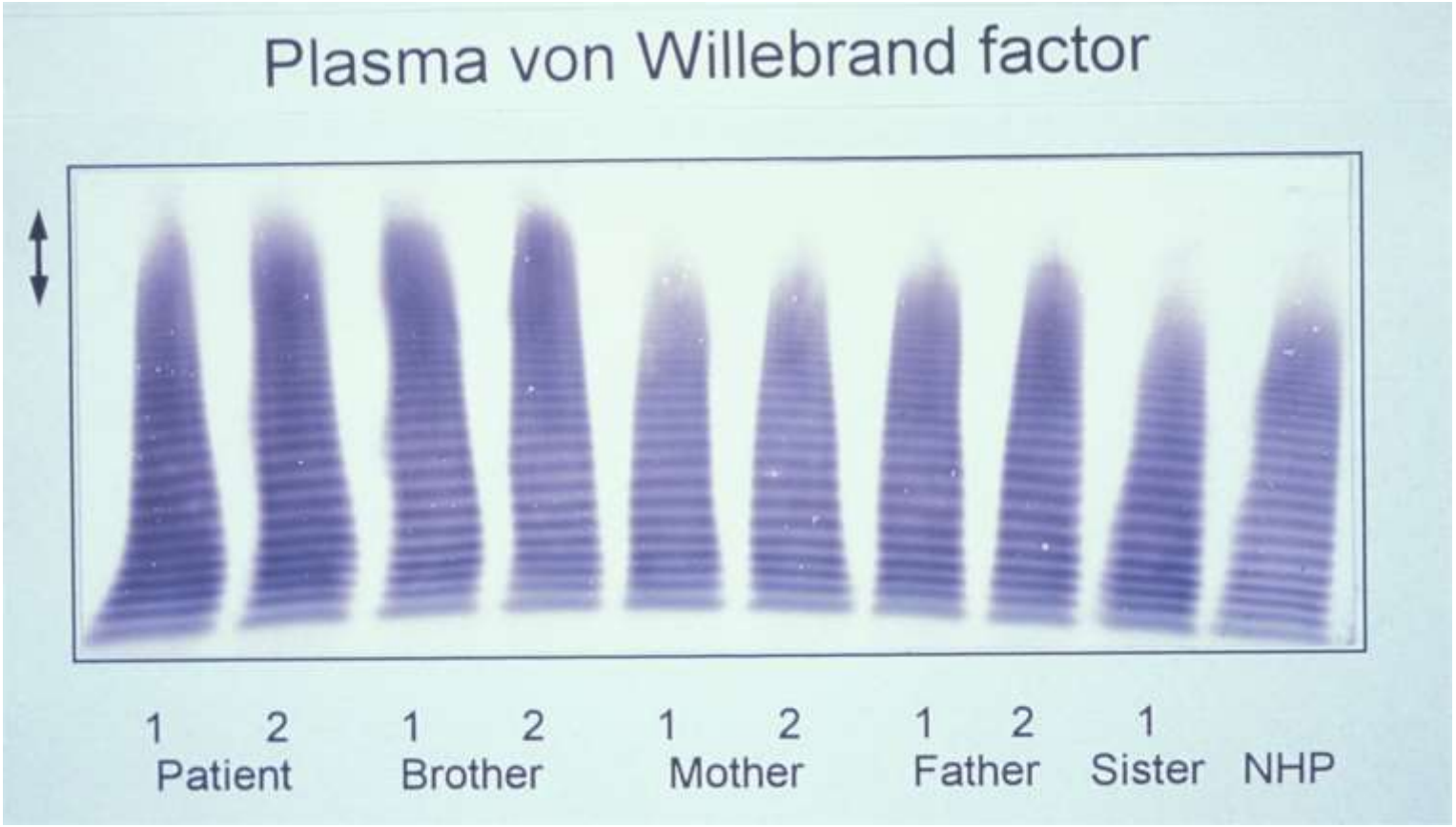
Outline

- **Historical Notes → Thrombotic Microangiopathies 2014**
- Laboratory investigations, assays of ADAMTS13

Historical Notes (I)

- 1924 Moschcowitz: New disease
- 1947 Singer et al.: Thrombotic thrombocytopenic purpura
- 1955 Gasser et al.: Hemolytic uremic syndrome
- 1966 Amorosi, Ultmann: Pentad defining TTP, Mortality 90%
- 1982 Moake et al.: ULvWF multimers in chronic relapsing TTP (in remission)
- 1997 Furlan et al.: Severe deficiency of VWF-cleaving protease in 2 brothers with chronic relapsing TTP
- 1998 Furlan et al., Tsai and Lian: Severe acquired (or constitutional) deficiency of VWF-cp in most patients with acute TTP

Historical Notes (II)

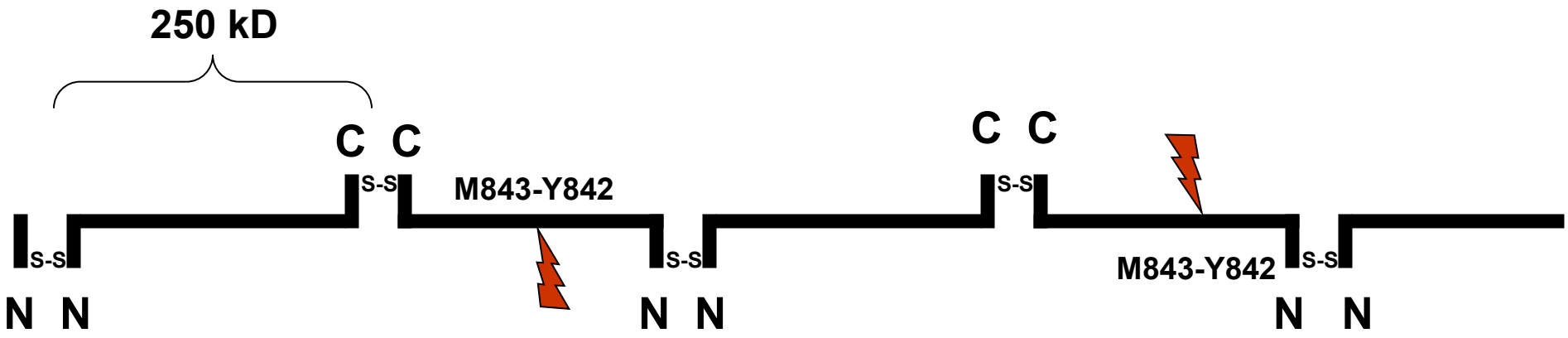


2 brothers with chronic relapsing TTP and ULvWF
(Furlan et al. Blood 1997;89:3097)

Historical Notes (III)

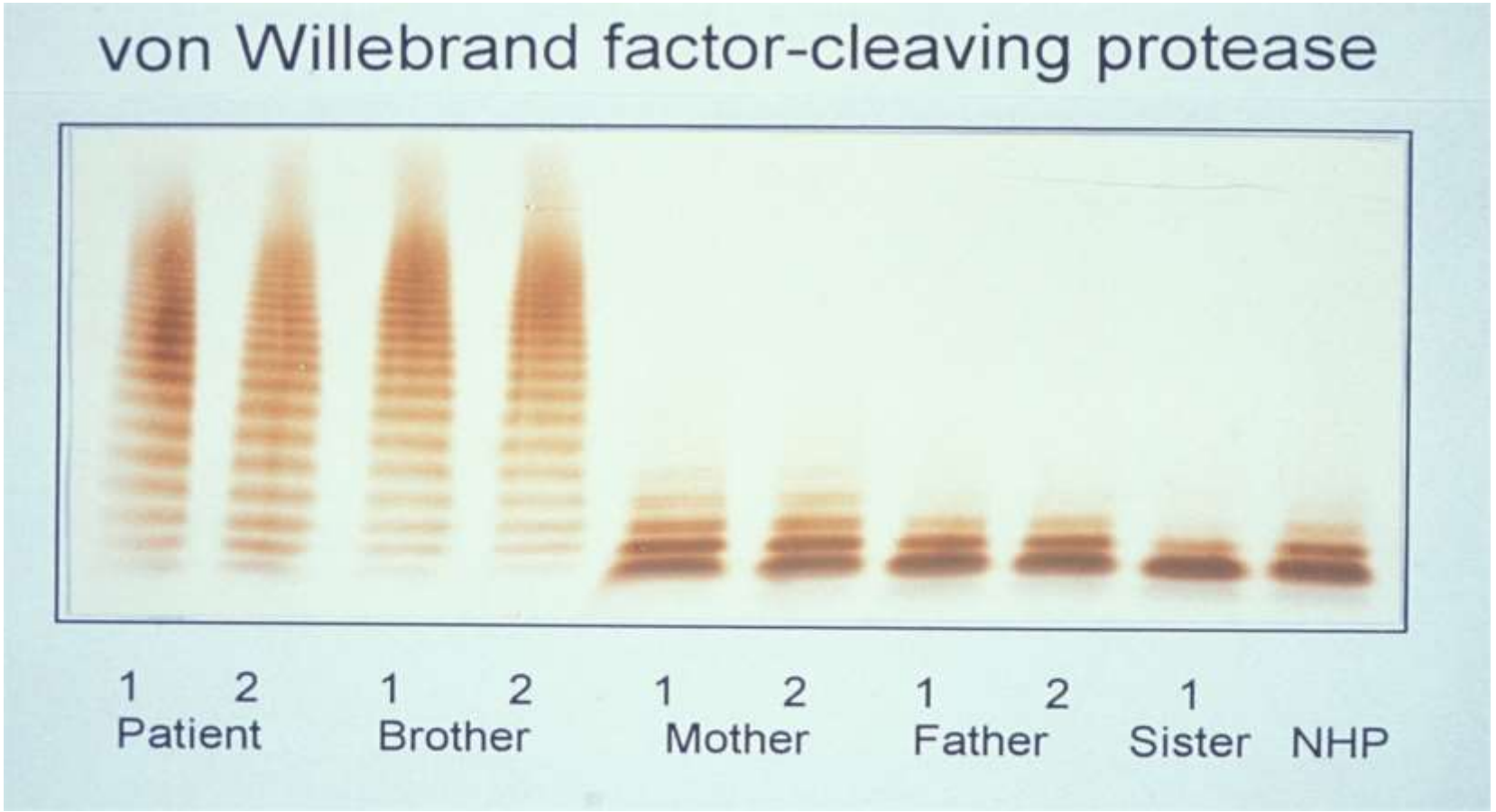
Detection of vWF-cleaving protease

(Furlan et al. Blood 1996; Tsai. Blood 1996)



Could a deficiency of this protease explain the presence of ULvWF multimers ?

Historical Notes (IV)

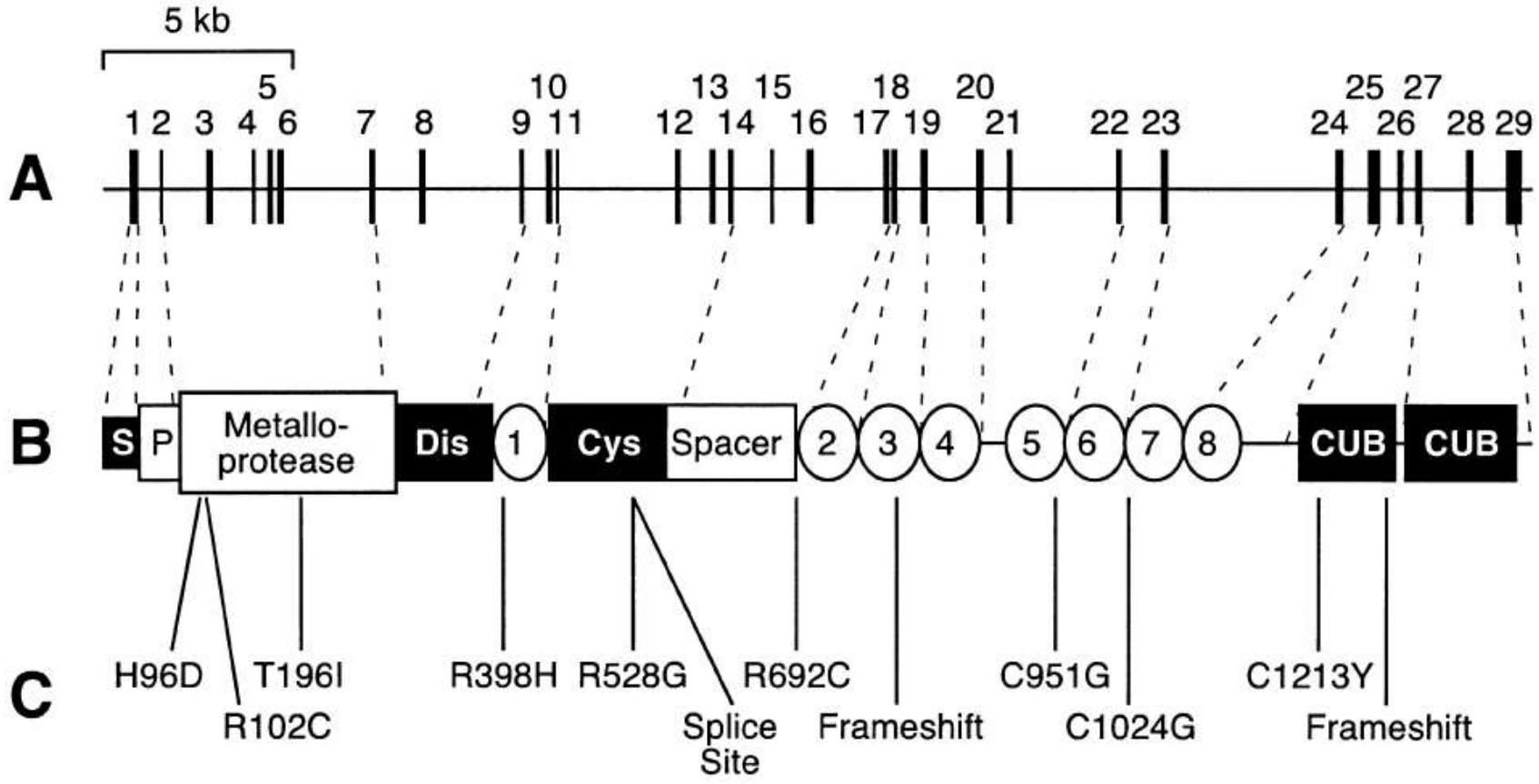


Lacking vWF-cleaving protease activity in the 2 brothers with chronic relapsing TTP (Furlan et al. Blood 1997)

Historical Notes (V)

- Gerritsen et al. (2001), Fujikawa et al. (2001), Soejima et al. (2001), Zheng et al. (2001): Protein purification from plasma, sequence analysis $\rightarrow$ Protein structure, chromosomal localization to 9q34: ***ADAMTS13***
- Levy et al. (2001): genome-wide linkage analysis in families with hereditary TTP $\rightarrow$ ***ADAMTS13***, 12 different mutations accounting for 14/15 disease alleles in their families
- Plaimauer et al. (2002): Expression of recombinant ADAMTS13

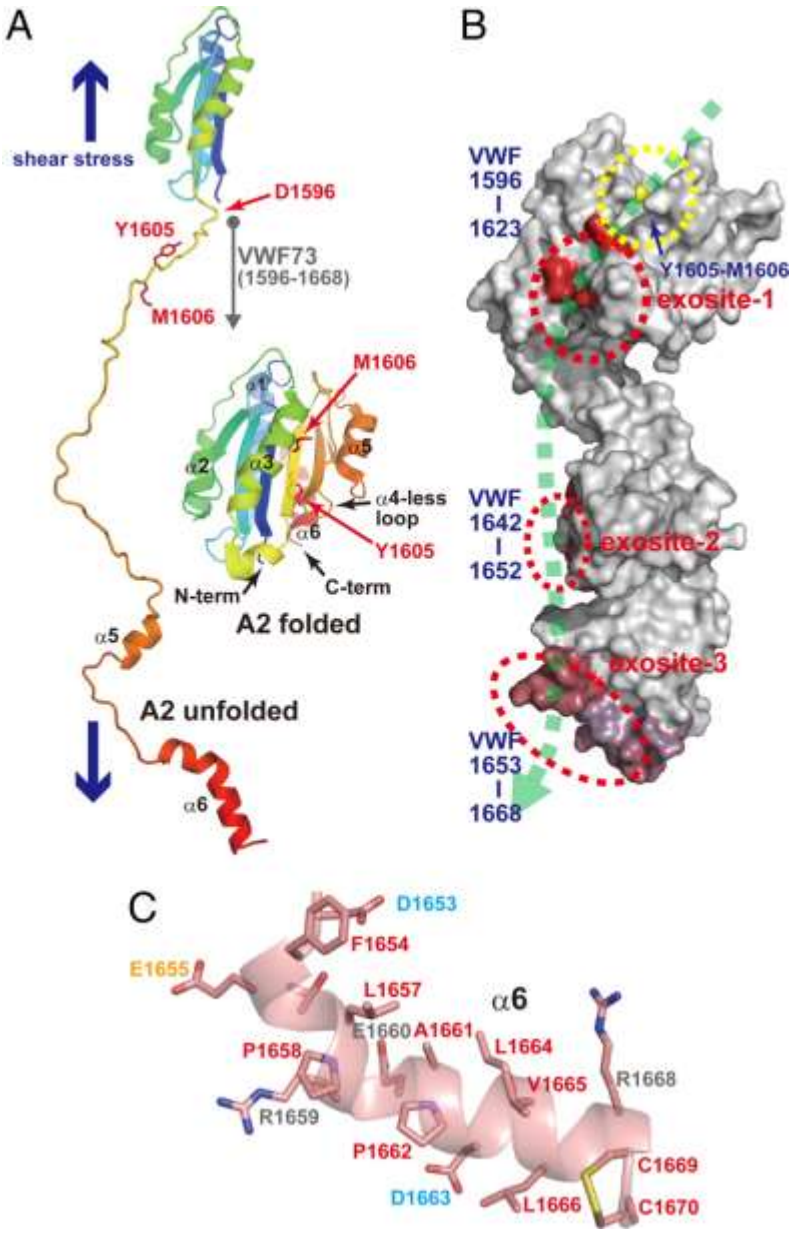
ADAMTS13 (VI)



ADAMTS13 gene, protein, mutations (Levy et al. Nature 2001). From Zheng et al. Curr Opin Hematol 2002

Update 2010: 76 mutations reported (Lotta et al. Human Mutation 2010)
Update 2011: Mutations in 43 Japanese patients (Fujimura et al. JTH 2011)

ADAMTS13 (MDTCS)-VWF interactions



Akiyama M et al.
PNAS 2009;106:19274-19279

Thrombotic Microangiopathies 2014

- **Hereditary TTP** with severe **constitutional ADAMTS13 deficiency** (Upshaw Schulman syndrome)
- **Acquired idiopathic TTP** with **severe acquired ADAMTS13 deficiency**
- Acquired idiopathic TTP **without** severe ADAMTS13 deficiency
- **TMAs** associated with hematopoietic stem cell transplantation, disseminated neoplasia, drugs (e.g. mitomycin C, ticlopidine, ciclosporin), pregnancy (including HELLP), HIV infection, severe hypertension, connective tissue disease (e.g. SLE)
- **Atypical HUS** (often regulatory protein defects or hyper-functional mutations of alternative complement pathway)
- **Typical HUS** (D+ HUS; E. coli O157:H7)
- → **Syndromes of Thrombotic Microangiopathy**.

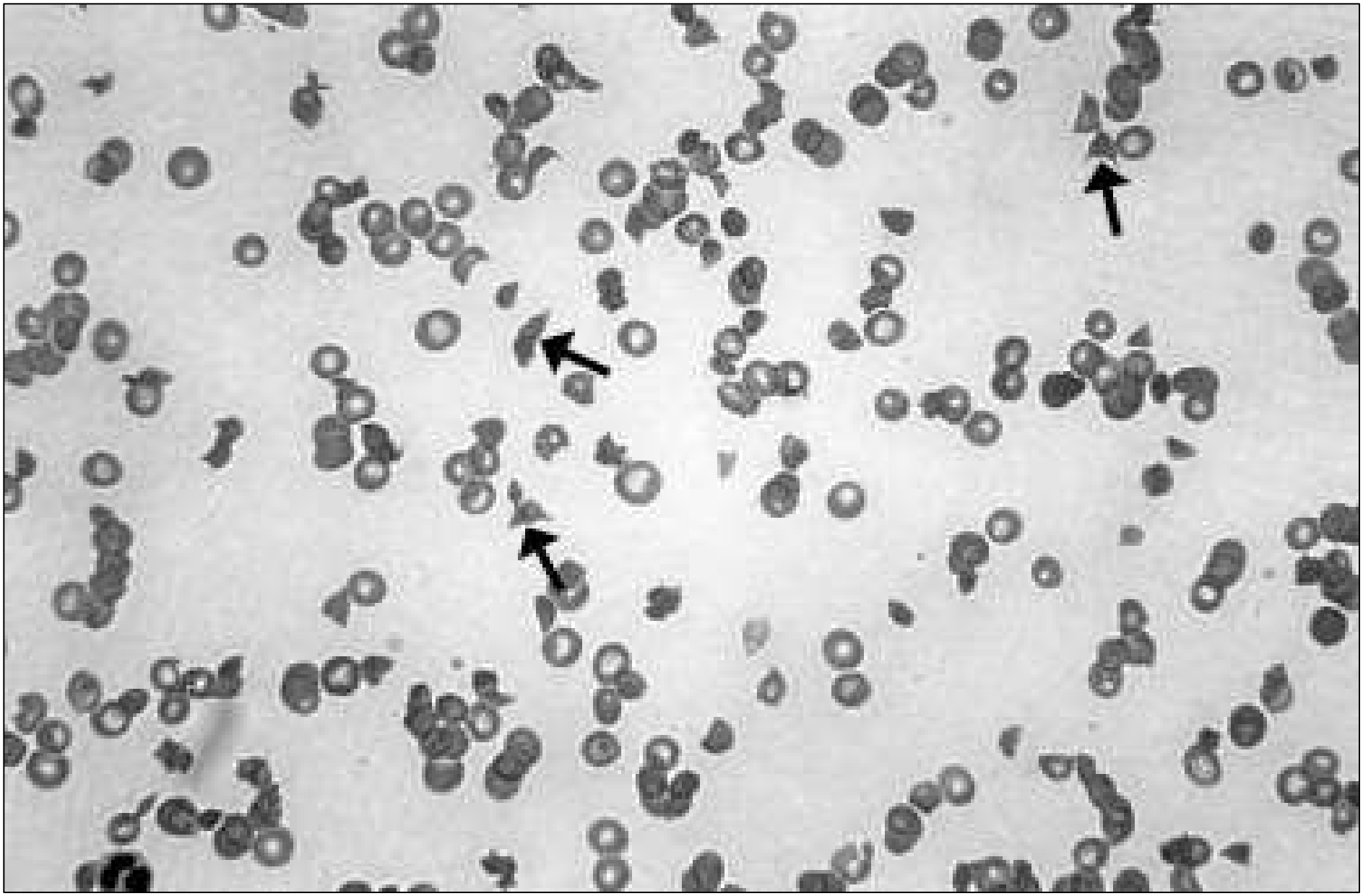
(J.N. George, C.M. Nester. N Engl J Med 2014;371:654-666)

Outline

- Historical Notes → Thrombotic Microangiopathies 2014
- **Laboratory investigations, assays of ADAMTS13**

Diagnostic Evaluation

- Blood count, blood smear
- LDH, haptoglobin, Coombs' test, reticulocytes, urinalysis, renal function, neuro-imaging
- Exclusion of neoplasia, drugs, SLE, allogeneic HSCT, HIV, enterohemorrhagic E. coli
- ADAMTS13 activity, functional inhibitor
- (Autoantibodies by ELISA, ADAMTS13 antigen, VWF multimer analysis)
- (Complement regulatory gene mutations: *CFH, CFHR1-5, MCP, CFI, CFB, C3, Thrombomodulin*)
- (*DGKE* mutations)



Many schistocytes (arrows), thrombocytopenia, patient with acute TTP

From B. Lämmle et al. J Thromb Haemost 2005;3:1663-1675

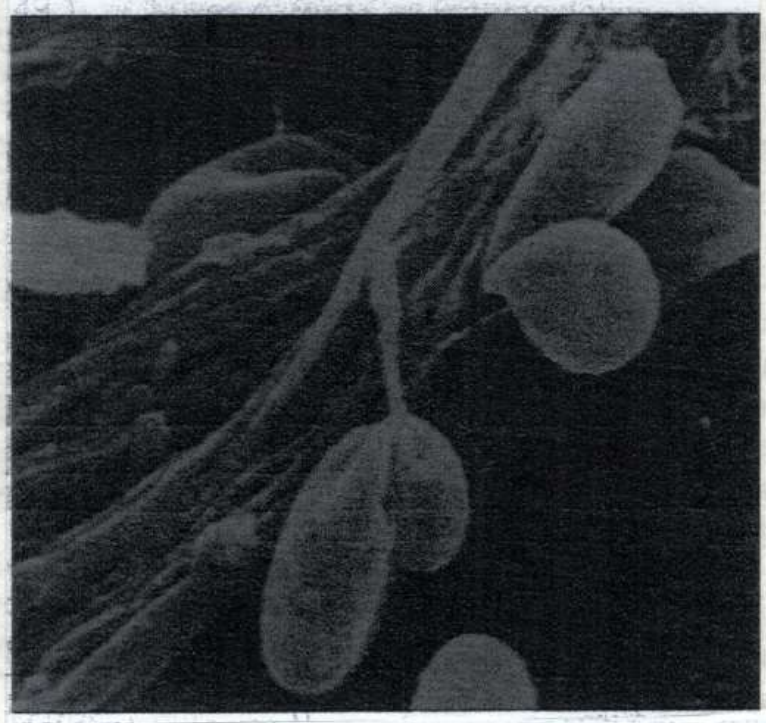


FIG. 49.3. Scanning electron micrograph. Red cells are "clotheslined" over fine fibrin strands (in vitro model). Other cells, moving past these trapped erythrocytes, may cause their fragmentation. Thicker fibrin strands in background do not cause this injury. (From Bull BS, Kuhn IH. The production of schistocytes by fibrin strands. Blood 1970;35:104.)

Is the model of erythrocyte fragmentation on fine fibrin strands adequate to explain schistocytes in TTP?

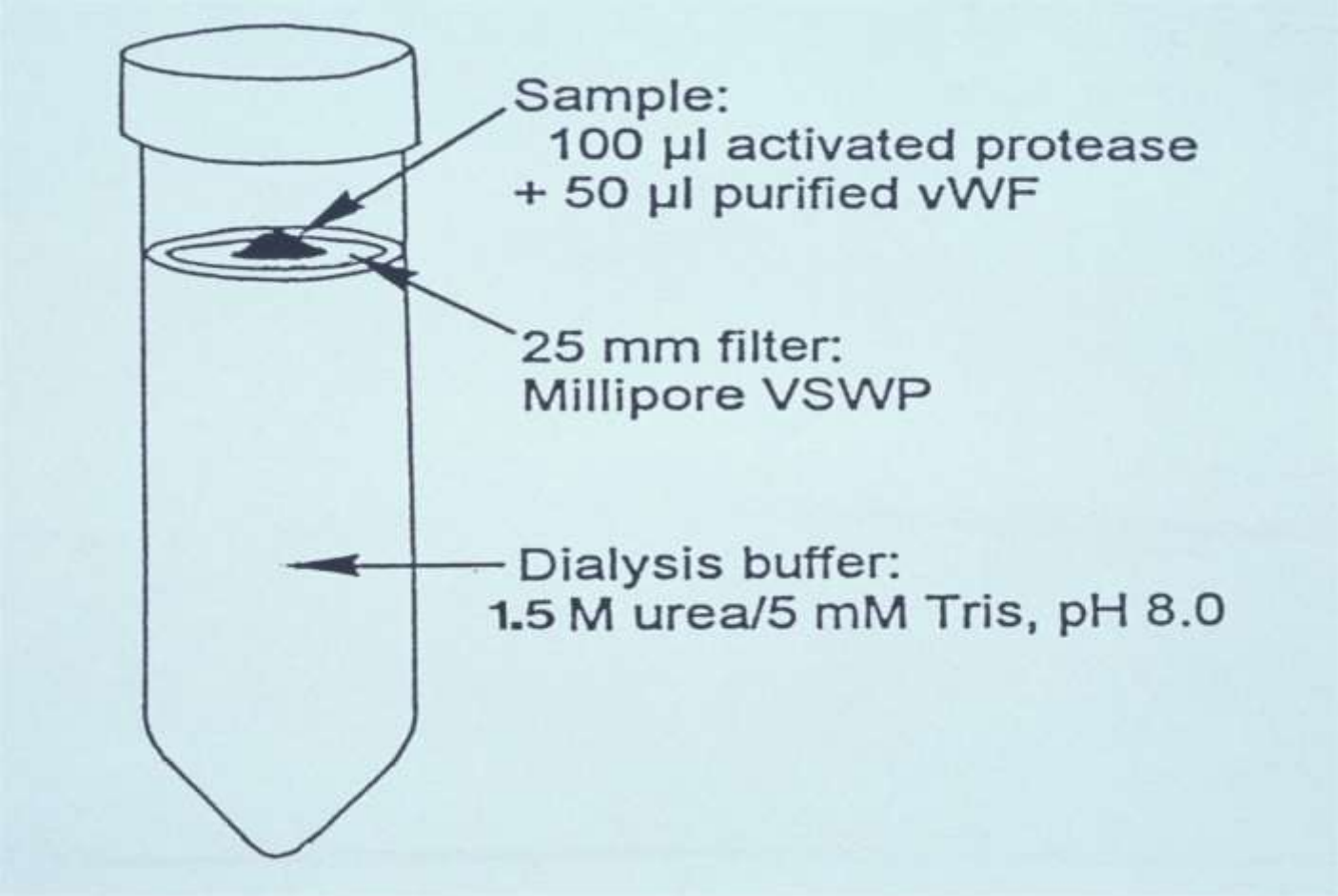
J. Foerster. Red cell fragmentation syndromes. In: Wintrobe's Clinical Hematology, 10th edition, 1999, pp. 1305ff

ADAMTS13 activity assays (I)

- **Heavy debate** over many years concerning the suitability of **ADAMTS13 assays**
- VWF multimer degradation assay (Immunoblotting assay) (1)
- SDS-PAGE analysis of 350 kD VWF fragment (2)

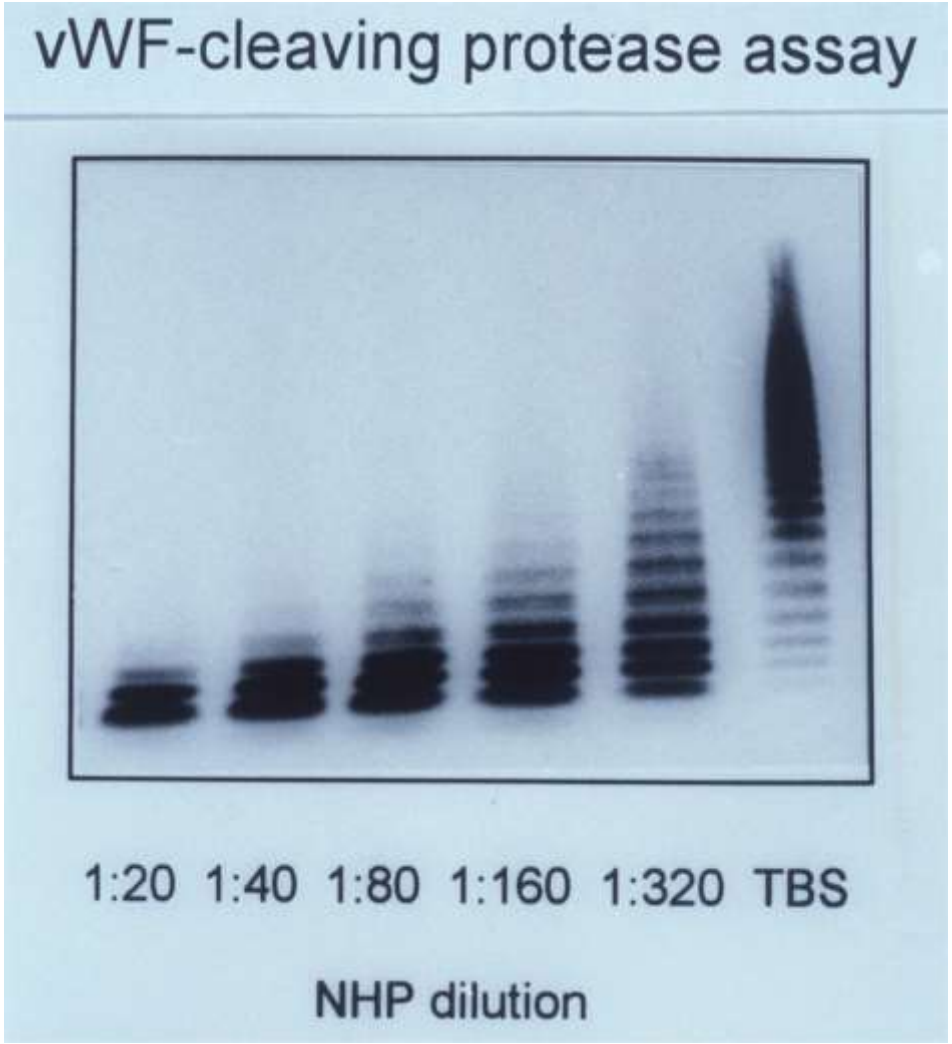
1) M. Furlan et al. Blood 1996;87:4223
2) H.M. Tsai. Blood 1996;87:4235

ADAMTS13 activity assays (II)



Immunoblotting assay (Furlan et al. Blood 1996)

ADAMTS13 activity assays (III)

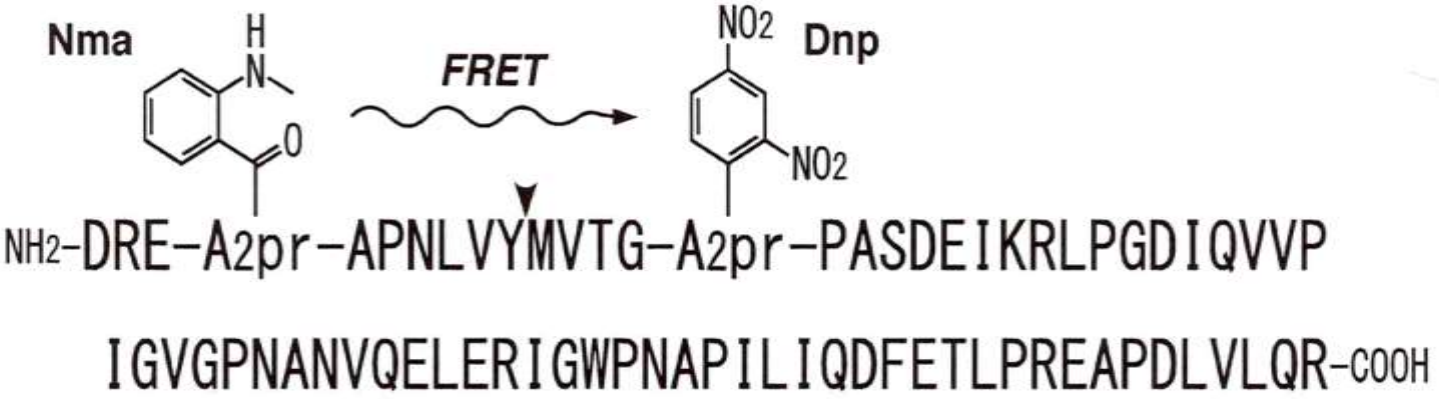


Immunoblotting assay,
Standard curve

ADAMTS13 activity assays (IV)

FRETS-VWF73, a first fluorogenic substrate for ADAMTS13

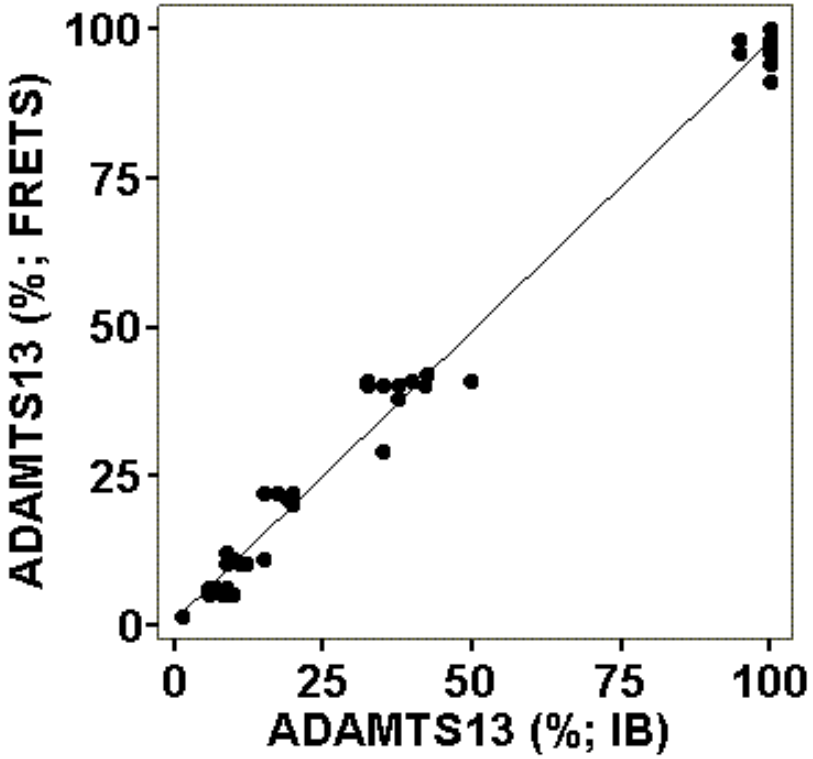
(Kokame et al. BJH 2005)



ADAMTS13 activity assays (V)

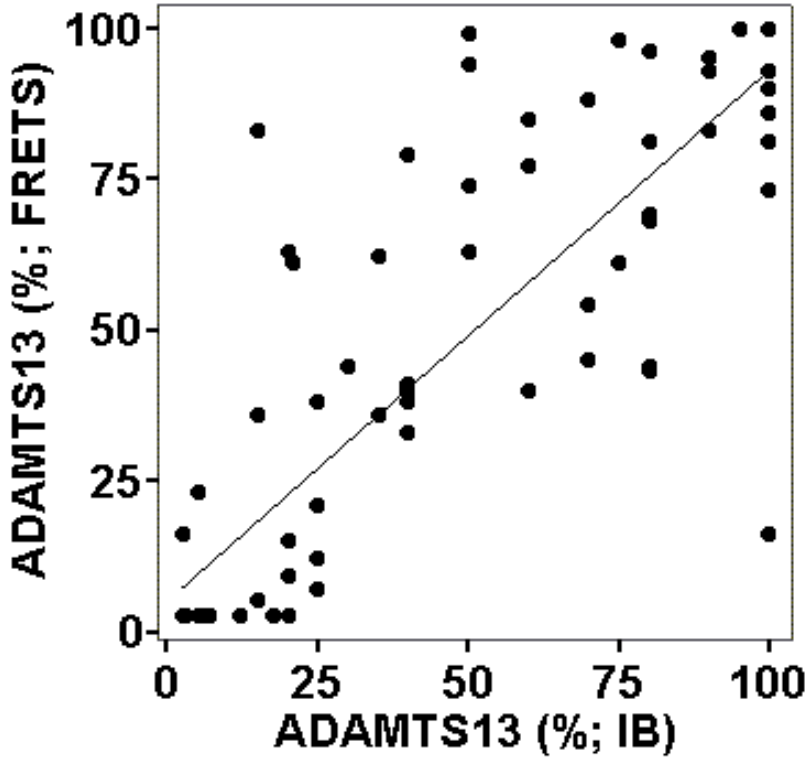
Comparison of different assays

ISTH Multicenter Study
JTH 2008;6:1534-41



Correlation coefficient ***Rs* = 0.995**

Idiopathic TTP-HUS patients, Oklahoma Registry



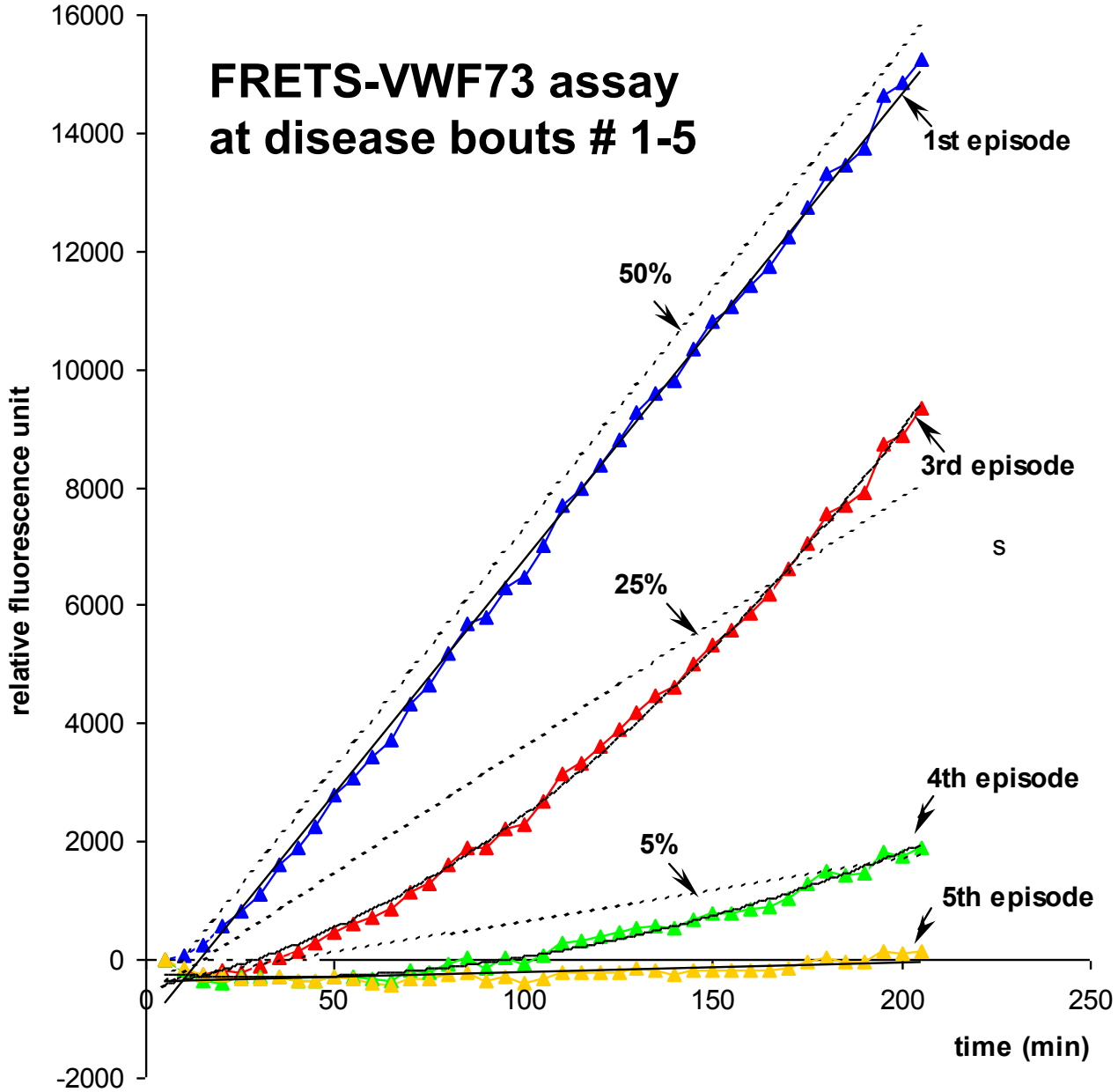
***Rs* = 0.854**

ADAMTS13 assays (VI)

HIV-positive patient with chronic relapsing TTP and evolution of a severe ADAMTS13 deficiency

Bout	ADAMTS-13 activity (%)			AD-13 inhibitor	AD-13 antibodies	VWF proteolysis
	IB	FRETS-VWF73	Flow-based assay	IB (BU/ml)	ELISA (AU/ml)	on multimer analysis
1	60	53	normal	none	26.8	normal
3	50	15	severe deficiency	none	73.5	severely impaired
4	6	<5	n.d.	traces	>88.3	severely impaired
5	<3	<5	severe deficiency	1	>88.3	impaired

R. Froehlich-Zahnd et al. Haematologica 2012;97:297-303

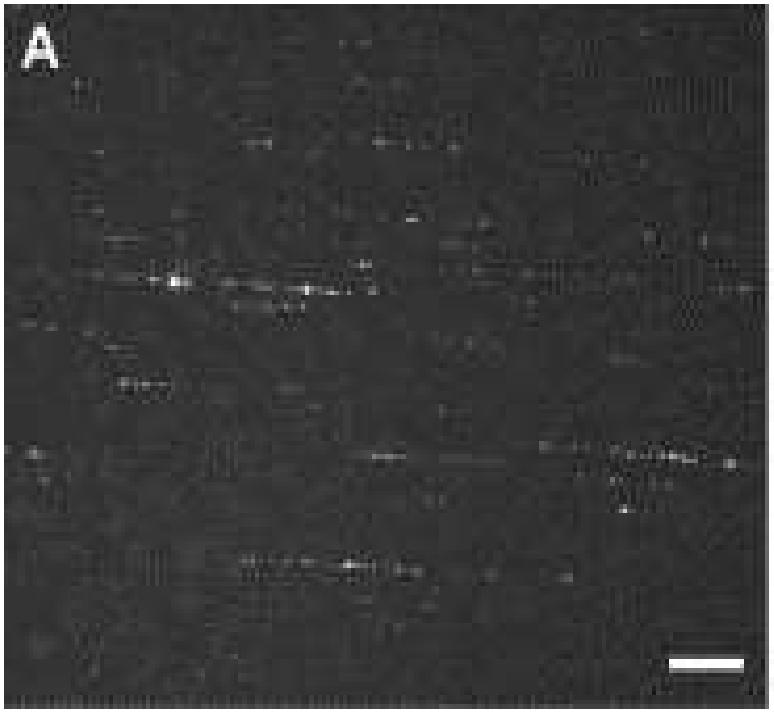

FRETS-VWF73 assay
at disease bouts # 1-5
1st episode
50%
3rd episode
s
25%
4th episode
5%
5th episode
relative fluorescence unit
time (min)
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
0
50
100
150
200
250

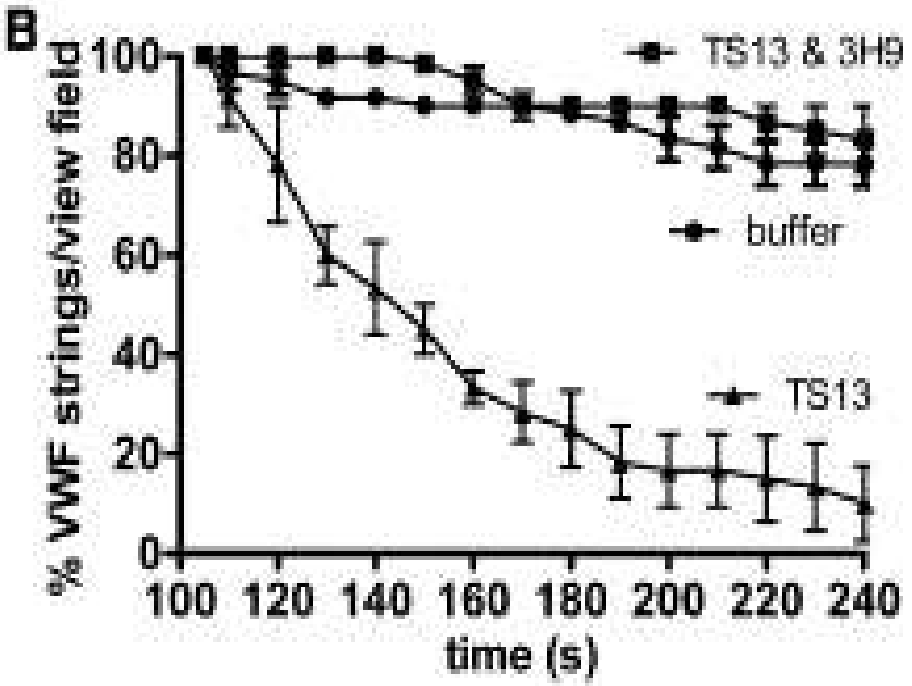
ADAMTS13 assays (VIII)

- Flow-based assay of UL-VWF strings on endothelium with attached fluorescent platelets (1,2)

1. J.F. Dong et al. Blood 2002;100:4033
2. **K. De Ceunynck et al. J Biol Chem 2011;286:36361**

ADAMTS13 activity assays (IX)





K. De Ceunynck et al. J Biol Chem 2011;286:36361

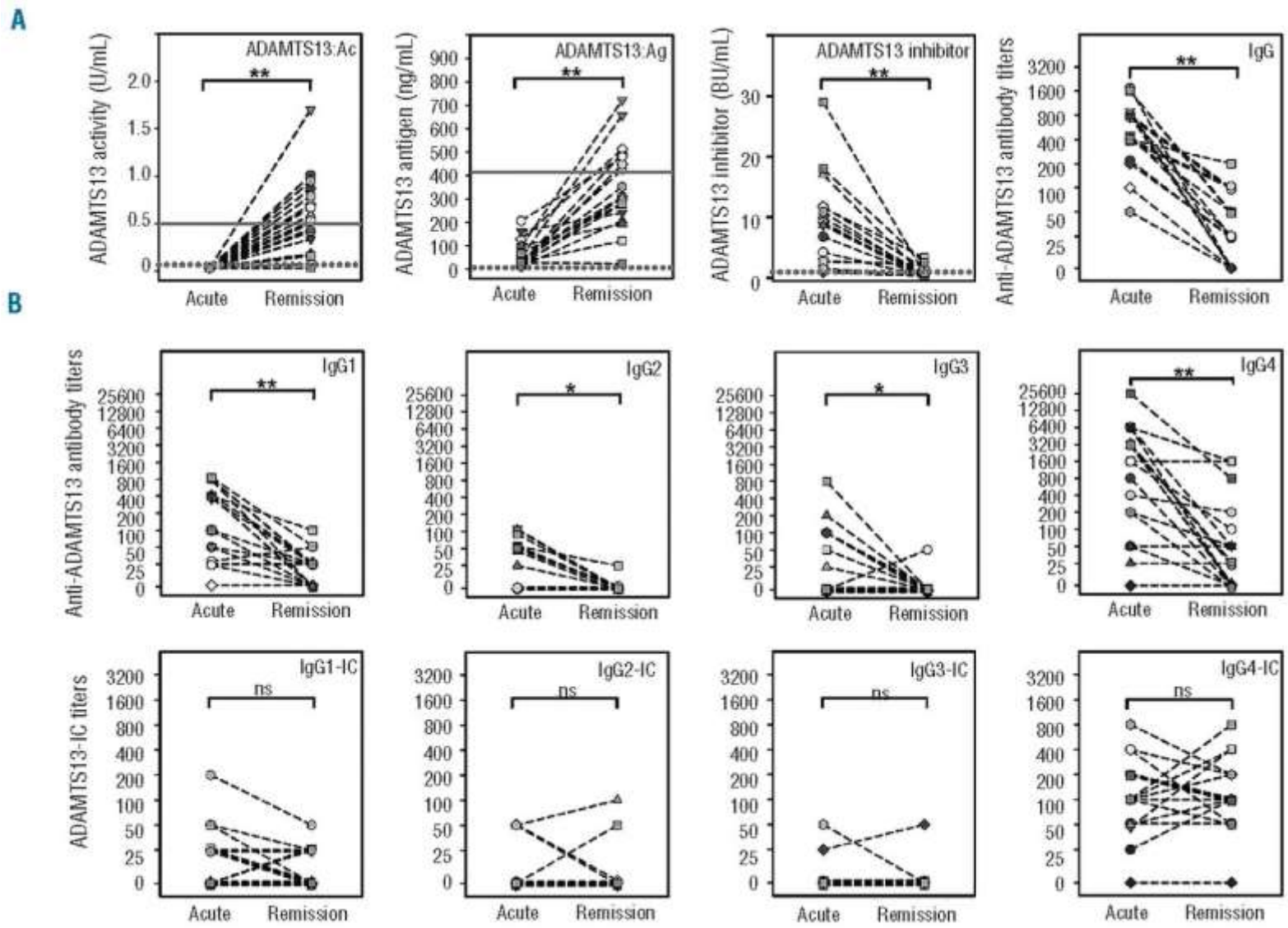
Possible advantage of **flow-based assay** using **endothelium-anchored VWF-cleavage**: may detect ADAMTS13 **«inactivated»** by **interleukin-6 or Shigatoxin-1/-2 (?)**
(Bernardo et al. Blood 2004;104:100; Nolasco et al. Blood 2005;106:4199)

ADAMTS13 assays (X)

- Inhibitor assay using heat-inactivated patient plasma mixed with NHP (2 hrs at 37°C) → residual activity (Bethesda units) (1)
- Autoantibodies by ELISA (2)
- ADAMTS13 antigen (3)
- Immune complexes of ADAMTS13/anti-ADAMTS13 IgG (4)

1) S. Kinoshita et al. Int J Hematol 2001;74:101-108
2) M. Rieger et al. Blood 2005;106:1262-1267
3) M. Rieger et al. Thromb Haemost 2006;95:212-220
4) S. Ferrari et al. Haematologica 2014;99:779-787

ADAMTS13 assays (XI)



Ferrari S et al. Haematologica 2014;99:779-787

Many additional research topics

- **Should we rethink about those?**
  - Endothelial injury, decreased prostacyclin production
  - Reduced fibrinolytic activity, anti-endothelial cell autoantibodies
  - Autoantibodies against GP IV (CD 36), microvascular endothelial cell apoptosis induced by TTP plasma
  - Platelet aggregating factors (37 or 59 kD proteins)
  - Calcium dependent cysteine protease (calpain) aggregating platelets
- **Composition of microvascular thrombi?**
- **Complement activation**
  - The important pathogenetic factor in D+HUS, aHUS and TTP (M. Noris et al. Nature Rev Nephrol. 2012;8:622) → Eculizumab for all patients??
- **Neutrophil activation (NETS)**
  - Trigger for acute disease? (T.A. Fuchs et al. Blood 2012;120:1157)

Conclusion/Perspective

- **Pathophysiology of TTP: Definite role of ADAMTS13 deficiency, clearly there are other players**
- **ADAMTS13 activity: useful assays available, different assays may give variable results**
- **Flow-based ADAMTS13 activity assay for routine use would be desirable**
- **Assays of functional inhibitors, ADAMTS13 antigen, autoantibodies, immune complexes diagnostically/prognostically important**
- **Recognize hereditary TTP → *ADAMTS13* mutations**

Acknowledgment

- **J.A. Kremer Hovinga, L. Alberio, M. Schaller, J.D. Studt**, S.C. Meyer, G. Mäder, C.R. Largiadèr, R.Fröhlich-Zahnd, **M. Mansouri**, I. Sulzer, **M. Furlan** (Bern)
- **Ch. Von Auer,** K. Jurk, T. Falter, H. Rossmann, I. Scharrer (Mainz)
- **J.N. George, S.K. Vesely**, D.R. Terrell (Oklahoma)
- **Y. Fujimura, M. Matsumoto, T. Miyata** (Japan)
- **D.D. Wagner, T.A. Fuchs** (Boston, Hamburg)
- **X.L. Zheng** (Philadelphia), **J.F. Dong** (Seattle)
- **A.S. von Krogh,** P. Quist-Paulsen, A. Waage (Trondheim)
- **U. Budde, R. Schneppenheim** (Hamburg), J. Voorberg, B.M. Luken (Amsterdam)
- **All physicians/hospitals having sent patient samples to Bern**
- **Swiss National Science Foundation**, Baxter Bioscience Grant (Hereditary TTP Registry)