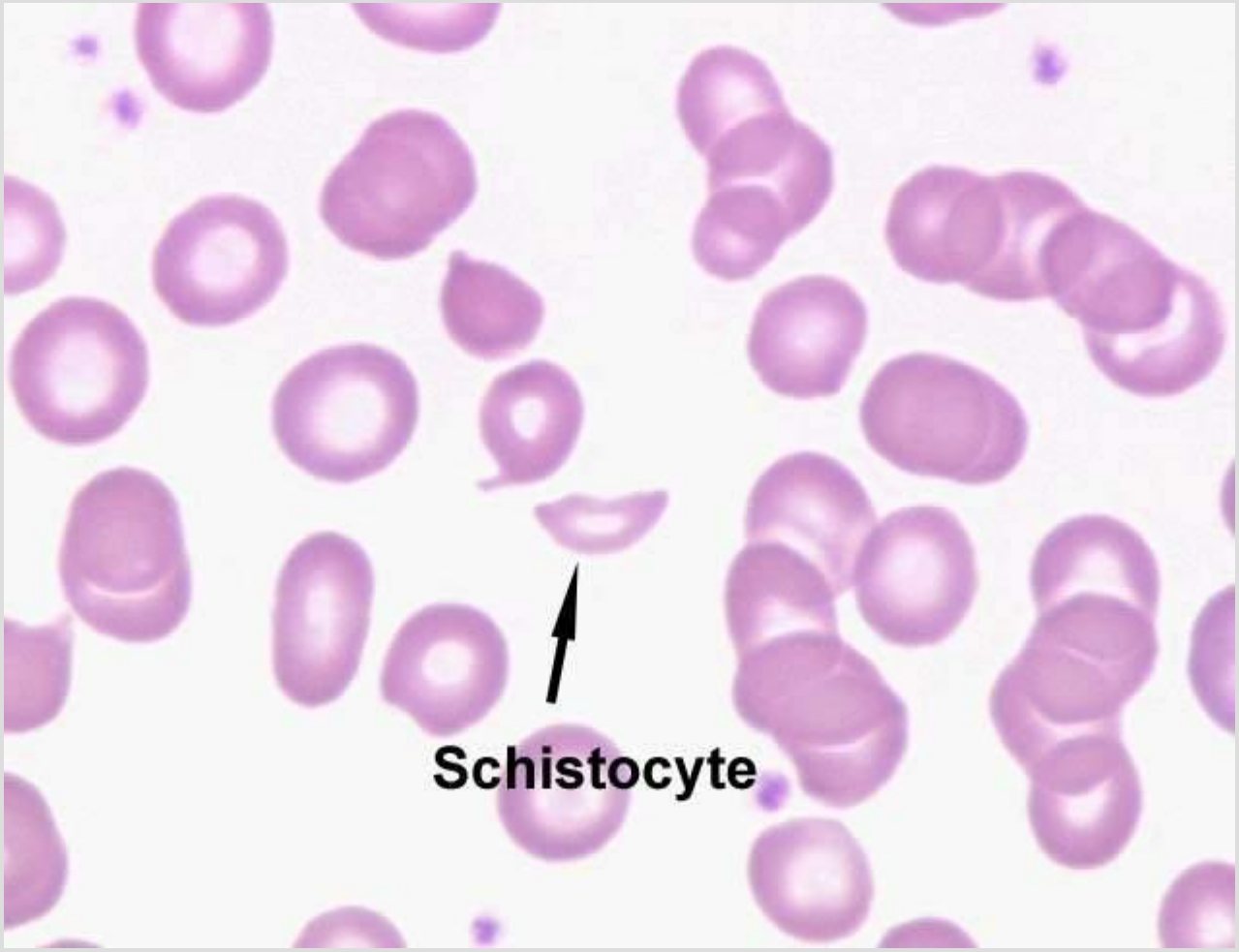
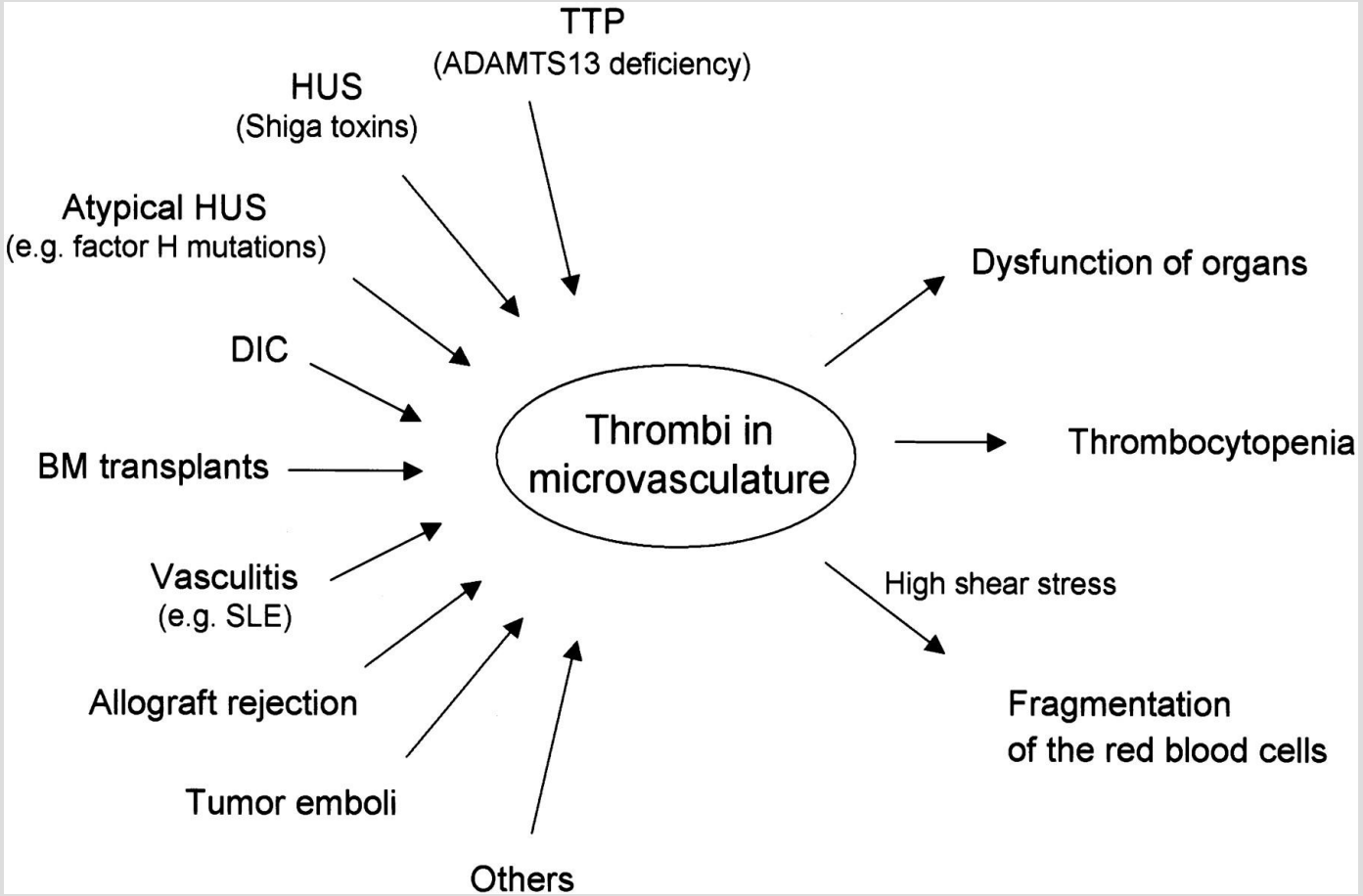
MicroAngiopatische Hemolytische Anemie – MAHA

Prof dr L Noens UZG



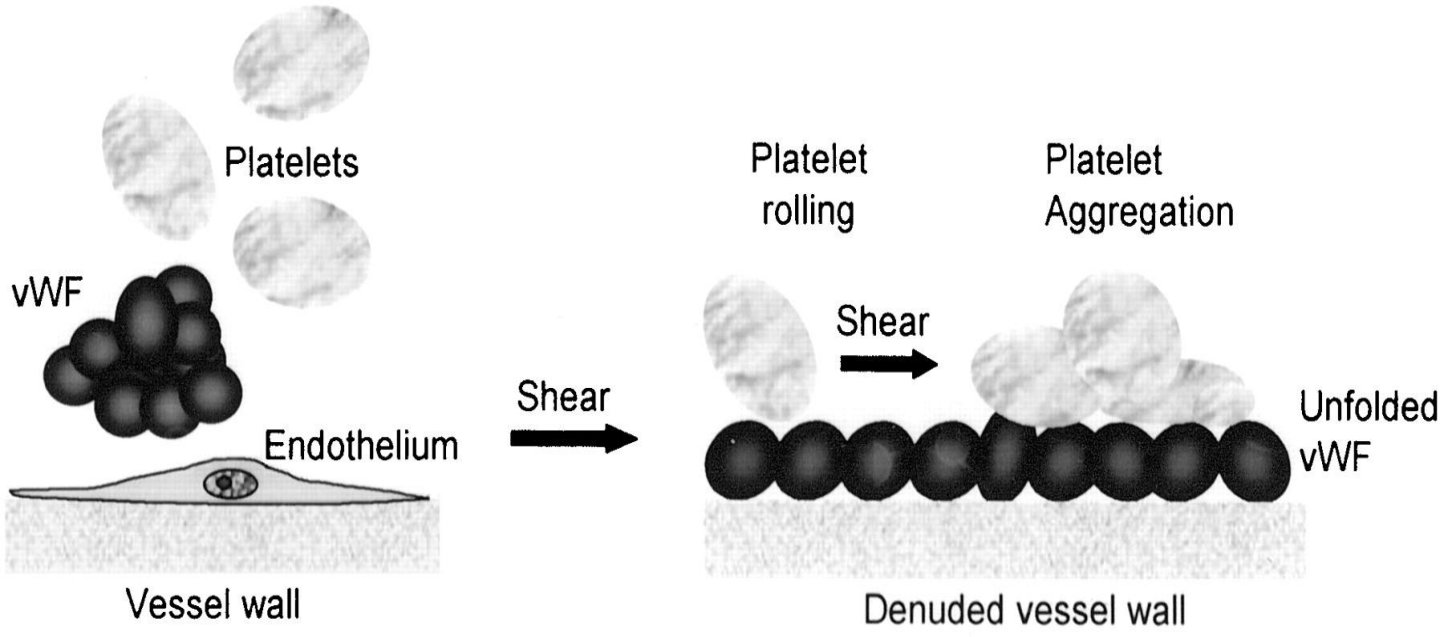
The syndrome of thrombocytopenia and microangiopathic hemolysis (thrombotic microangiopathy) has multiple causes



Tsai, H.-M. Arterioscler Thromb Vasc Biol 2003;23:388-396

Arteriosclerosis, Thrombosis, and Vascular Biology

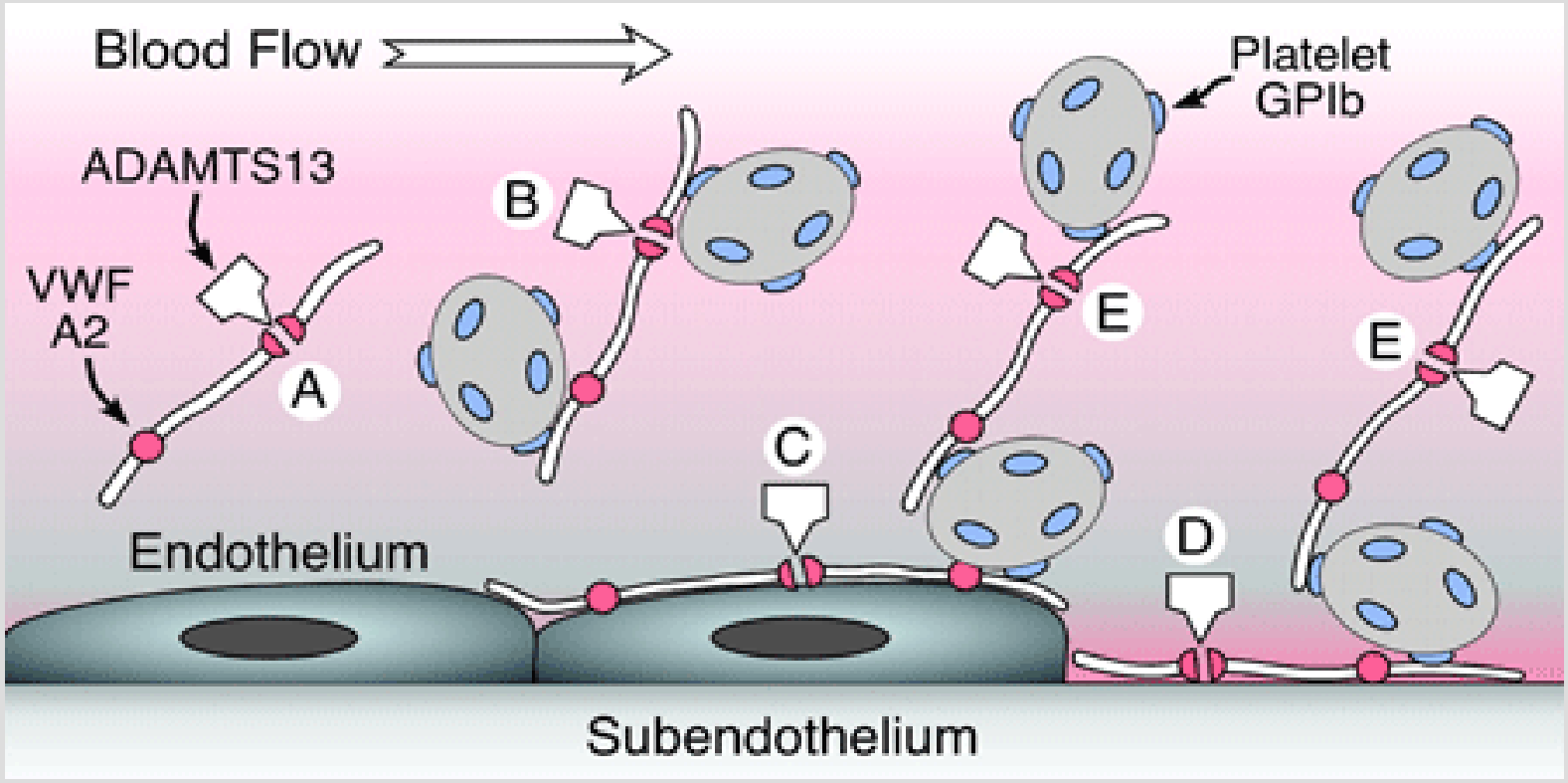
Scheme showing how shear stress may promote vWF-mediated platelet aggregation at sites of vessel injury



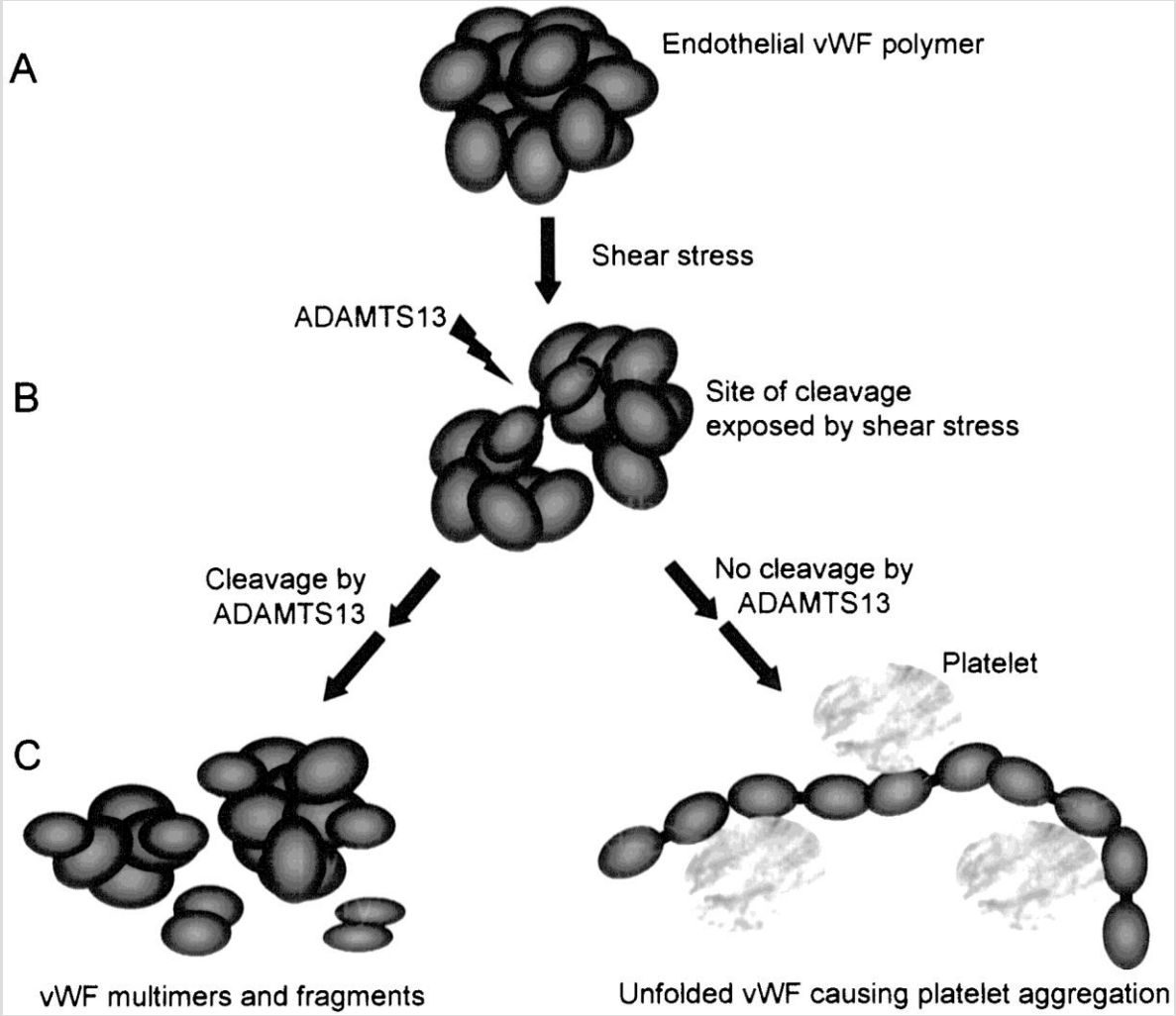
Tsai, H.-M. Arterioscler Thromb Vasc Biol 2003;23:388-396

Arteriosclerosis, Thrombosis, and Vascular Biology

ADAMTS13 physiology



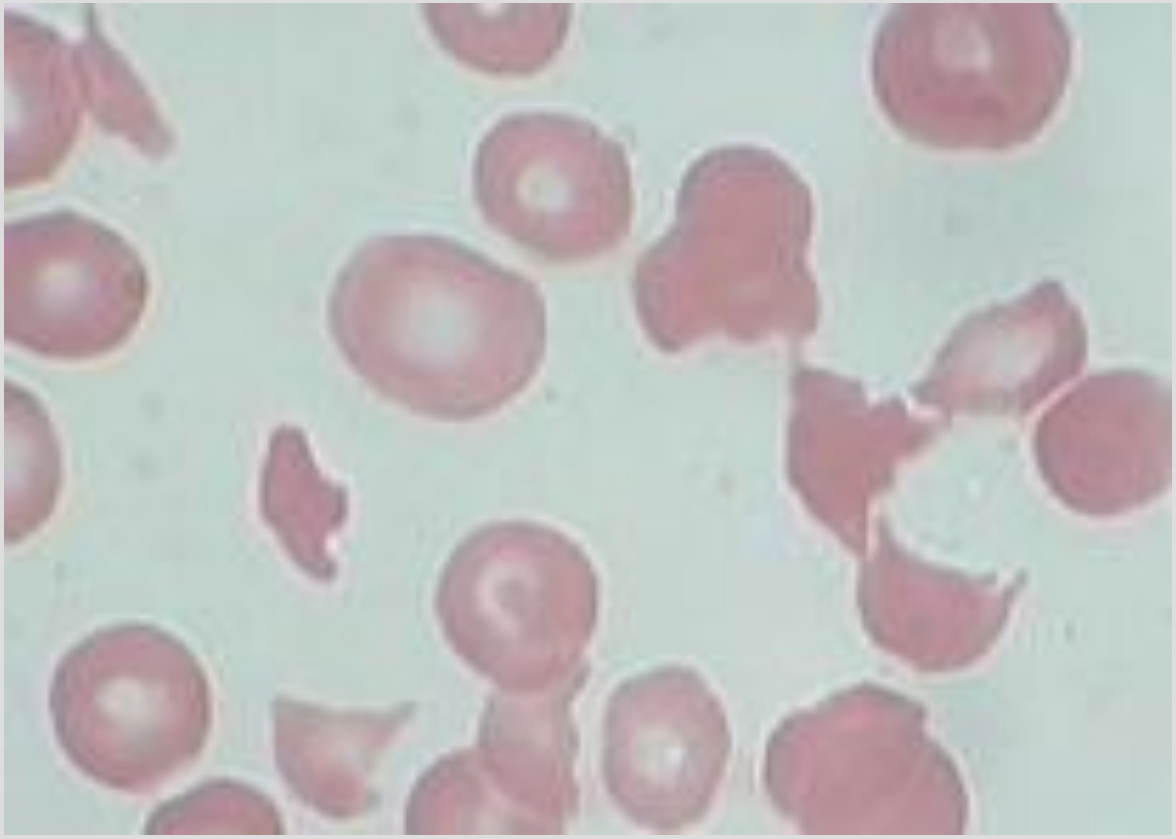
Deficiency of ADAMTS13 causes platelet thrombosis



Tsai, H.-M. Arterioscler Thromb Vasc Biol 2003;23:388-396

Arteriosclerosis, Thrombosis, and Vascular Biology

Schistocytes in TTP



blood
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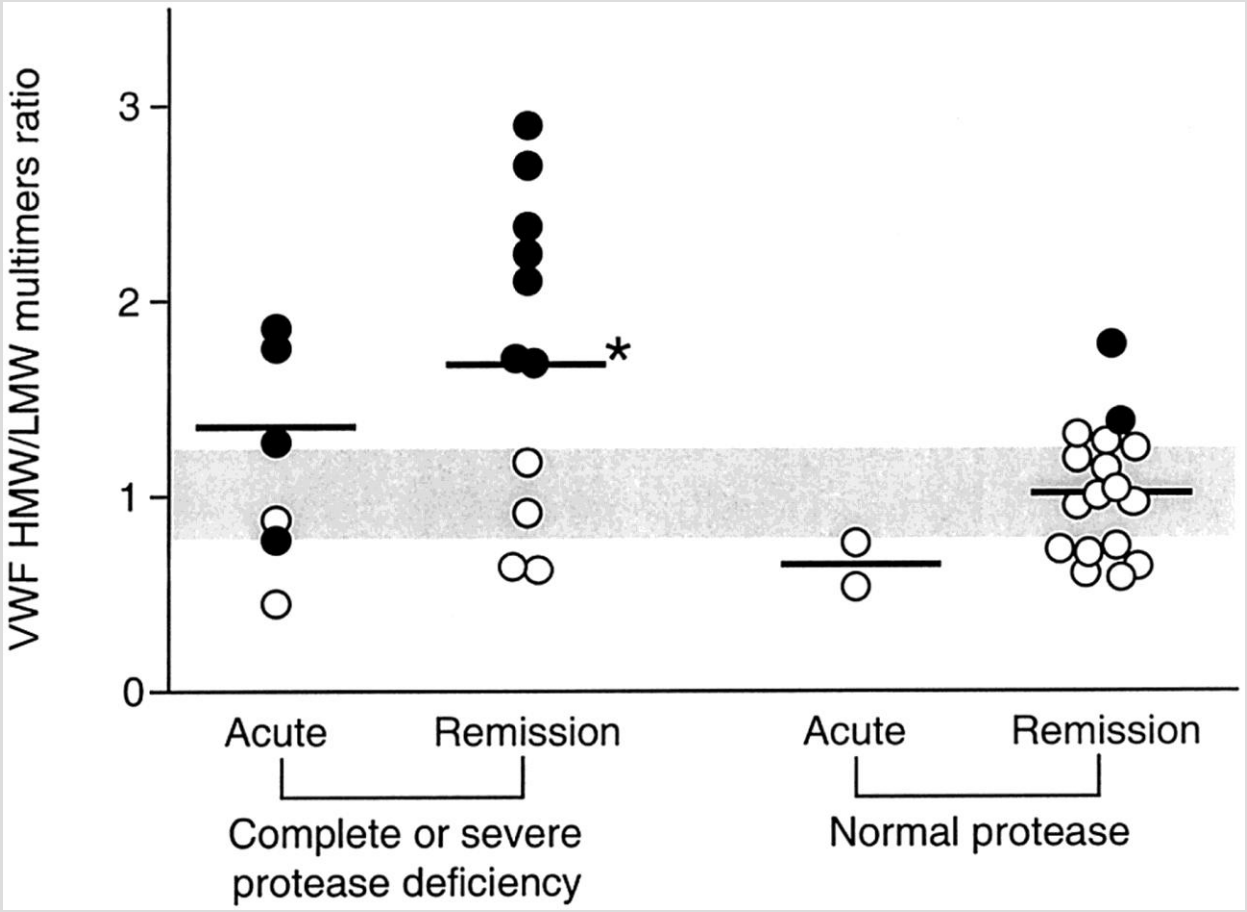
VWF multimers



Remuzzi, G. et al. Blood 2002;100:778-785

blood
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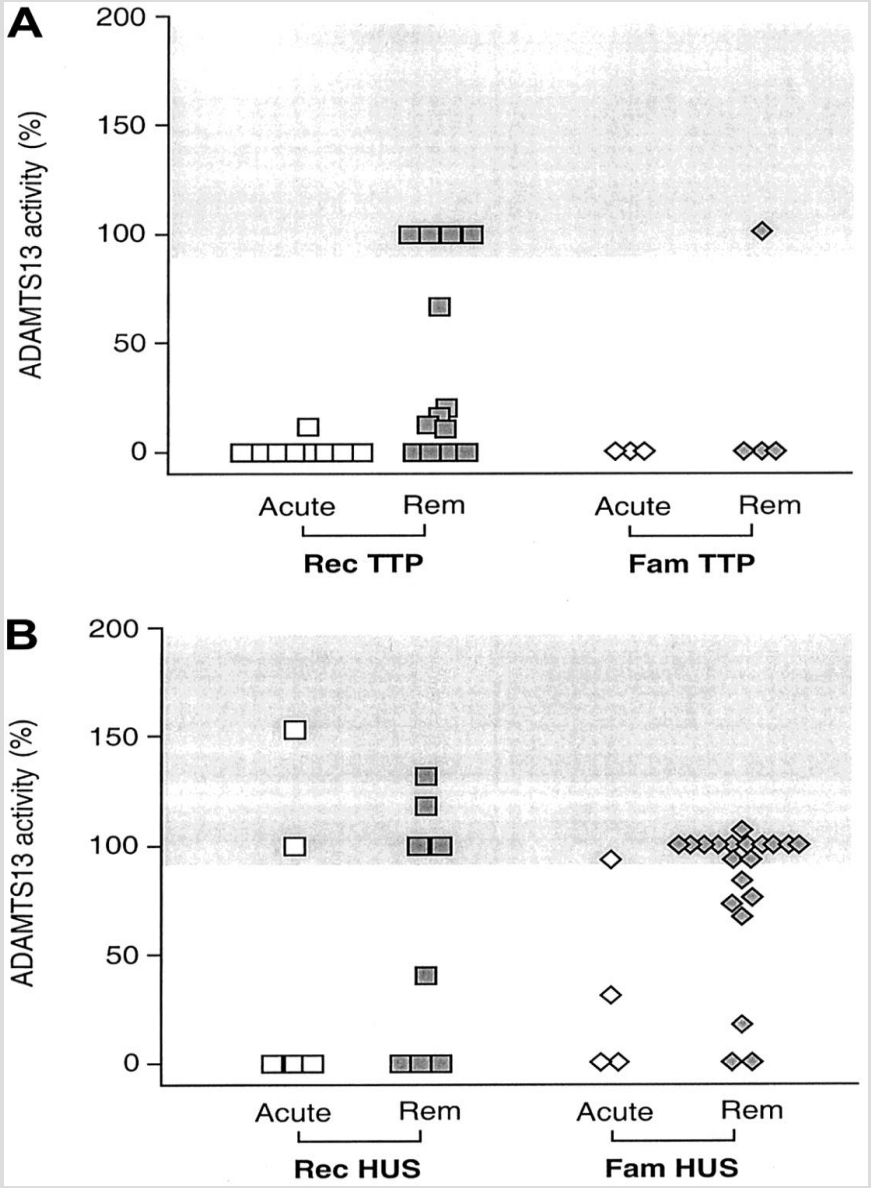
ADAMTS13 activity measurements



Remuzzi, G. et al. Blood 2002;100:778-785

DIAGNOSIS AND MANAGEMENT OF TTP

- Disease group 1 :
  - Hemolytic Uremic Syndromes/HUS
  - Thrombotic Microangiopathy/TMA
  - Transplant Associated Microangiopathy/TAM
- Disease group 2 :
  - Thrombotic Thrombocytopenic Purpura/TTP

HUS

- d-HUS/typical HUS
- Diagnosis:
  - Following bloody diarrhea (Shiga-toxin/ E.Coli strain) mainly in children
  - Acute renal failure, spontaneous recovery,low mortality in children
- Treatment in children:
  - Supportive (dialysis,transfusions,renal TX) but not TPE

- a-HUS/atypical HUS
- Diagnosis:
  - Familial cluster or sporadic in adults
  - w/o diarrhea, severe symptoms, high mortality
  - Familial(w/o mutated complement regulating genes):relapses,ESRD, mortality rate above 50%
  - Sporadic:drug induced, pregnancy,infections, autoimmune diseases or transplant related (HSC/organ)

a-HUS/TMA/non-id.TTP

- Diagnosis:
  - Clinics and Lab Features are indistinguishable from TTP: ‘TTP-HUS’ or ‘Non-idiopathic TTP or TMA’
  - ADAMTS13 or –inhibitor assays : ?
  - Multiple co-factors : ‘injury’ to the microviscular endothelial cells

a-HUS/TMA/non-id.TTP

- Treatment :
  - TPE is reasonable option in children
  - TPE appropiate under certain conditions : id. TTP is not excluded
  - If early response is poor : Add corticosteroids and intensivate TPE
  - Transplant-associated TAM : TPE no longer standard of care (effectiveness not proven)
  - Familial HUS : preventive plasma transfusions?

Disease group 1 : TPE

- Rationale:
  - Undefined in most cases (aHUS:Type II-1 – TMA: Type II-2 strength of evidence)
  - Replacement of specific deficiencies in familial a-HUS
  - Replacement of ADAMTS13 and/or other unidentified factors in a-HUS
- Technical notes:
  - Duration of daily TPE until CR : variable
  - **CR : platelets>150k/µl and LDH normal and no neurologic deficits if initially present.**
  - Recurrence : < 30 days / Relapse : >30 days.
  - Taper or stop abruptly : ?

Disease group 2 : TTP

- Diagnosis :
  - **MAHA + Thrombocytopenia** +
  - Mostly ADAMTS13 deficiency: congenital or acquired (neutralising Ab)
  - Incidence : 0.37 / 100 000 / y
- Treatment :
  - **Emergency TPE :** mortality < 10%
  - Eventually Plasma infusions first (30-40 ml/kg)
  - Adjuvant treatments : corticoids/rituximab/vincristin/splenectomy/IVIg/…
  - Platelet transfusions relatively contraindicated

Table 8-2. Controlled Trial of TPE vs. Plasma Infusion for TTP

	TPE	Plasma Infusion	
Baseline			
Number of patients	51	51	
Platelets ($\times 10^{-9}$/L)	22.4	24.1	
Hematocrit	0.26	0.25	
Lactate dehydrogenase (U/L)	1407	1248	
Results after 9 days of treatment			
Success[a]	24 (47%)	13 (25%)	
Failure[a]	27 (53%)	38 (75%)	
Survived	49 (96%)	43 (84%)	
Died	2 (4%)	8 (16%)	
Results after 6 months		*no TPE*	*with TPE*[b]
Number of patients	51	32	19
Success[a]	40 (78%)[a]	10 (31%)	15 (79%)
Failure[a]	11 (22%)[a]	22 (69%)	4 (21%)
Overall survival			
Number of patients	51	20	31
Survived	40 (78%)	10 (50%)	22 (71%)
Died	11 (22%)	10 (50%)	9 (29%)

[a] $p < 0.025$ between groups.

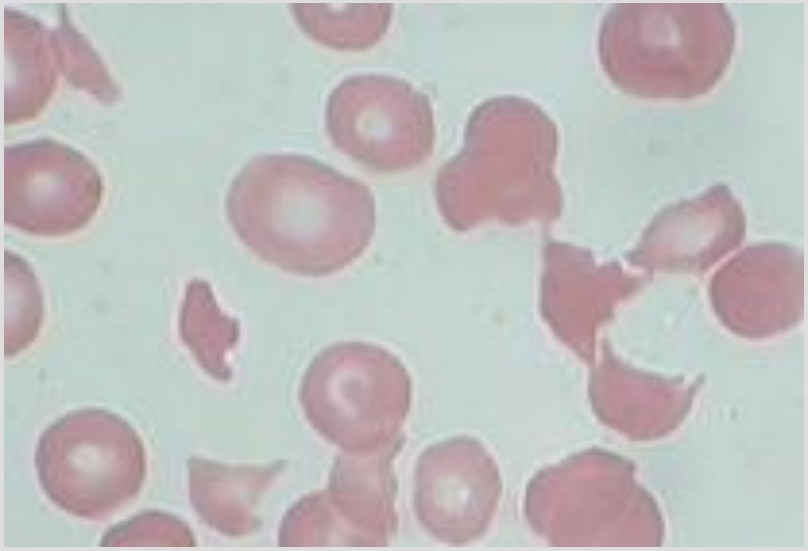
[b] Patients who failed plasma infusion after 9 days were offered TPE.

SOURCE: Adapted from Rock GA, Shumak KH, Buskand NA, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. N Engl J Med 1991;325:393.

TTP treatment

- Rationale :
  - Removal of Ab / restoring ADAMTS13 protease activity (RCT 3 - CT 2 – Type I evidence strength)
- Technical notes :
  - Median 7-8 daily TPE before hematological restoration; on average 12 sessions (large internat.survey)
  - Variable pattern of platelet recovery
  - Duration : until CR for 2-3 days
  - Persistence of schistocytes does not preclude discontinuation

TTP : TPE with FFP : SD- or MB- plasma ?



The apheresis machine
(with half-filled bag of plasma)

Virus inactivation of plasma : MB vs SD

MB-method

- Target:viral nuclear proteins and DNA/RNA are damaged
- Single unit
- Every unit is different
- Active on encapsulated and non encapsulated virusses(parvovirus B19, HAV)

SD-method

- Lipid membrane is destroyed by organic solvent (tri-n-butylfosfaat) and detergent (triton X-100)
- Pool of 350 l
- Product is QControled
- Active only on encapsulated virusses

SD- vs MB-Plasma : a concern ?

- Retrospective comparisons and anecdotal data from different sources raised a concern on clinically important outcome differences in TTP-patients who were treated with TPE.
- **MB-treated plasma seemed to be less effective in this context.**
- No prospective comparative study data are available yet.
- Preclinical data : a recent coöperative study :

Comparison of von Willebrand factor, FVIII and ADAMTS13 in blood components used for treatment of thrombotic thrombocytopenic purpura

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[1]Coagulation Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium

[2]Laboratory for Thrombosis Research, IRC, K.U.Leuven Campus Kortrijk, Kortrijk, Belgium;

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Introduction

Attention has recently turned to the composition of plasma products used in the treatment of thrombotic thrombocytopenic purpura (TTP). In Belgium, two virus inactivated fresh-frozen plasma (FFP) products are available: solvent/detergent (SD) treated pooled plasma (SD-FFP) and methylene blue/light (MB) treated single plasma units (MB-FFP). The use of MB-FFP in TTP patients is only anecdotal and seems less effective.

Materials and Methods

Plasma levels of FVIIIc, Von Willebrand factor antigen and activity (VWF:act) were measured with routinely used methods. ADAMTS13 antigen (ADAMTS13ag) was measured with a commercial polyclonal (Imubind® ADAMTS13 ELISA, American Diagnostica Inc, Stamford, CT, USA) and a non-commercial monoclonal antibody-based ELISA[a]. ADAMTS13 activity (ADAMTS13act) was measured by proteolysis of FRETS-VWF73[b], a fluorogenic substrate for the metalloprotease (Peptides International Inc, Louisville, KY).

Five production batches of SD-FFP (Octaplas, Octapharma, Vienna, Austria) and 198 single donor units of MB-FFP(Red Cross-Flanders Blood Services, Brugge, Belgium) of the different ABO blood groups were analysed. The plasma of 40 healthy volunteers was analysed in parallel. The pooled plasma of these 40 healthy volunteers was used as reference for selected activity assays, whereas standard human plasma, as provided by the manufacturer, was referred to when using the commercial ADAMTS13 ELISA kit.

The authors thank J. Coene from Red Cross-Flanders Blood Services and Octapharma for providing the plasma samples.

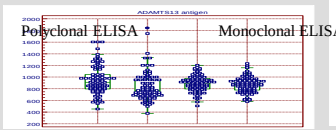
[a] *Feys HB., Liu F., Dong N., Pareyn I., Vauterin S., Vandeputte N., Noppe W., Ruan C., Deckmyn H., Vanhoorelbeke K. ADAMTS-13 plasma level determination uncovers antigen absence in acquired thrombotic thrombocytopenic purpura and ethnic differences. J Thromb Haemost 2006, 4: 955-962.*

[b] *Kokame K., Nobe Y., Kokubo Y., Okayama A., Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. British Journal of Haematology, 2005, 129, 93-100.*

Results

MB treatment influences the levels of all measured plasma proteins. Although statistically significant, the differences were minor. SD-FFP contains less VWF:act, ADAMTS13act and ADAMTS13ag (only with the monoclonal-based ELISA[a]) compared to the normal volunteers and MB-FFP. ADAMTS13ag measured with the polyclonal ELISA, shows higher levels.

Effect of MB treatment on FFP (*P* <0.05 paired Wilcoxon test, before and after MB treatment)



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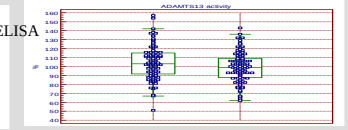

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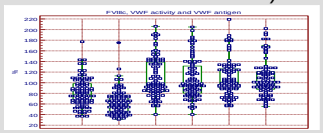


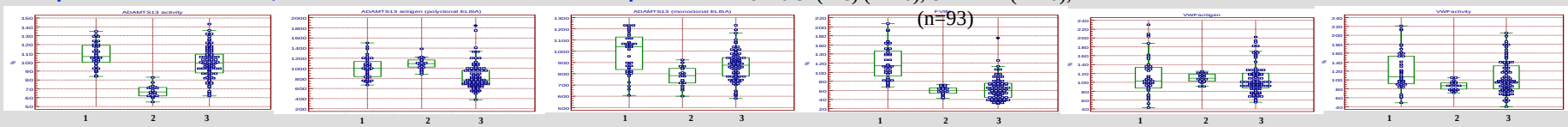
P=0.0000 *P*=0.0067 *P*=0.0198

mean ± sd	before MB*	after MB	Percentage change due to MB
FVIIIc (%)	81,8 ± 26,6	63,8 ± 23,4	-32 ± 28,19
VWFag (%)	108,4 ± 34,2	107,7 ± 32,5	-1 ± 11,8
VWFact (%)	108,6 ± 37,6	107,2 ± 36,4	-3 ± 16,0
ADAMTS13 ag (polyclonal)	944,4 ± 238,7	830,2 ± 246,0	-2 ± 8,9
ADAMTS13 ag (monoclonal)	889,2 ± 136,6	872,2 ± 128,0	-17 ± 25,3
ADAMTS13 act	104,7 ± 18,7	99,7 ± 16,5	-6 ± 19,1

* Virus inactivation with methylene blue/light treatment

Comparison of ADAMTS13, FVIIIc and VWF in virus inactivated plasmas

Normals[1] (Nls) (n=40); SD-FFP[2] (n=20); MB-FFP[3] (n=93)



	ADAMTS13 act (%)			ADAMTS13 ag (polyclonal) (ng/ml)			ADAMTS13 ag (monoclonal) (ng/ml)			FVIIIc (%)			VWFag (%)			VWFact (%)		
	Nls	SD-FFP	MB-FFP	Nls	SD-FFP	MB-FFP	Nls	SD-FFP	MB-FFP	Nls	SD-FFP	MB-FFP	Nls	SD-FFP	MB-FFP	Nls	SD-FFP	MB-FFP
mean ± sd	108.6 ± 13.5	67.5 ± 6.5	99.7 ± 16.5	1004.4 ± 204.6	1090.5 ± 118.2	830.2 ± 246.0	984.2 ± 177.5	777.6 ± 85.6	872.2 ± 128.0	121.3 ± 40.0	59.7 ± 8.7	63.8 ± 23.4	112.5 ± 42.6	109.7 ± 10.0	107.7 ± 32.5	120.2 ± 42.5	87.1 ± 9.8	107.2 ± 36.4
MB-FFP	0,0037	0,0000		0,0000	0,0000		0,0007	0,0010		0,0000	0,7932		0,6811	0,1447		0,0763	0,0284	
Nls		0,0000			0,0439			0,0000			0,0000			0,3884			0,0003	

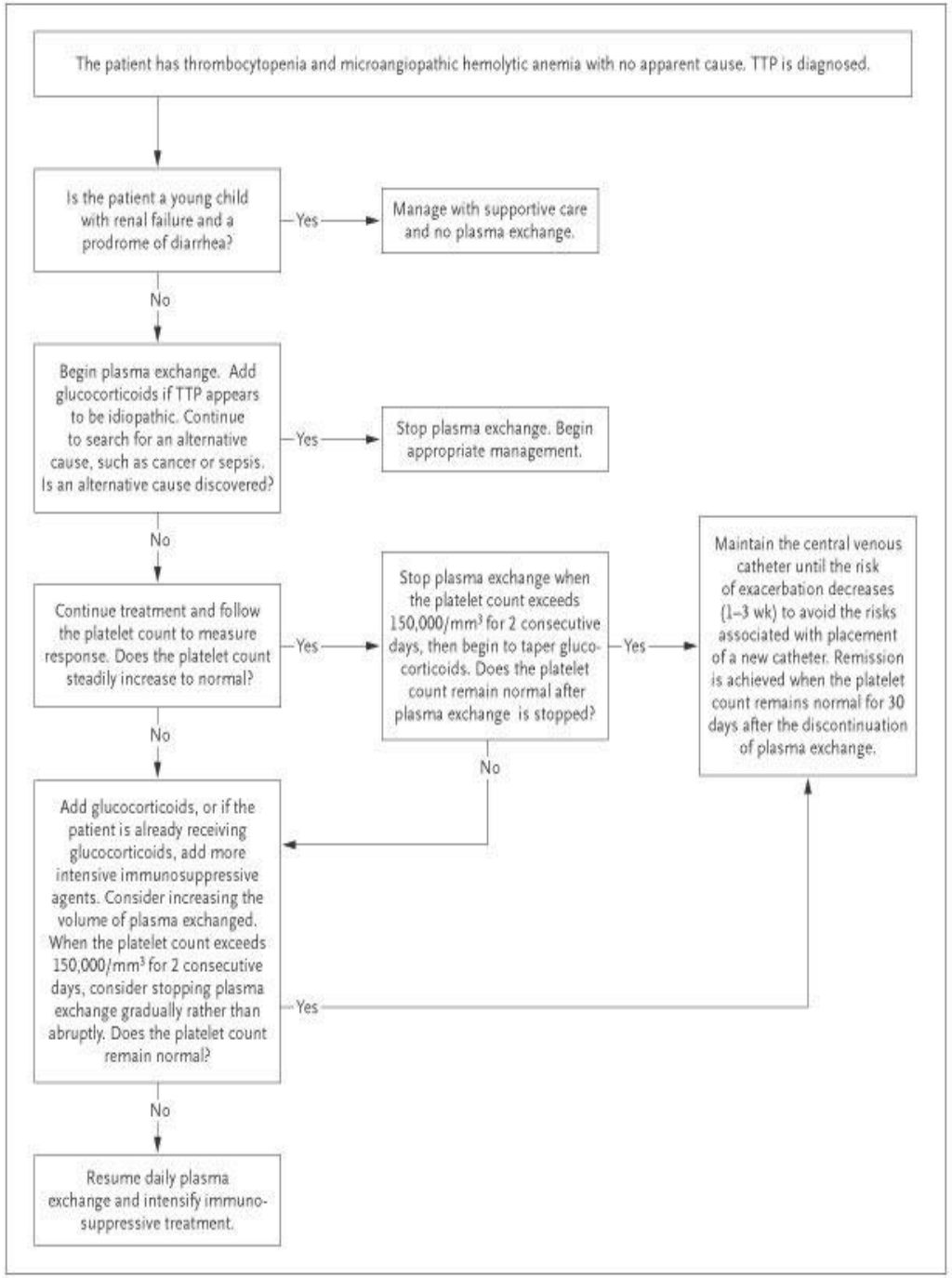
P <0.05 Wilcoxon test

Conclusion: The quantitative differences in ADAMTS13ag levels with both methods give rise to further investigations regarding the protein structure of ADAMTS13 in both plasma products. Although the ADAMTS13 activity in SD-FFP is lower than in MB-FFP, the clinical outcome in TTP patients has been shown to be better with SD-FFP. Hitherto unidentified plasma proteins may contribute to the pathophysiology of TTP and explain the reported difference in clinical efficacy. A randomized clinical trial should be conducted to find out which plasma product is best suited to treat TTP patients.

Alvarez-Larran A., Del Rio J., Ramirez C., Albo C. et al. Methylene blue-photoinactivated plasma vs. fresh-frozen plasma as replacement fluid for plasma exchange in thrombotic cytopenic purpura. Vox Sanguis, 2004, 86, 246-251;

De La Rubia J., Arriaga F., Linares L., Larrera et al. Role of methylene blue-treated or fresh-frozen plasma in the response to plasma exchange in patients with thrombotic thrombocytopenic purpura. Br. J. of Haematology, 2001, 114: 721-723.

A Plan for the Management of an Initial Episode of Clinically Diagnosed Thrombotic Thrombocytopenic Purpura (TTP)



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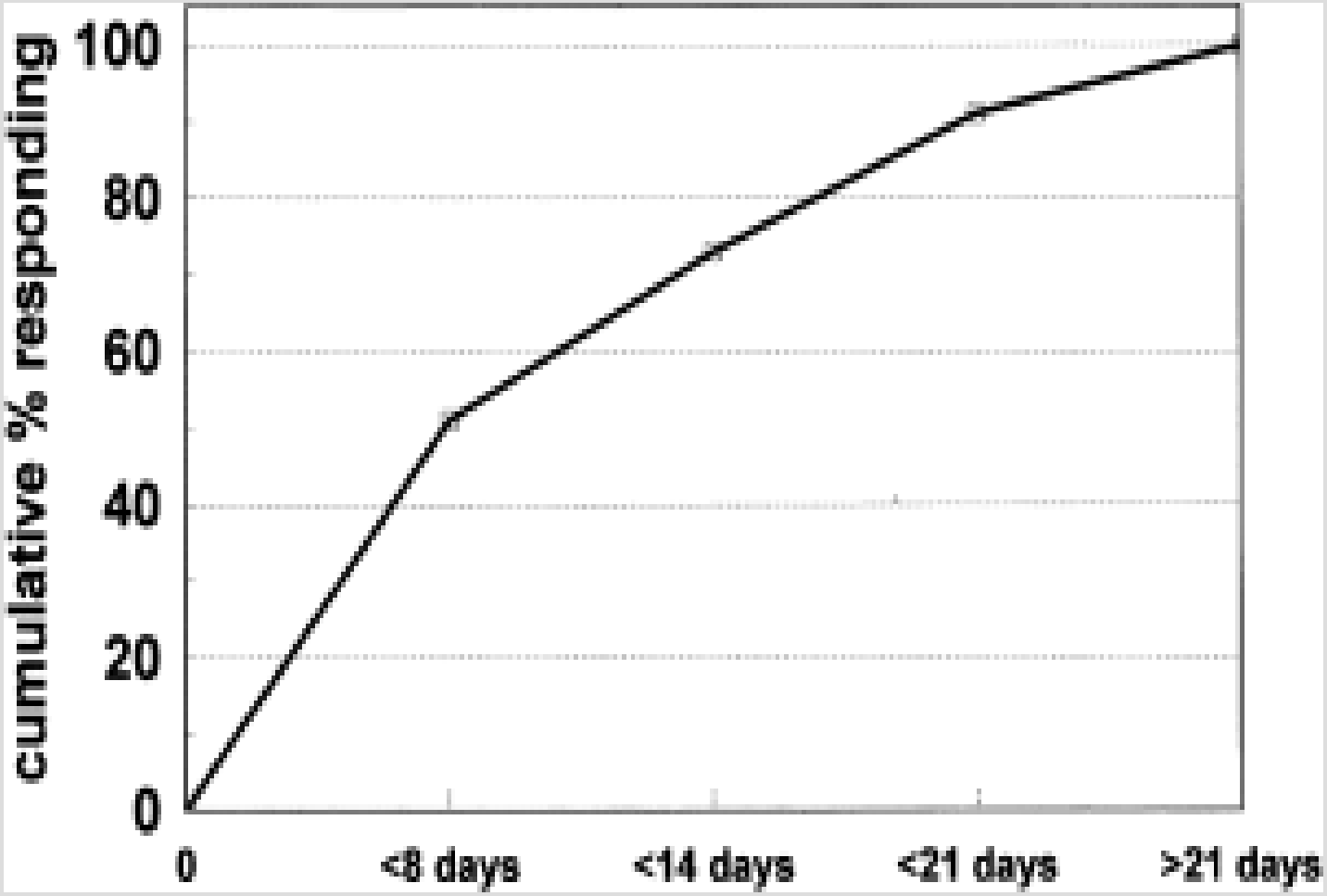
George J. N Engl J Med 2006;354:1927-1935

TTP : TPE therapy duration in 122 patients



ADAMTS13

- Specificity and sensitivity in id.TTP :
  - Undetectable or low <5% : rare in non id.TTP
  - In id.TTP : <5% varies from 33 to 100% of patients!
- Prognostic significance :
  - Severe deficiency = good TPE response rate, but higher probability of relapse, most in patients with detectable inhibitors.
  - Inhibitors = high mortality risk factor (17-25%)
- Biomarker of disease :
  - Not always! (mutated ADAMTS13/…)

TTP clinical strategy

- **Empiric TPE for all id.TTP**
- ADAMTS13 testing, if rapid test (almost) available?
  - ADAMTS13 as biomarker in relapsing TTP?
  - More sensitive tests, also for inhibitors (antibodies) needed
- Primary or secundary immunosuppression?
  - W/o Rituximab in trial?

TPE in TTP Patients : Questions :

- Discussion :
  - SD- or MB-Plasma?
  - **Current ‘guideline’** (e.g.UK) : **SD-plasma**
- Treatment algorithms : available
- Study proposals : ?
  - SD- vs MB-Plasma
  - TPE w/o Rituximab
  - Other combinations