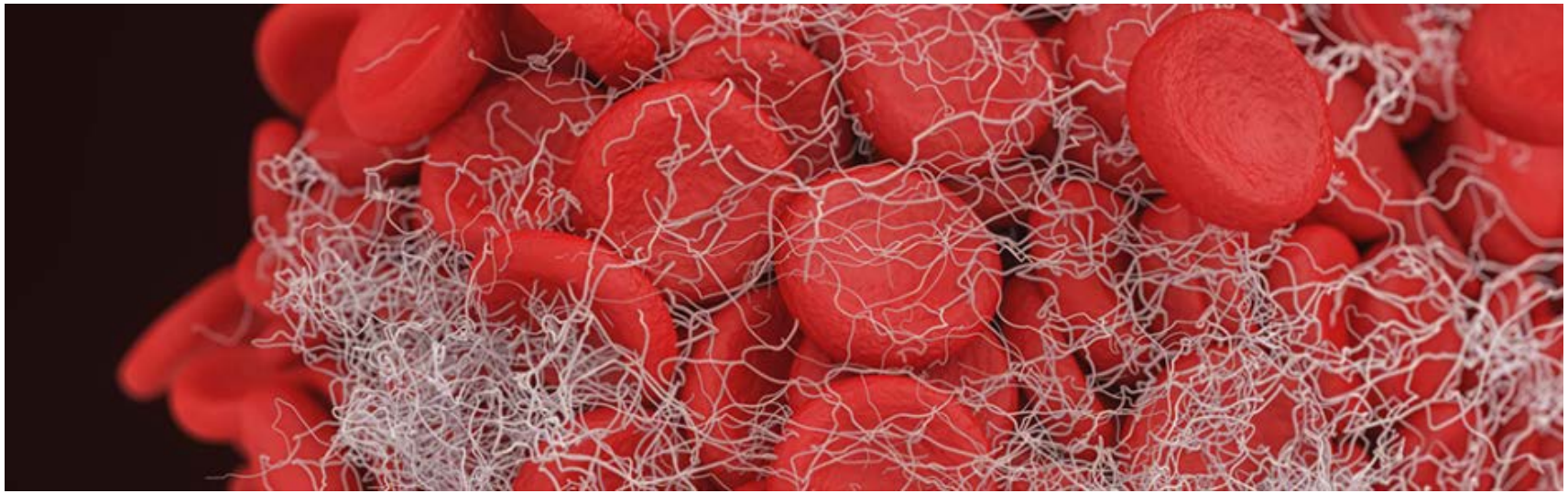
Laboratory investigation and diagnosis of thrombotic thrombocytopenic purpura (TTP)



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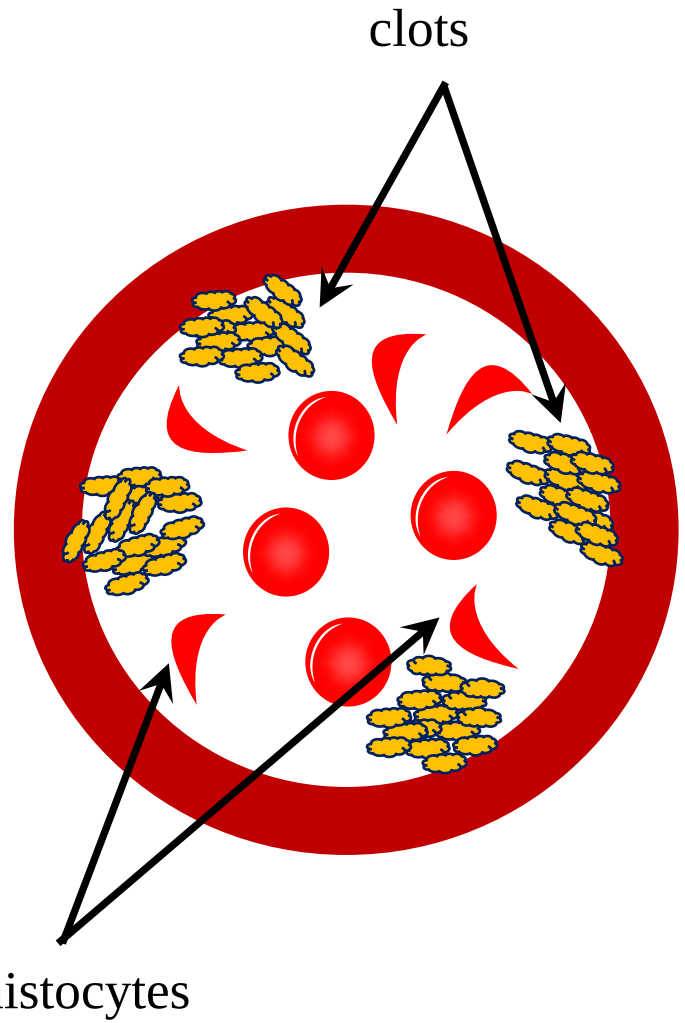
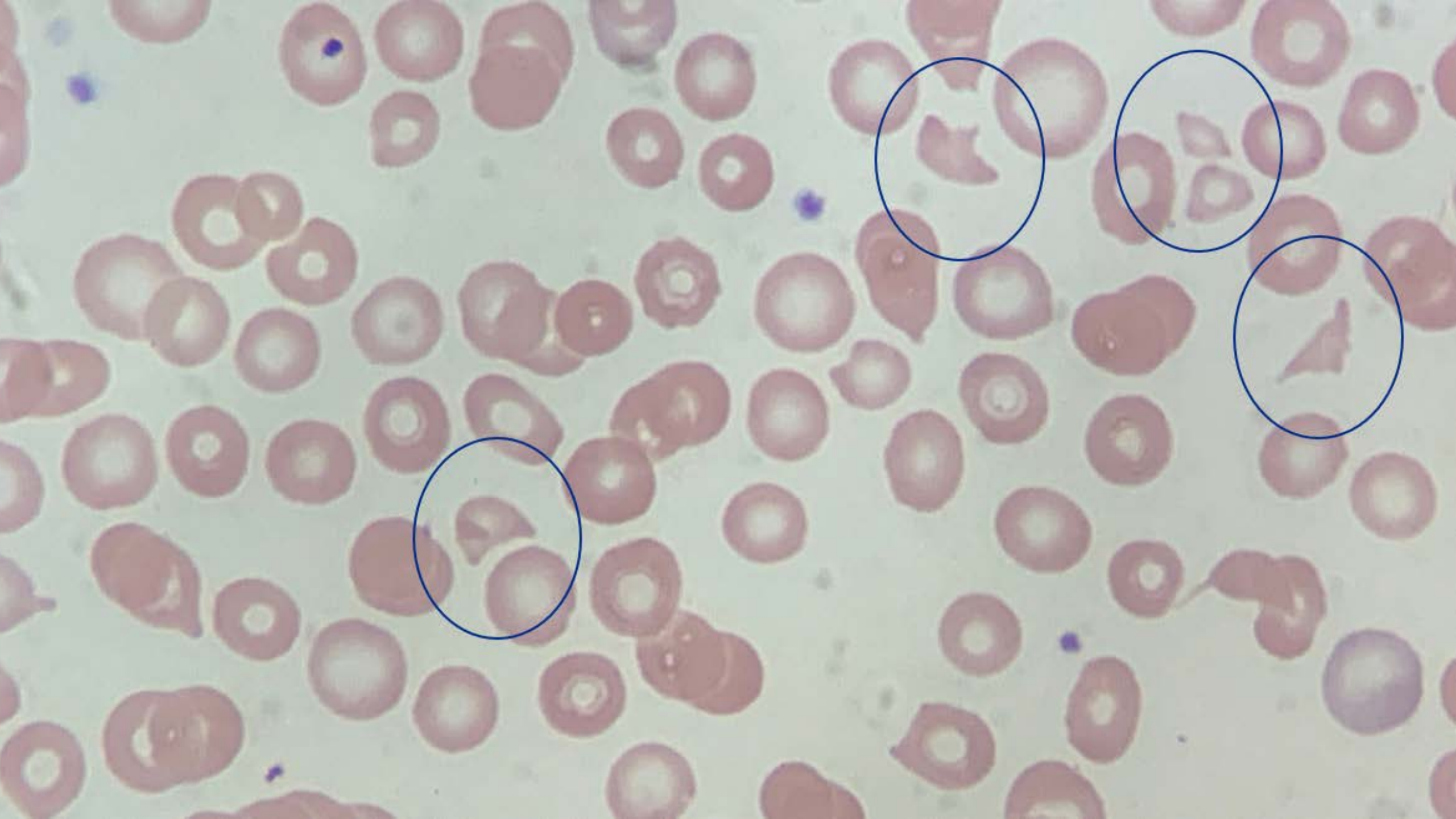
Department of Clinical Biochemistry,
Bispebjerg Hospital, Copenhagen, DK

TTP: a trombotic microangiopathy (TMA)

- trombosis

- micro (=small) + angio (=vessel)





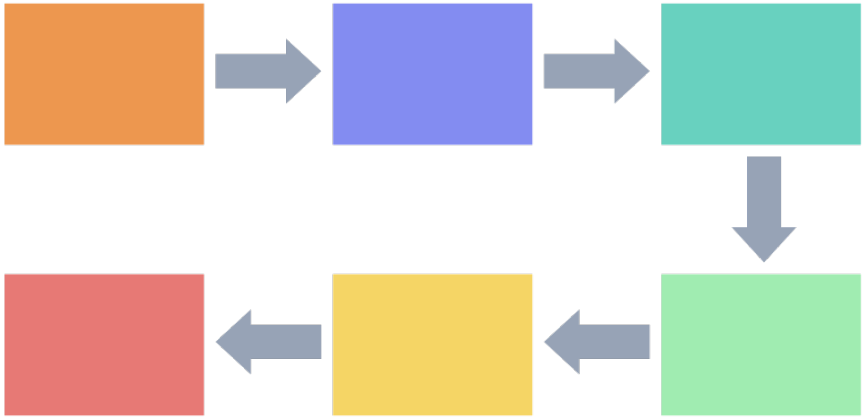
TMA: a laboratory-based diagnosis?

1) hemolytic anemia (↓ haptoglobin, ↑ LDH, ↑ bilirubin)

2) schistocytes > 1% (**not always…!**)

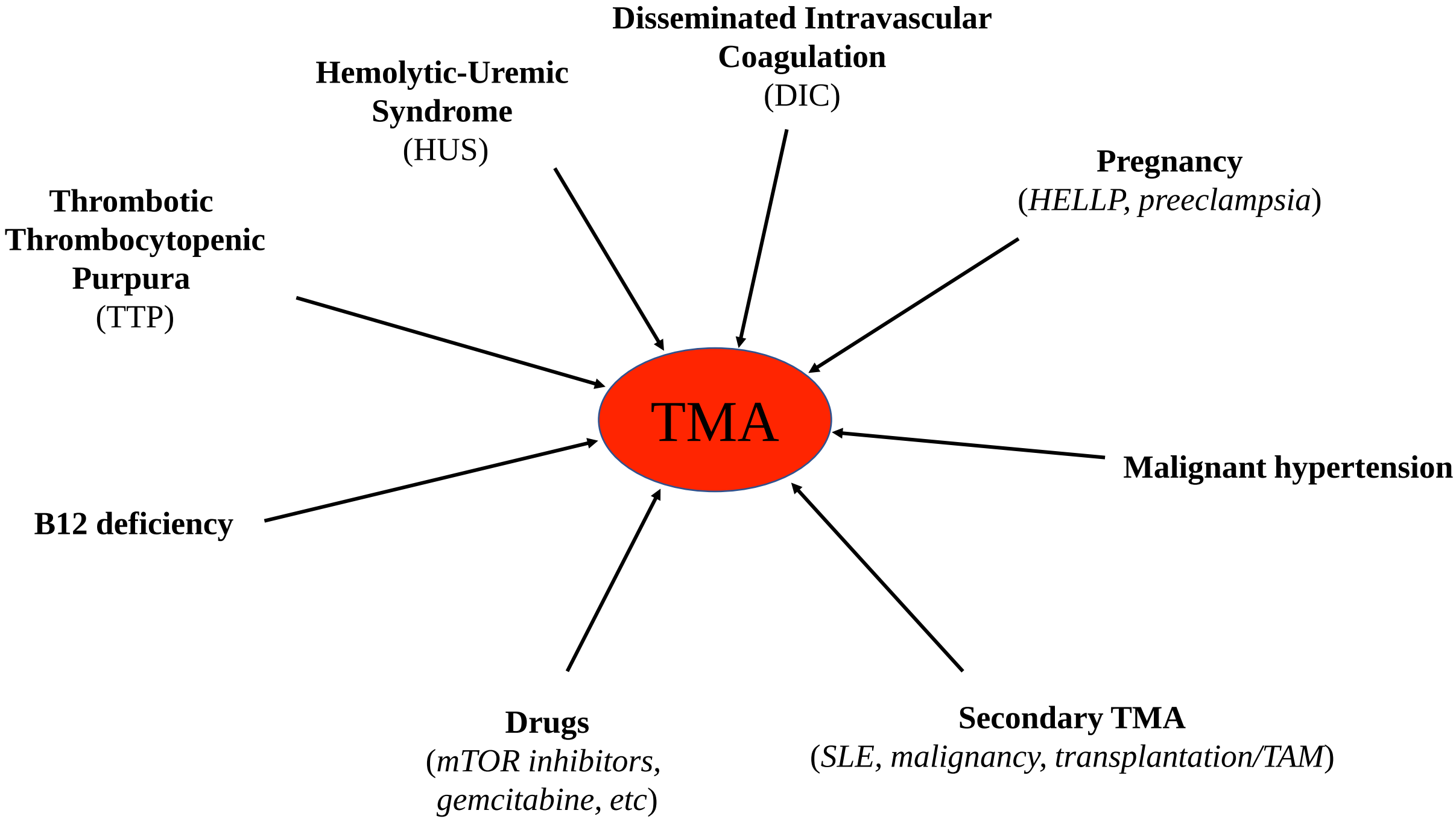
3) thrombocytopenia

TMA: and then what?



- **<u>the easy part</u>**: the patient has TMA

- **<u>the tricky part</u>**: which TMA? (***read***: which treatment…?)


Disseminated Intravascular Coagulation (DIC)
Hemolytic-Uremic Syndrome (HUS)
Pregnancy (*HELLP, preeclampsia*)
Thrombotic Thrombocytopenic Purpura (TTP)
TMA
Malignant hypertension
B12 deficiency
Drugs (*mTOR inhibitors, gemcitabine, etc*)
Secondary TMA (*SLE, malignancy, transplantation/TAM*)

Thrombotic thrombocytopenic purpura (TTP)

- the most lethal TMA
  - *(90% mortality if left untreated)*

- the only TMA responsive to plasmapheresis
  - *mortality reduced to <10%*

plasmapheresis or not?


You get a plasmapheresis!
And you get a plasmapheresis!
And you get a plasmapheresis!

Plasmapheresis complications

Complication	**TTP (N = 68)**	**No TTP (N = 274)**
Death	4 (4.4%)	4 (1.5%)
Catheter-related (*infection*, *pneumothorax*, *etc*)	28 (41%)	41 (15%)
Plasma-related (*hypotension*, *anaphylaxia*, *hypoxia*)	8 (12%)	14 (5%)

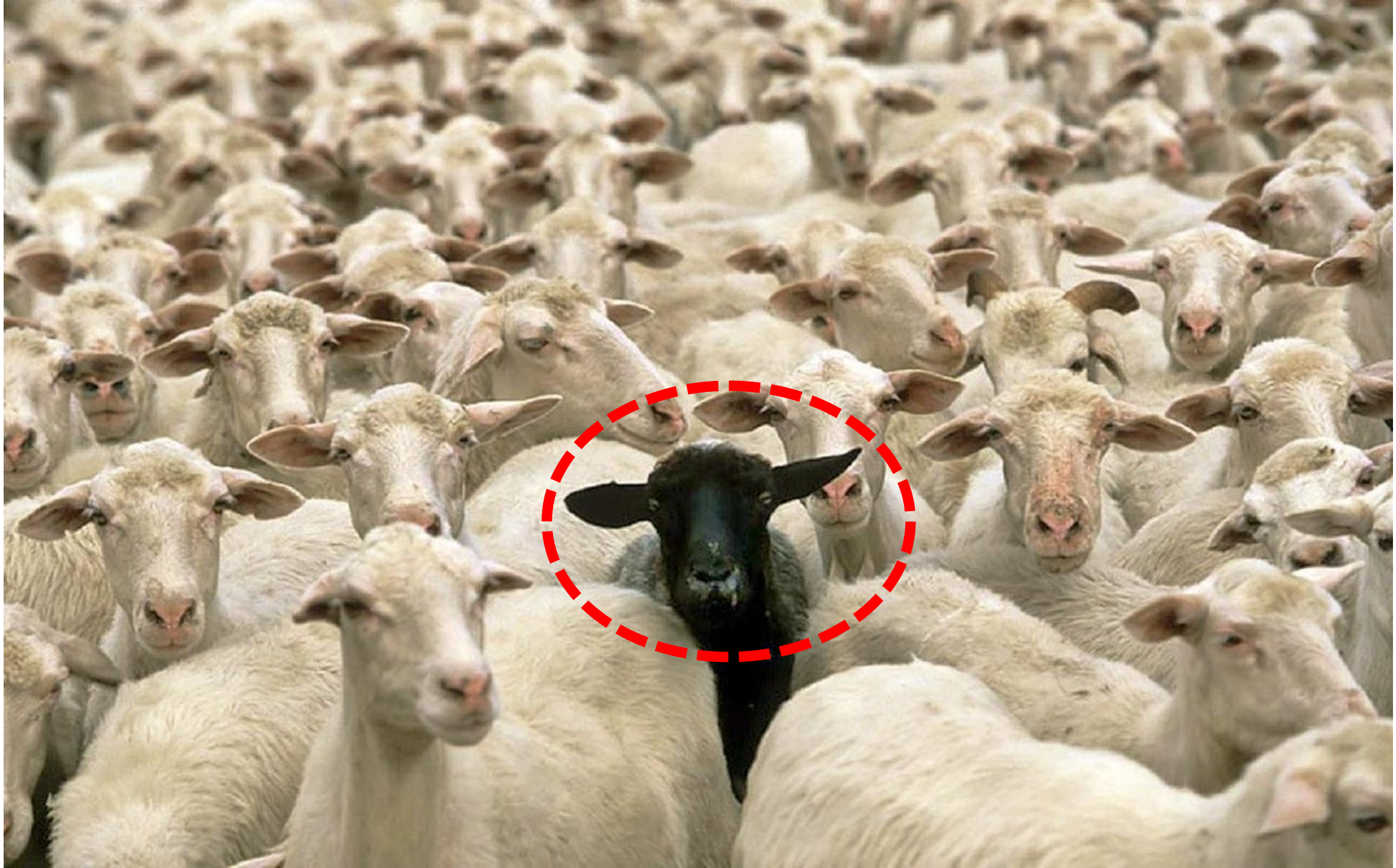
Page E, *et al*. Blood Adv. (2017) Apr 11; 1(10): 590–600

TTP statistics

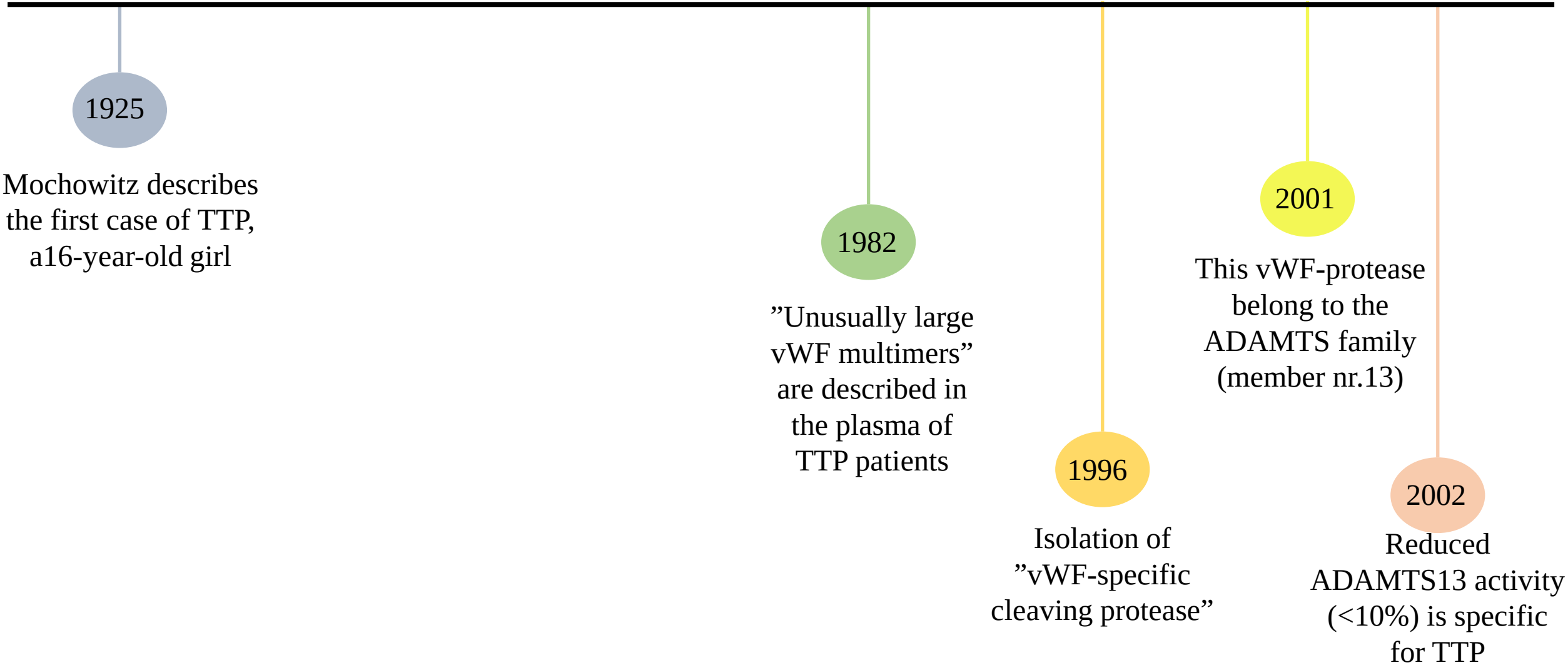
- **Incidence in the US**: 3-6 per 1.000.000 yearly

- **Incidence in DK**: 10-20 cases per year
  (~2 per 1.000.000 yearly)

Mariotte E, *et al*. Lancet Haematol (2016) May;3(5):e237-45
Page E, *et al*. Blood Adv. (2017) Apr 11; 1(10): 590–600



TTP: the (hi)story…

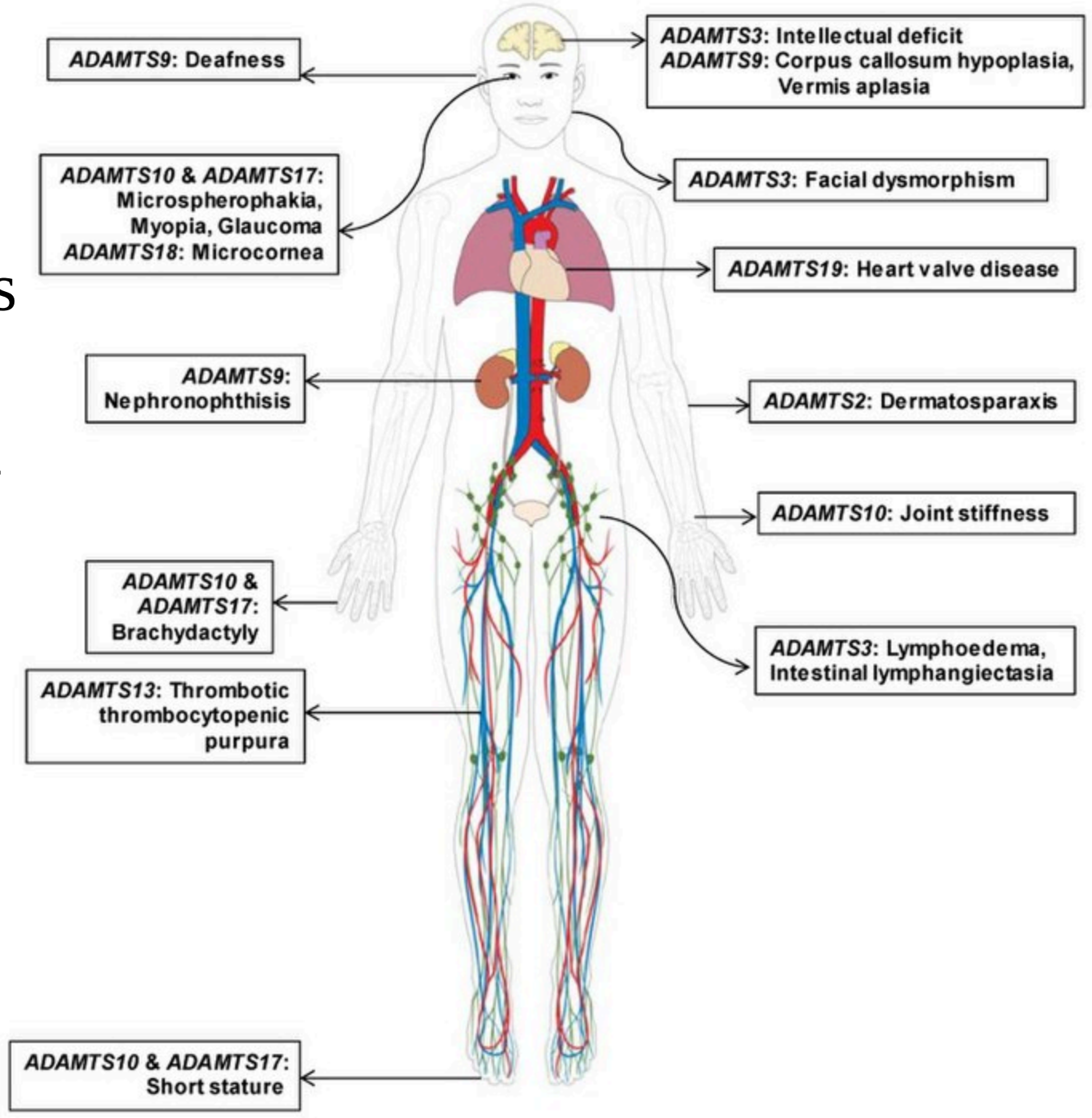


Metalloprotease

- metallo = metal (*greek*)
- protease = cleaves proteins

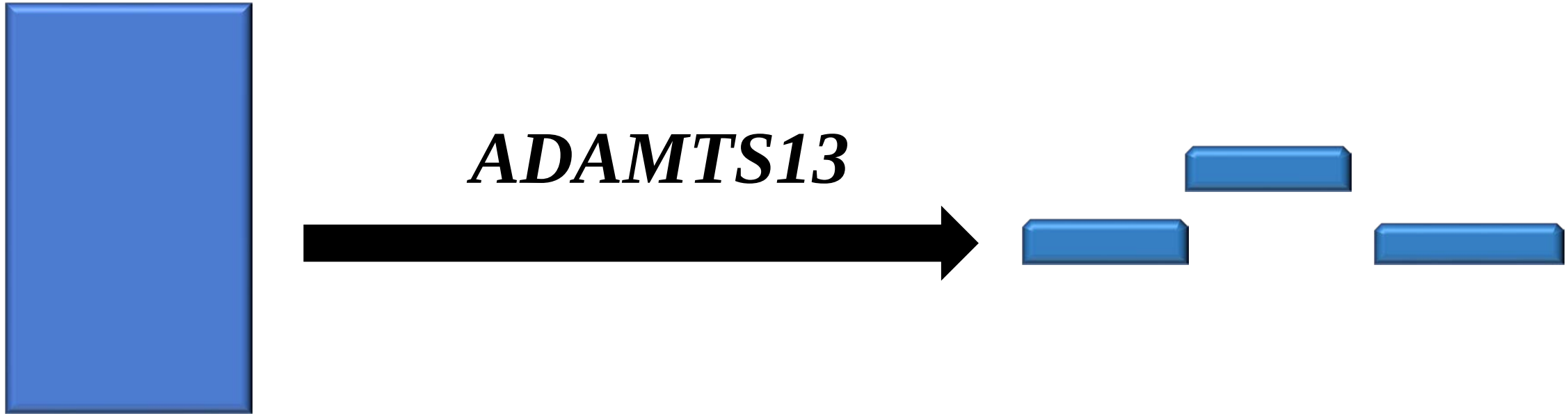
ADAMTS: cleaves proteins + requires metal
(*in vivo*: zink)

Extracellular matrix remodelling



ADAMTS13

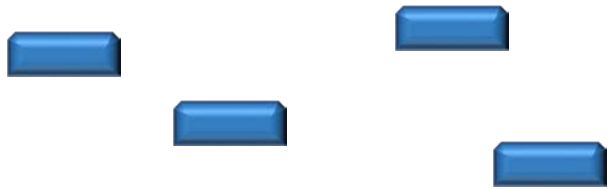
(***a d****isintegrin* ***a****nd* ***m****etalloproteinase with a* ***t****hrombo****s****pondin type 1 motif, member* ***13***)

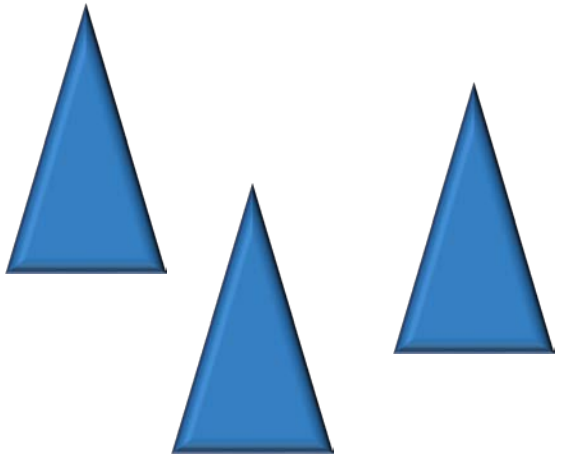


”Pre-vWF”

Normal vWF multimers

von Willebrand Factor



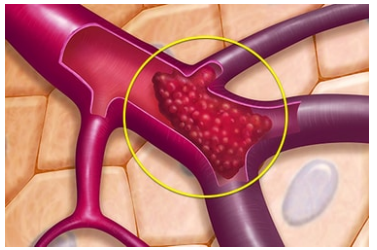


Small multimers

vW disease Type2A

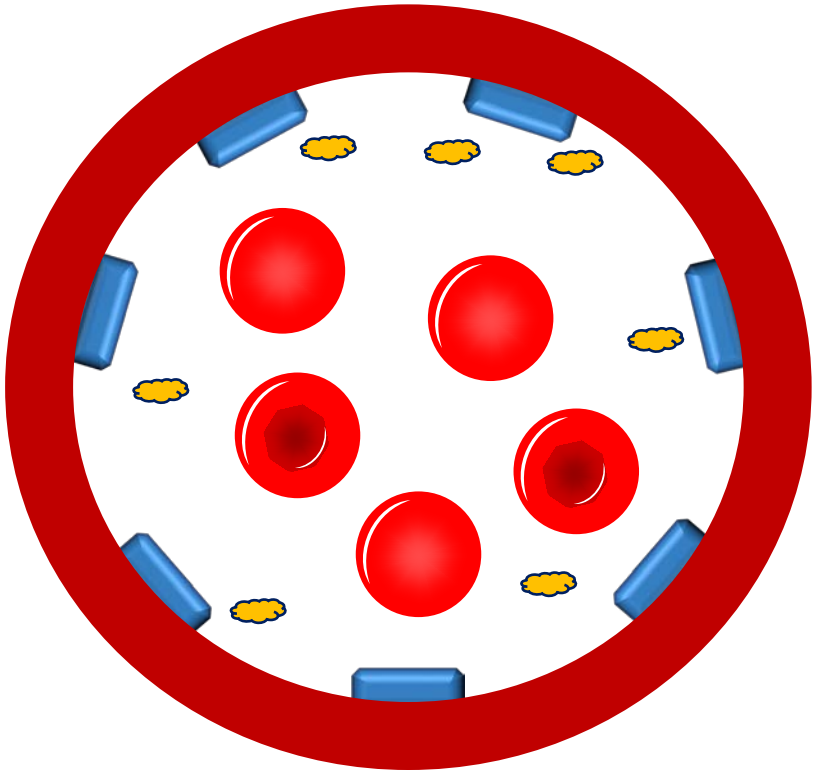
Normal multimers

Big multimers



TTP

Normal ADAMTS13 activity



Reduced ADAMTS13 activity (TTP)

Reduced ADAMTS13 activity: why?

- **<u>Mutations (biallelic)</u>**
  - Congenital TTP (cTTP or Upshaw-Schulman syndrome)
  - Rare (~ 5% of all cases)

- **<u>Antibodies against ADAMTS13</u>**
  - Acquired TTP or immune-mediated TTP (iTTP)
  - Common (~ 95% of all cases)

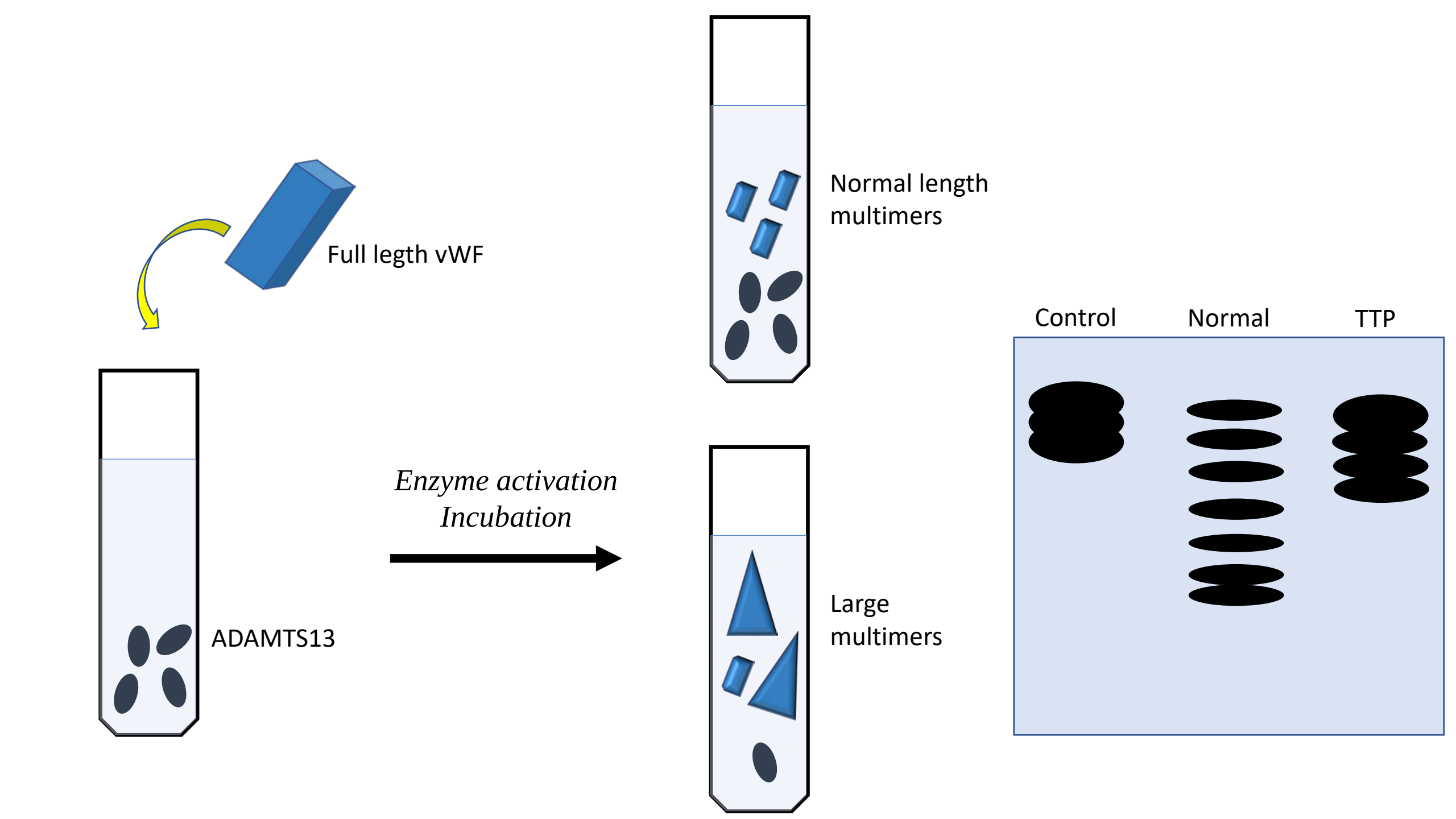
Mariotte E, *et al*. Lancet Haematol (2016) May;3(5):e237-45
Page E, *et al*. Blood Adv (2017) Apr 6;1(10):590-600

TTP = ADAMTS13 deficiency!!!

- **ADAMTS13 activity < 10%** specific for TTP

- Most effective diagnostic tool for TTP!

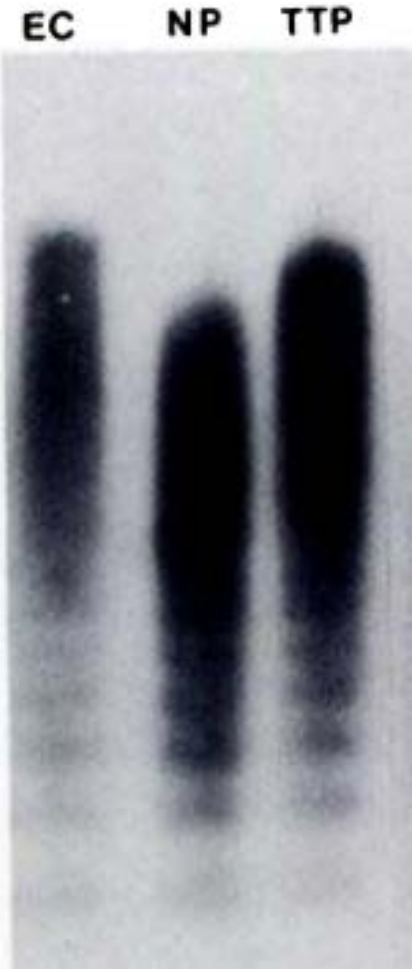
Furlan M, *et al*. N Engl J Med (1998) Nov 26;339(22):1578-84
Bianchi V, *et al*. Blood (2002) Jul 15;100(2):710-3


Full legth vWF
ADAMTS13
Enzyme activation
Incubation
Normal length
multimers
Large
multimers
Control
Normal
TTP

First assay for TTP (vWF multimers)

- electrophoresis: 5 hours
- wash: 1 hour
- incubation (with I^{185}): 18 hours
- wash: 6 hours
- drying: 6 hours
- film production: 24 – 48 hours

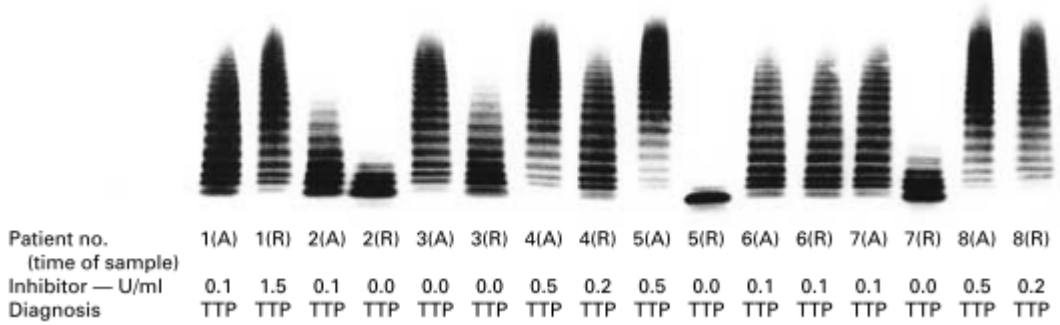
*Positive control: endothelial cells from umbilical cord veins. Isolated and grown for 2 days ➔ culture medium used!



Moake J, Byrnes J, *et al*. Blood (1984) Sep;64(3):592-8.

vWF multimers: revised

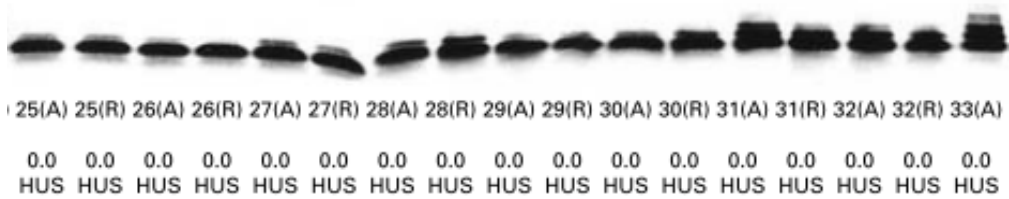
- sample - enzyme activation: metal (Ba^{++})
- substrate: large vWF cryoprecipitate



➔ mix + incubate: 24 hours

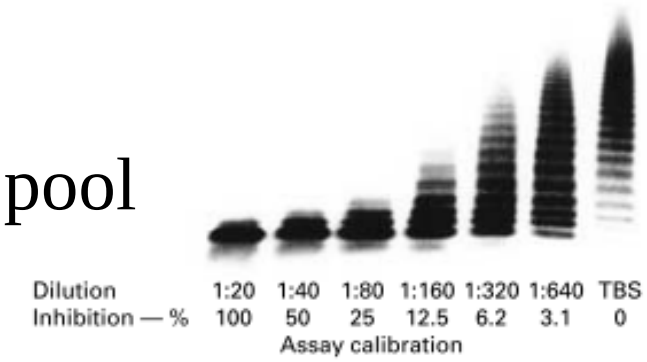
➔ Stop reaction: EDTA (minutes)

➔ Western blot: ~ 24 hours



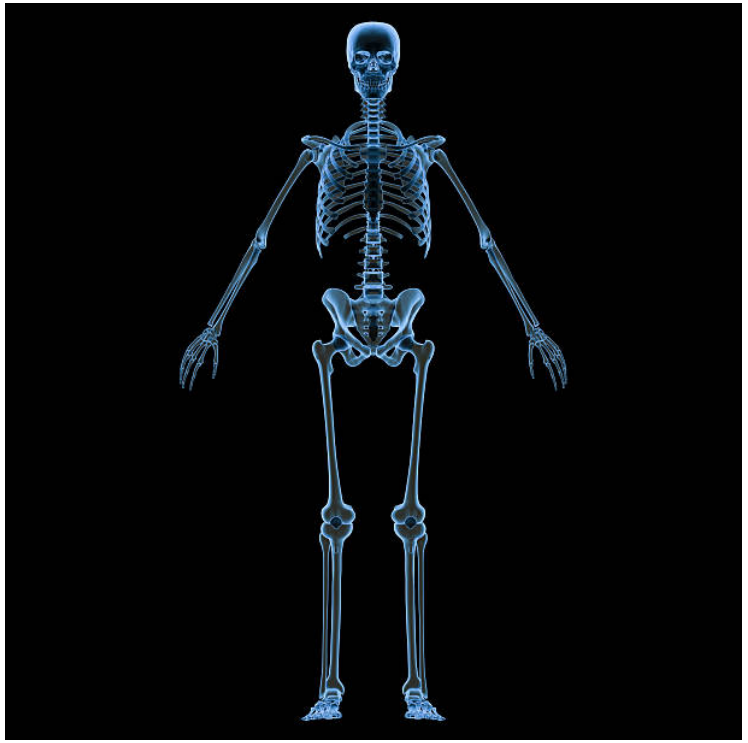
”Calibration curve”:

dilutions of normal plasma pool



Furlan M, *et al*. N Engl J Med (1998) Nov 26;339(22):1578-84

ADAMTS13 activity assays



First generation:

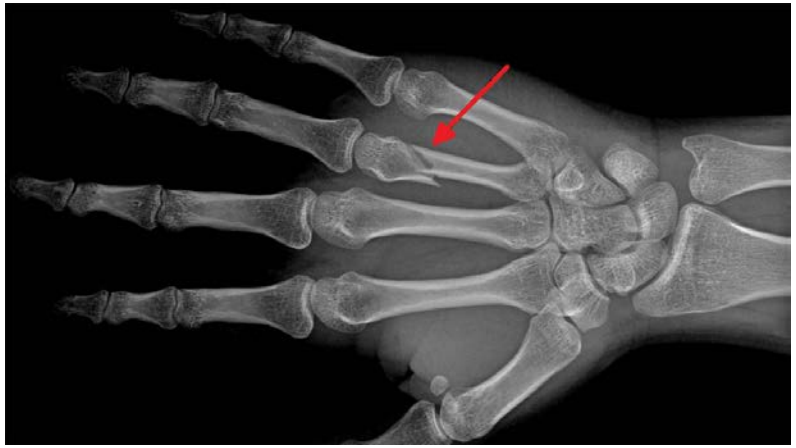
full-length vWF (**2813 aa…!!!**)

2-3 days…!

Second generation:

vWF peptides (most often VWF73)

30min – 4 hours



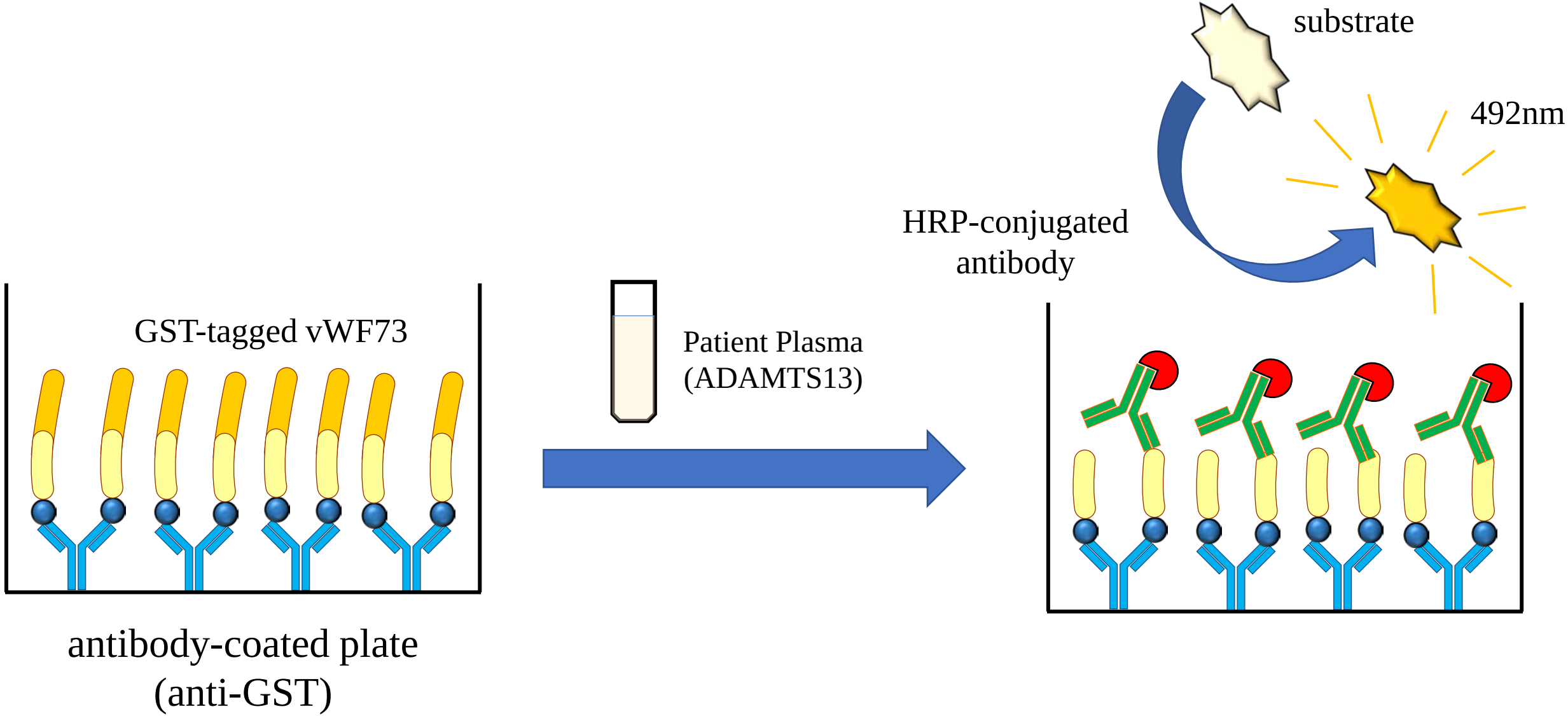
ADAMTS13 activity assays

Assay	Generation	Substrate	Measurement	Time
wWF multimers (IB)	First		vWF multimers	2-3 dage
Collagen-binding (CBA)			% bound in collagen	24 h
Ristocetin-cofactor			vWF:Rco [%]	24 h

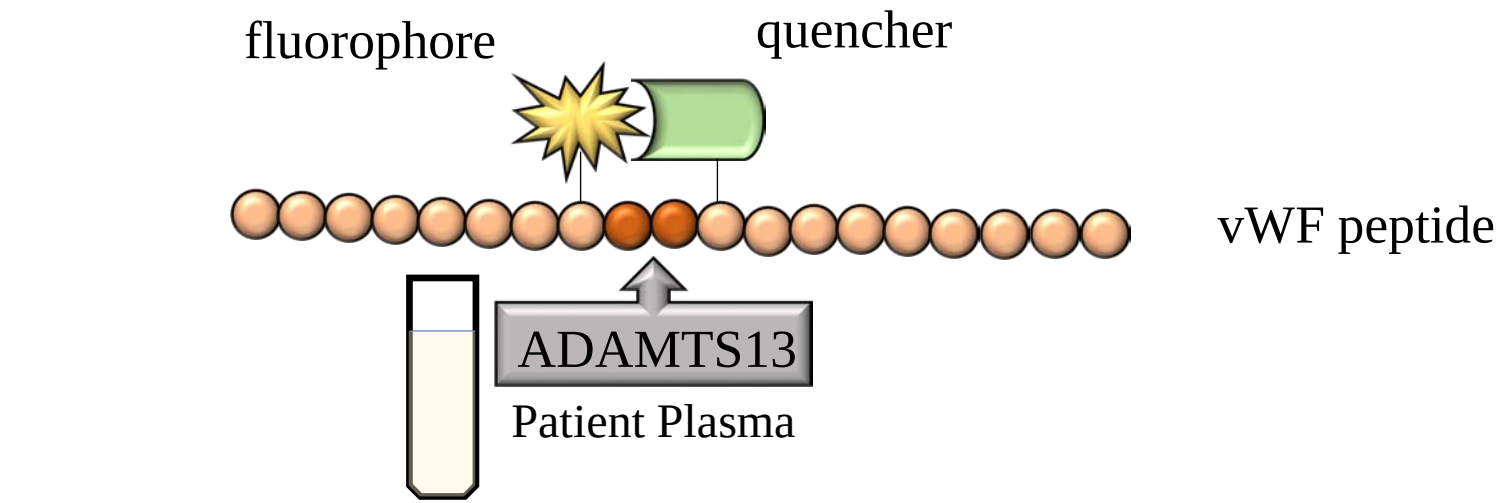
ADAMTS13 activity measurement

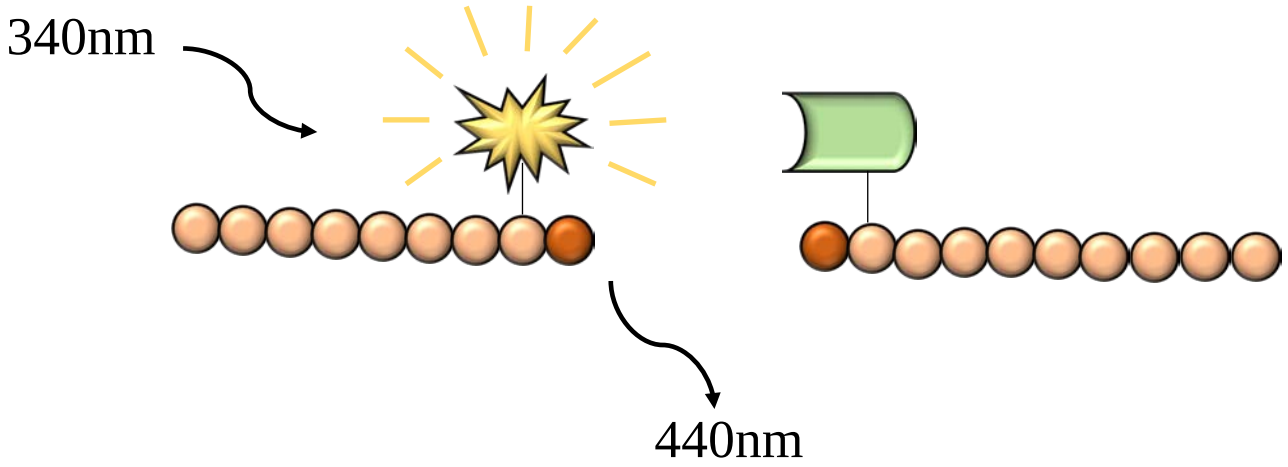
- TECHNOZYM® (ELISA)
- FRET
- HemosIL Accustar®

TECHNOZYM (ELISA-based)



FRET (fluorescence resonance energy transfer)

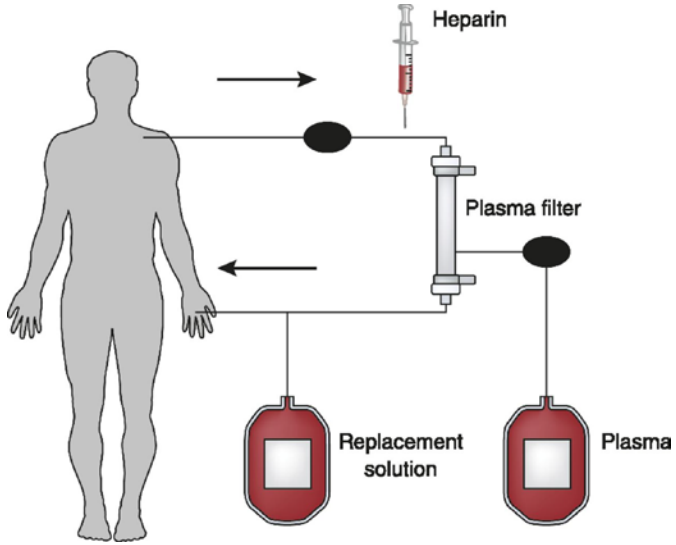





TIME GOES BY
SO SLOWLY…

Suspicion of TTP

Plasmapheresis



ADAMTS13

Zheng X, *et al.* (2020): *Journal of Thrombosis and Haemostasis*

TTP suspicion → Probability → Confirmation!

Basic tests

- Hgb
- Platelets
- Haptoglobin
- LDH
- schistocytes

Clinical scores

- PLASMIC
- French TMA
- Bentley

ADAMTS13 activity

PLASMIC score

Variable	Points
Thrombocytes < 30 x 10^9 /L	1
Hemolysis*	1
No active cancer	1
No history of solid-organ or stem-cell transplant	1
MCV < 90 fL	1
INR < 1.5	1
Creatinine < 2.0 mg/dL (177 µmol/L)	1

* *reticulocytosis, low haptoglobin, high bilirubin*

Sensitivity: 90 – 100%
Specificity: 46.2 – 92%
PPV: 50 – 72%
NPV: 98 – 100%

Score = 0-4 ➔ low probability for TTP
Score = 5 ➔ intermediate probability
Score = 6-7 ➔ high probability

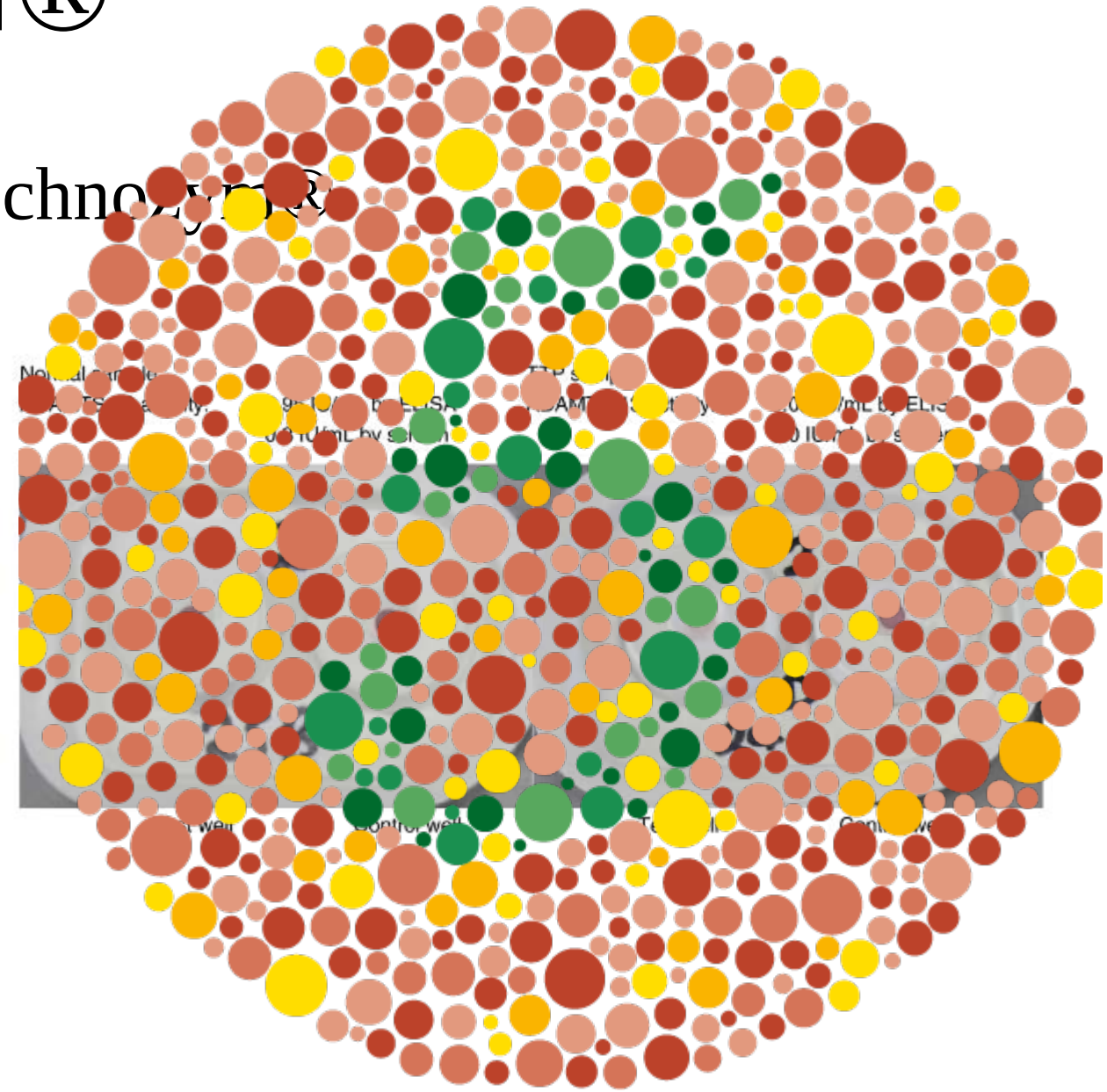
Bendapudi P, *et al*. Lancet Haematol (2017) Apr;4(4):e157-e164
Jajosky R, *et al*. Transfus Apher Sci (2017) Aug;56(4):591-594
Li A *et al*. J Thromb Haemost (2018) Jan;16(1):164-169

PLASMIC score

- not validated in children or pregnant women (cTTP)
- unreliable with increasing age!
- **cannot be used to diagnose TTP!**

TECHNOSCREEN®

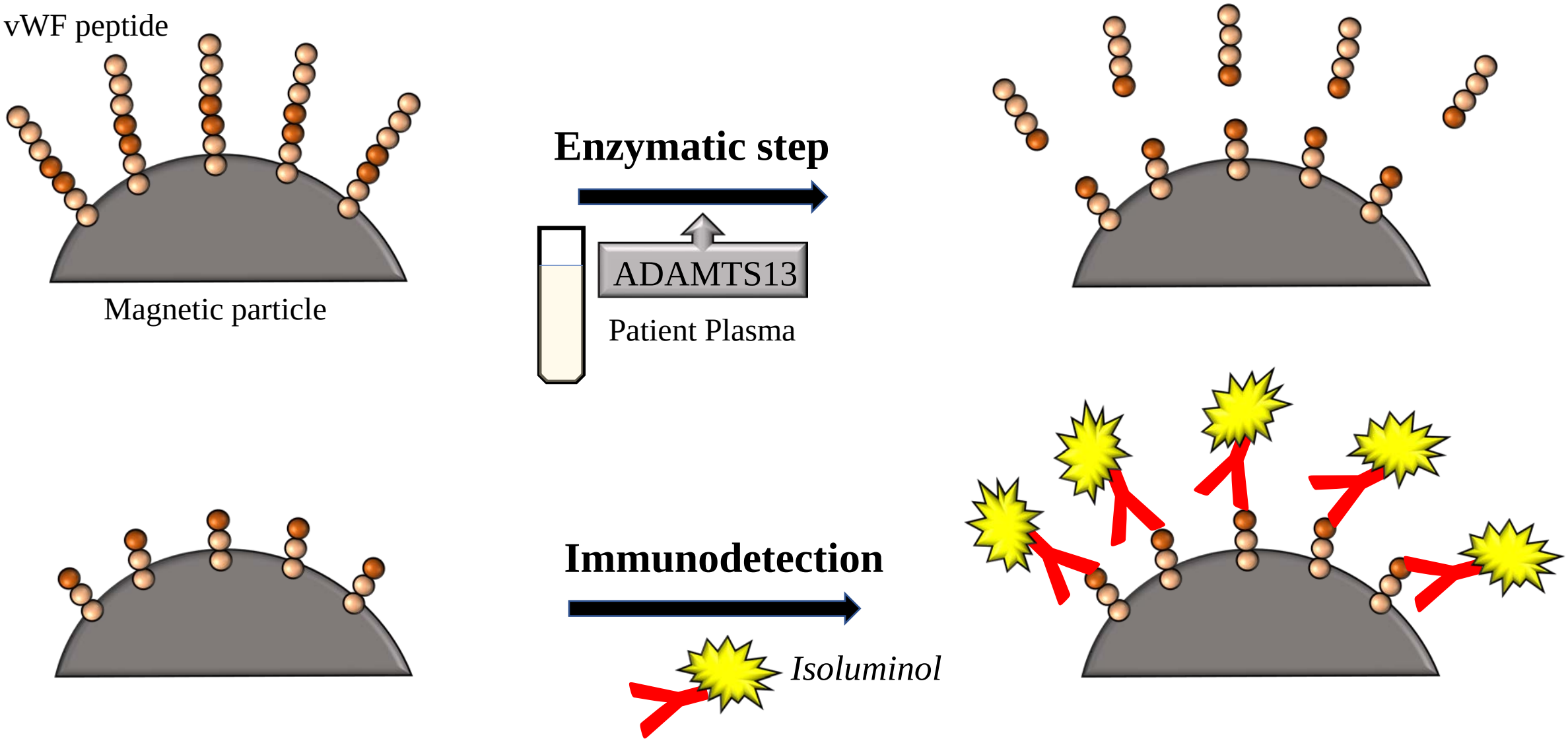
”pregnancy test” equivalent of Technozym®



Sensitivity: 88.7%
Specificity: 90.4%
PPV: 74.6%
NPV: 96.2%

Moore G, *et al. J Thromb Haemost (2020) Jul;18(7):1686-1694*

HemosIL Acustar® ADAMTS13 assay

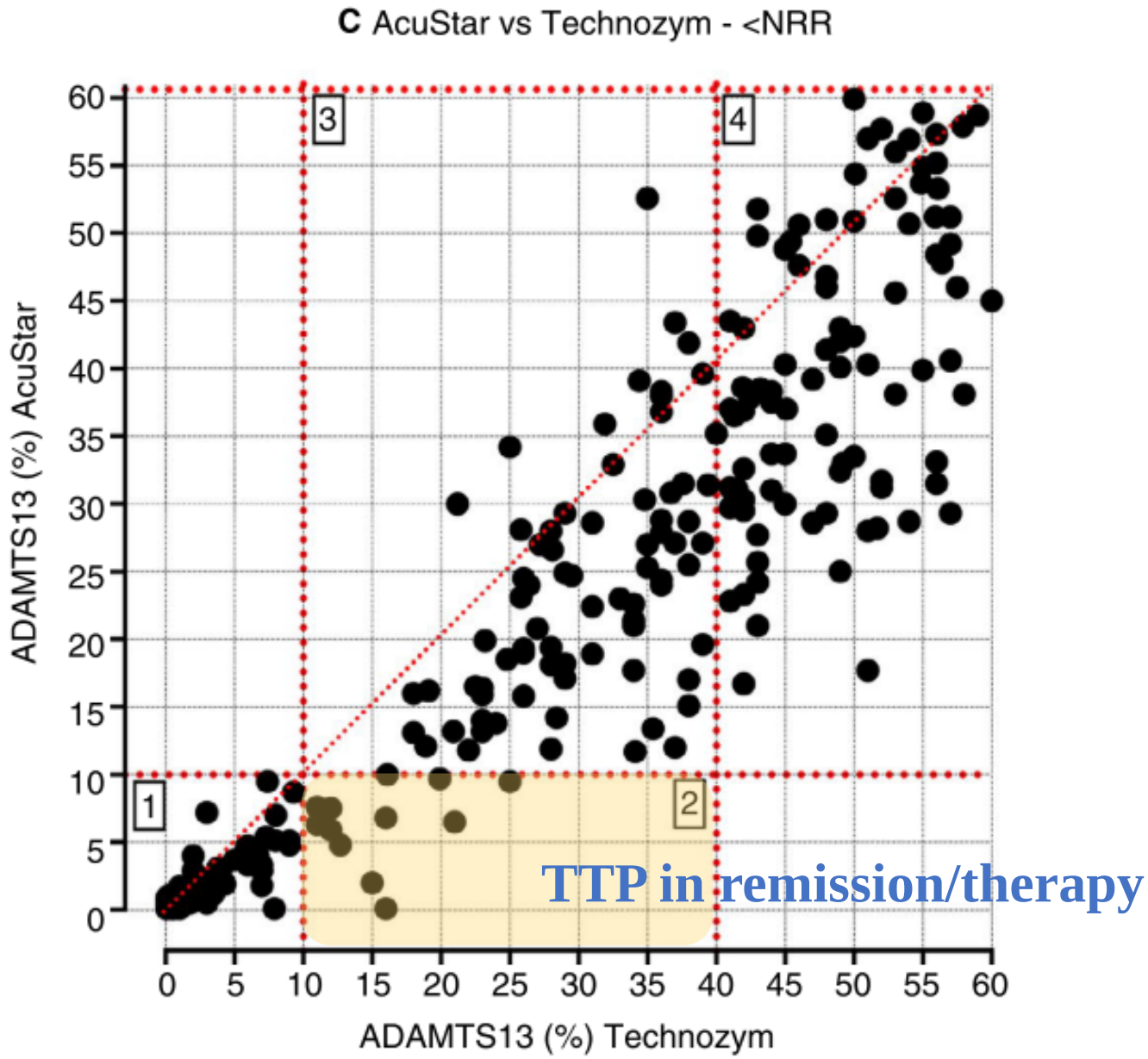


FRET vs Accustar

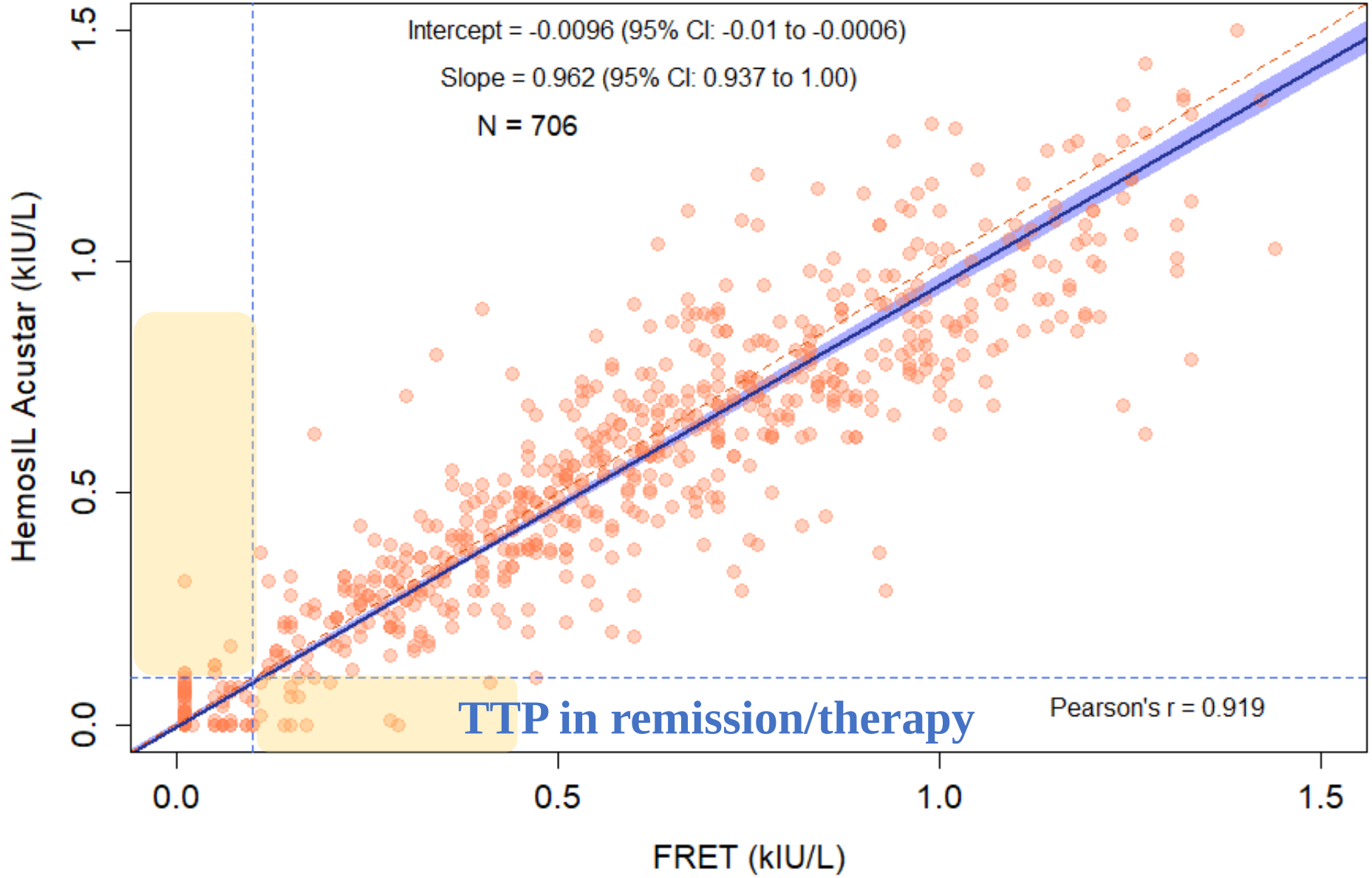
- manual (FRET) vs fully automated (Accustar)
- Running time: 4-5 hours (FRET) vs **33 min** (Accustar)
- Interference: bilirubin vs rheumatoid factor

HemosIL Acustar® vs the rest

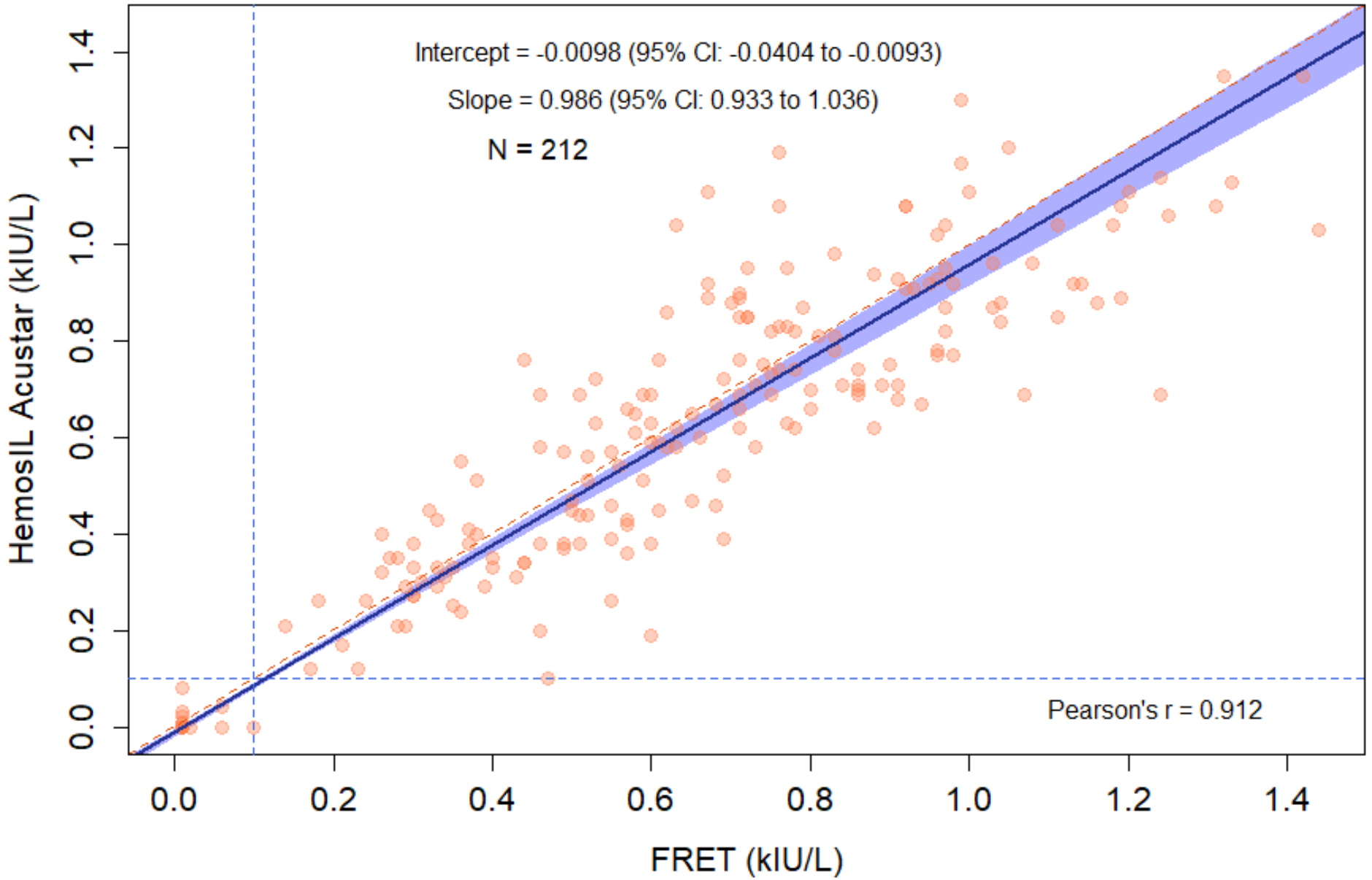
Study	**Assay comparison**	**Samples (*N*)**	**Conclusions**
Favresse, *et al* (2018); *Thromb and Haemost*	Technozym®	38	No misclassifications
Valsecchi, *et al* (2019); *Thromb and Haemost*	FRET Technozym®	176	**2 FN** with HemosIL (incl. one congenital TTP)
Stratmann, *et al* (2020); *Journ of Thromb and Hemol*	Technozym®	24	No misclassifications
Favaloro, *et al* (2021); *Journ of Thromb and Haemost*	Technozym®	733	12 discrepancies: most TTP in remission/therapy
Beranger, *et al* (2021); *Res and Pract Thromb and Haemost*	FRET	539	14 discrepancies – incl. **1 FN** with congenital TTP
Dimopoulos, *et al* (2022); *Journ of Applied Lab Med*	FRET	706	**2 FP** with HemosIL (one DIC, one SLE)



Favaloro E, *et al.* J Thromb Haemost (2021) Feb;19(2):417-428

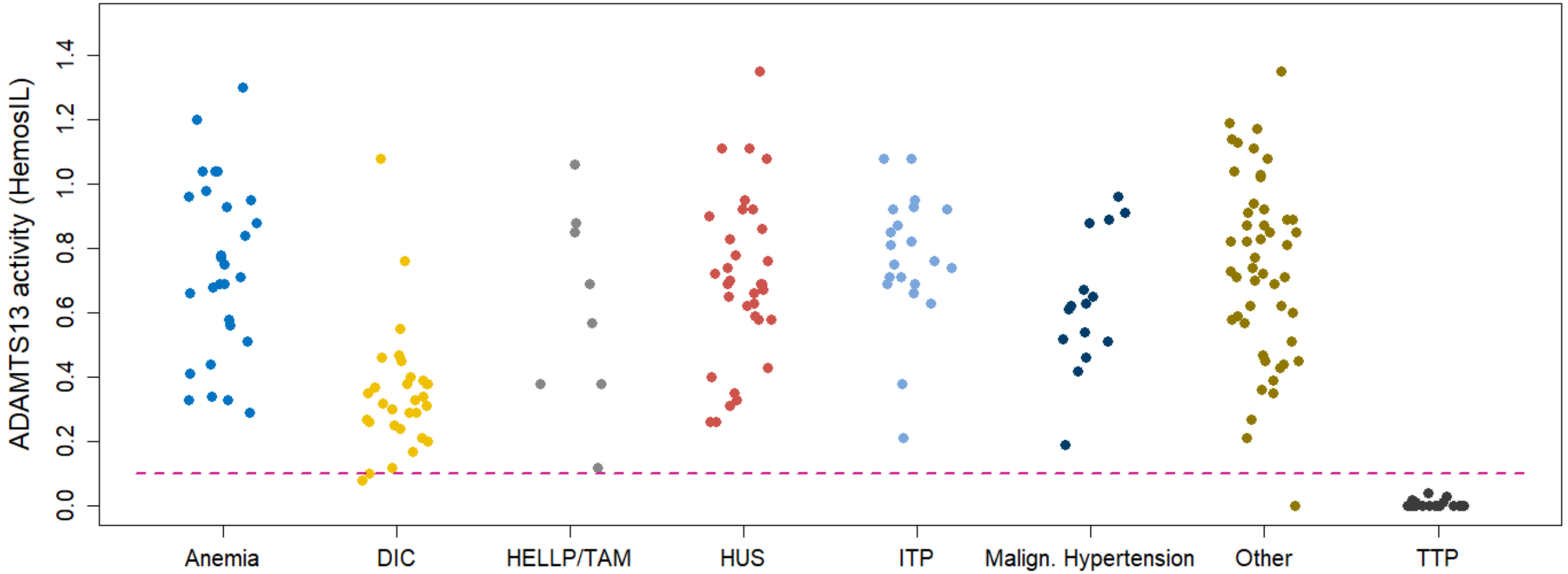


Dimopoulos K, *et al.* J Appl Lab Med (2022) May 4;7(3):637-649



Dimopoulos K, *et al.* J Appl Lab Med (2022) May 4;7(3):637-649

N = 212 (diagnostic samples)



Dimopoulos K, *et al.* J Appl Lab Med (2022) May 4;7(3):637-649

ADAMTS13 assays: no-one's perfect…

Assay	Studies	N	Sensitivity	Specificity	PPV	NPV
FRET	*7*	*1268*	95.4%	99.7%	99.2%	98.0%
Technozym®	*2*	*276*	89.0%	97.7%	95.7%	94.0%
HemosIL Acustar®	*2*	*612*	92.0%	99.5%	97.7%	98.2%

Dimopoulos K, *et al.* Crit Rev Clin Lab Sci (2023) Dec;60(8):625-639

Thank you

Questions?

(preferably some I can answer…)