

# Results of the Workshop on Platelet Function Testing

**Chris M. Hackeng**

Dpt of Clinical Chemistry, St Antonius Hospital,  
Nieuwegein, the Netherlands

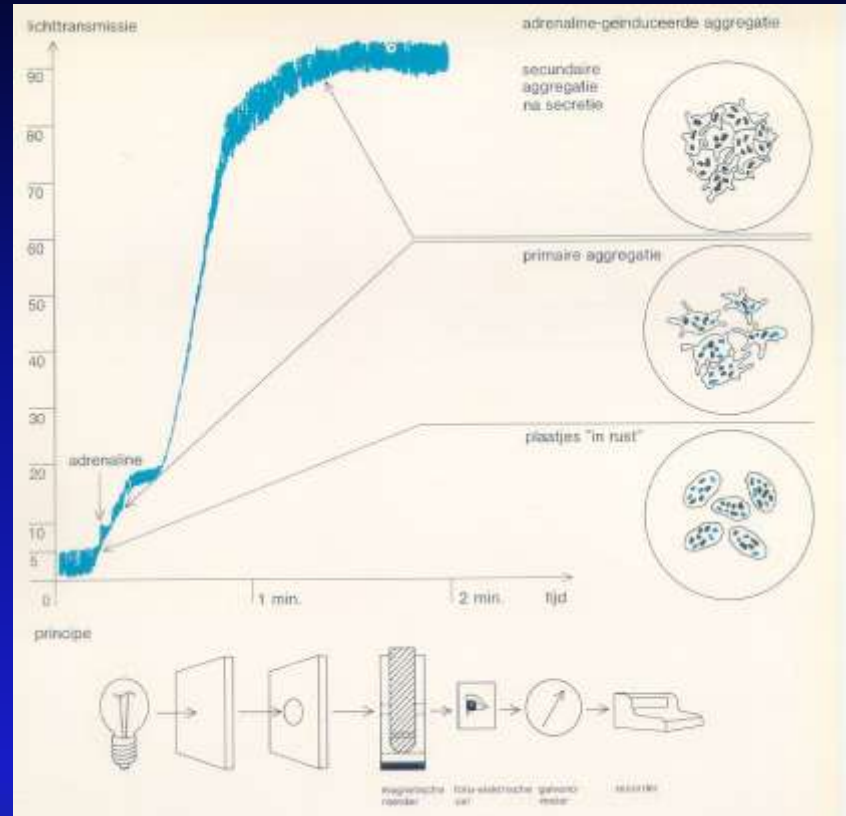
**Dr. J.C.J Eikenboom, Leiden University Medical Center**

**Dr. H.W. Verbruggen, Nijmegen**

**Dr. P. Meijer, ECAT**



# Light Transmission Aggregometry

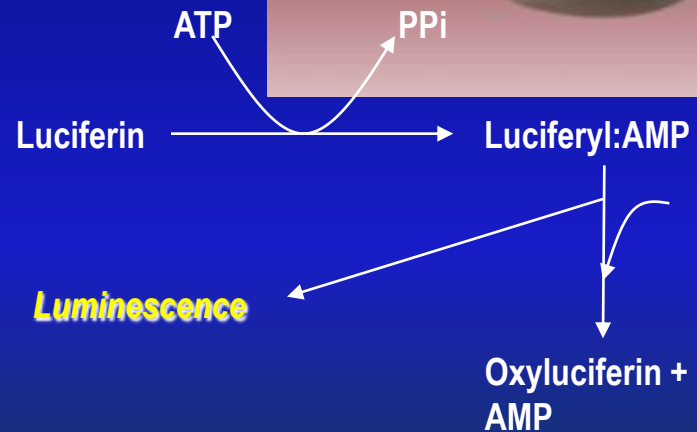
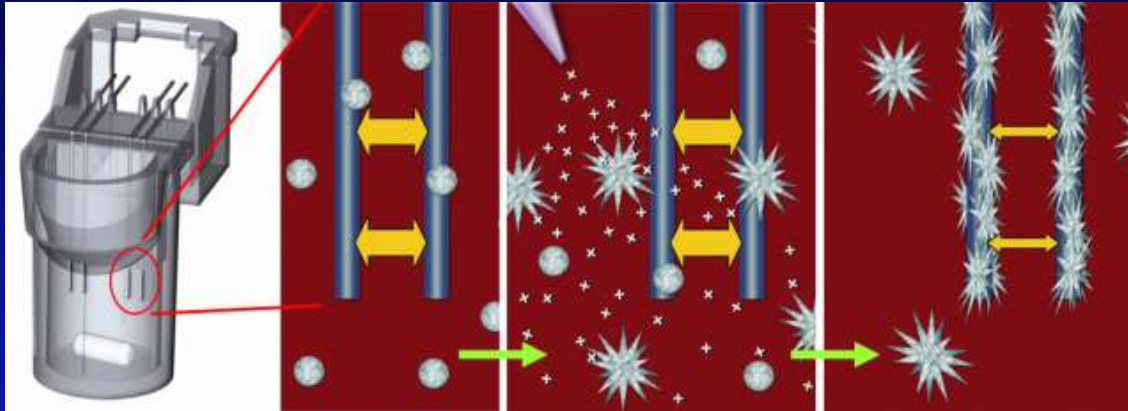


- Light transmittance through Platelet Rich Plasma (PRP)
- Time consuming, poorly standardized, centrifugation artifacts

# Workshop Platelet Function Testing

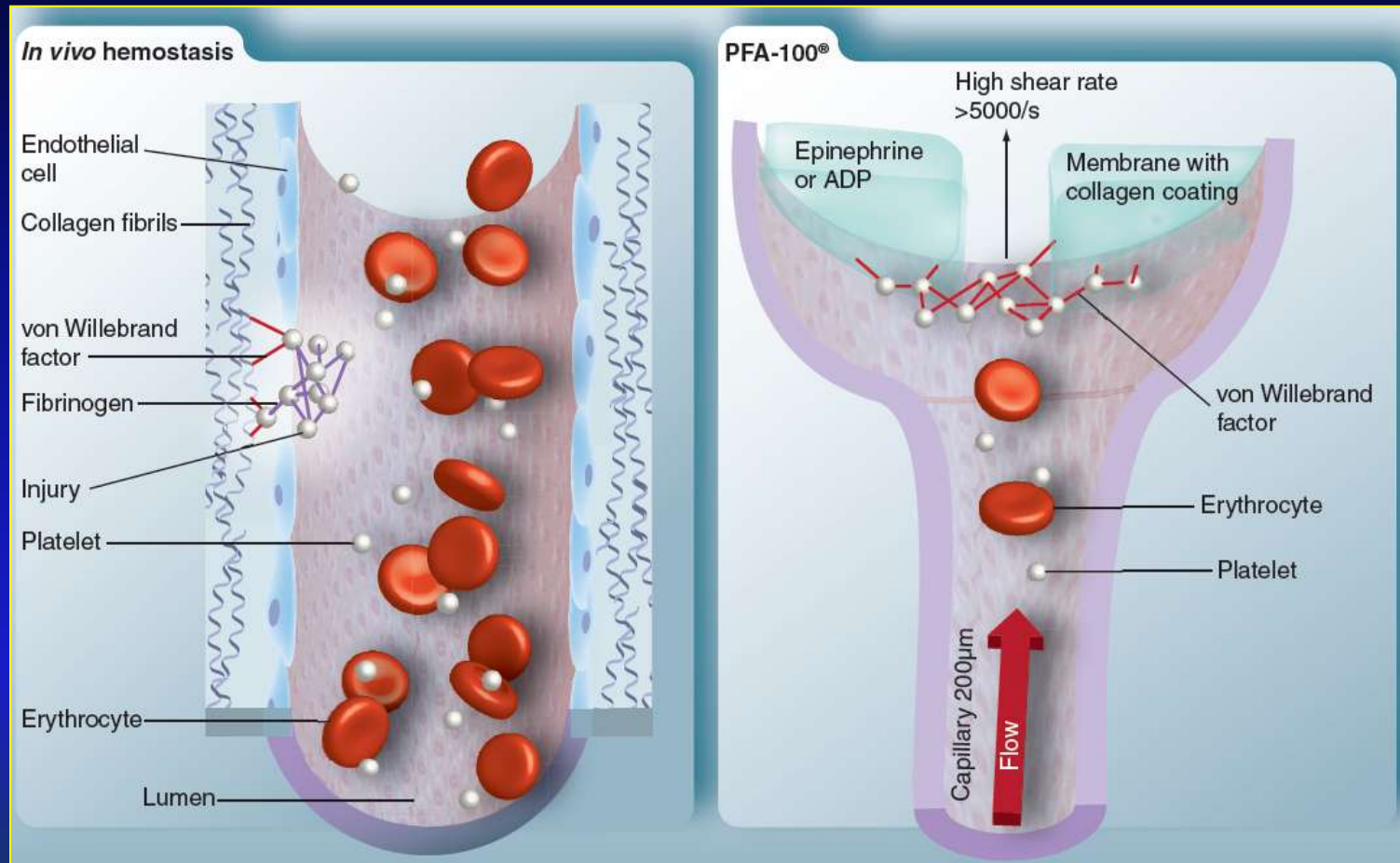
- 20 participants, 5 Groups
- 8 Patients
- 4-5 Patients per group
  
- Platelet function measurements in whole blood with:
  - Chronolog Impedance and Luminescence
  - Multiplate
  - PFA-100
  - VerifyNow
  
- Participants were given patient and family history
- Participants were blinded to hemostatic laboratory parameters (i.e. thrombocyte count, vWF, etc)

# Impedance aggregometry Chronolog/Multiplate<sup>®</sup>



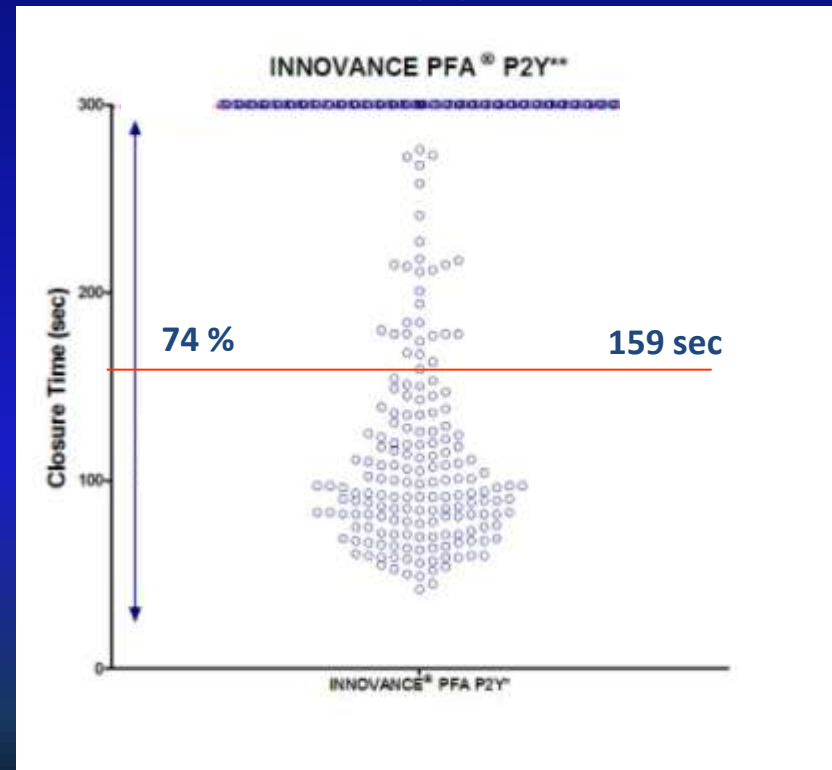
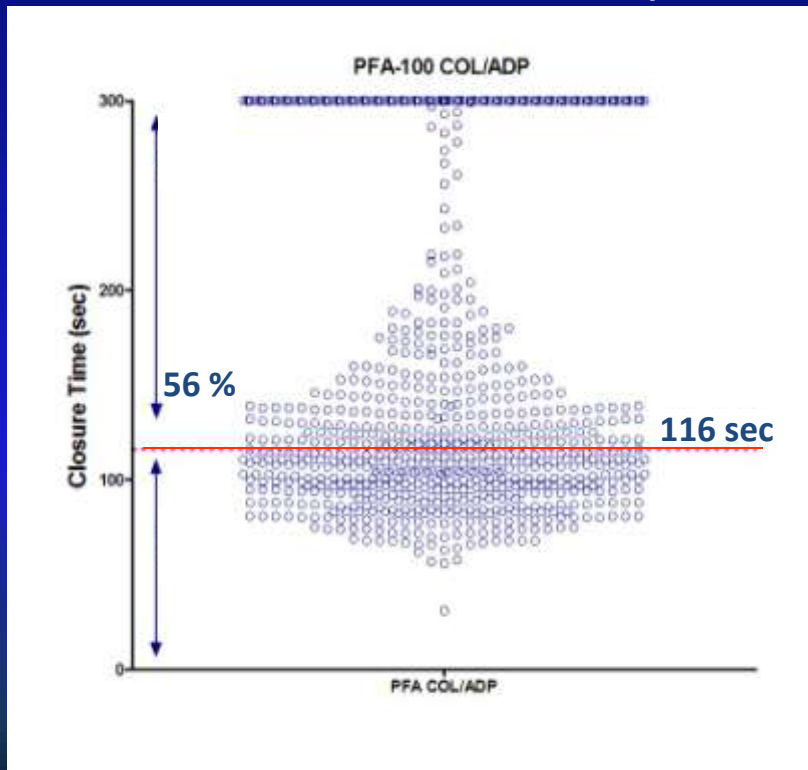
- Whole Blood Measurement
- No reversible aggregation (primary-secondary wave)
- Reported as  $\Omega$  (Ohm) (chronolog), or U (10AU.min) (Multiplate)

# Platelet Function Analyzer, PFA-100<sup>®</sup>

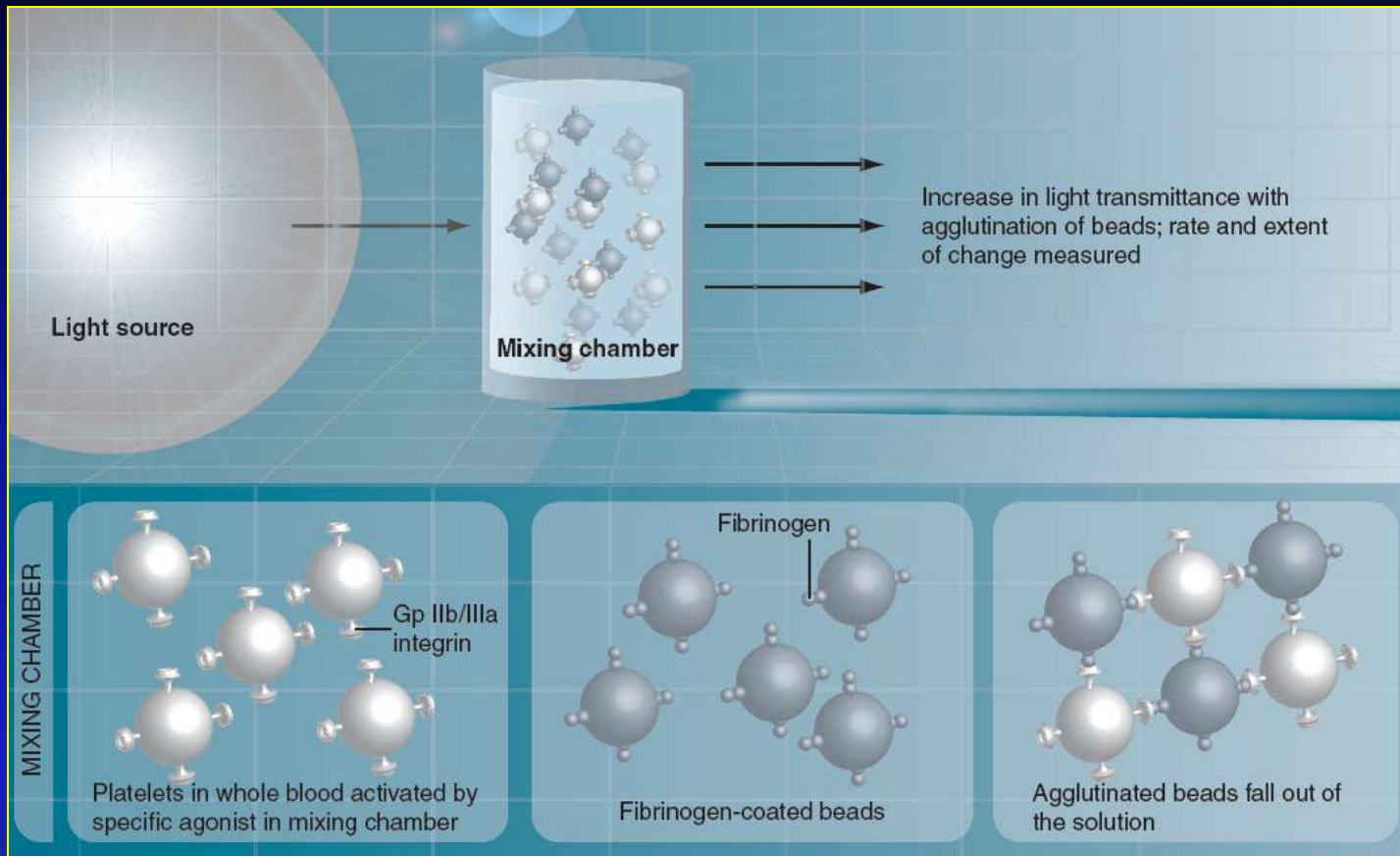


# PFA-100®

- Reported as Closure Time (CT, in seconds)
- Three cartridges: Collagen/ADP (Col/ADP), Collagen Epinephrin (Col/EPI) and clopidogrel specific Innovance P2Y\*
- Col/ADP and Col/EPI prolonged in VWD, P2Y (?)

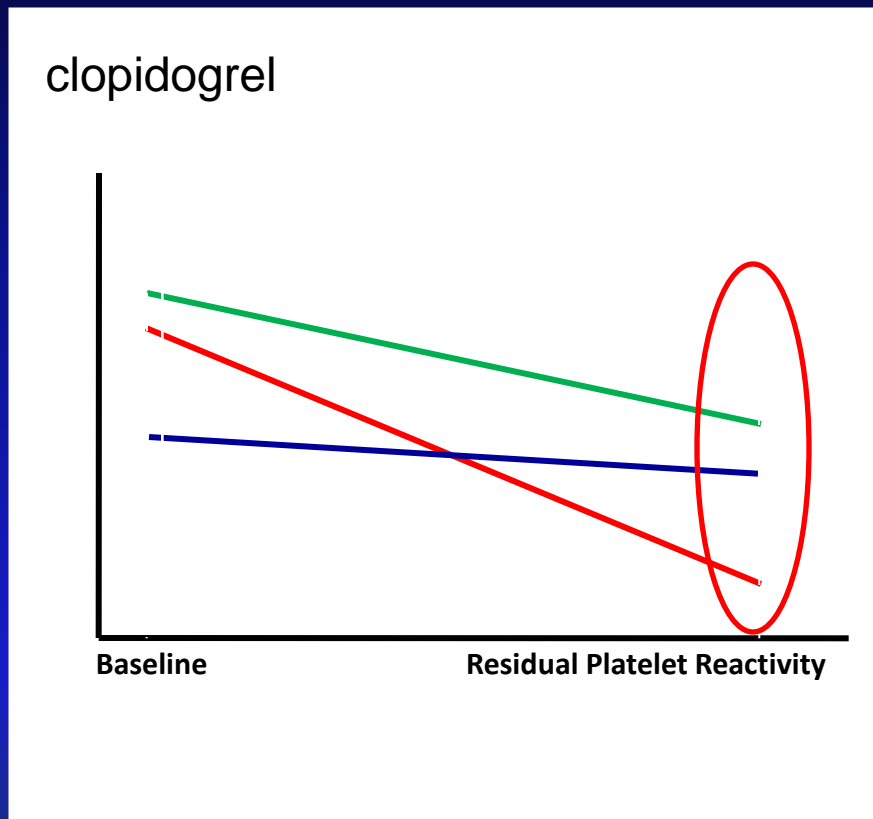


# VerifyNow<sup>®</sup>



- Whole Blood assay, 100% Point-Of-Care
- Arachidonic acid (VN-aspirin) or balance ADP, PGE1 (VN-P2Y12)
- Reported in Aspirin Reactivity Units (ARU) or Platelet Reactivity Units (PRU)

# On-treatment platelet reactivity



VN-P2Y12 also gives a “BASE”-value (TRAP) to calculate the % inhibition

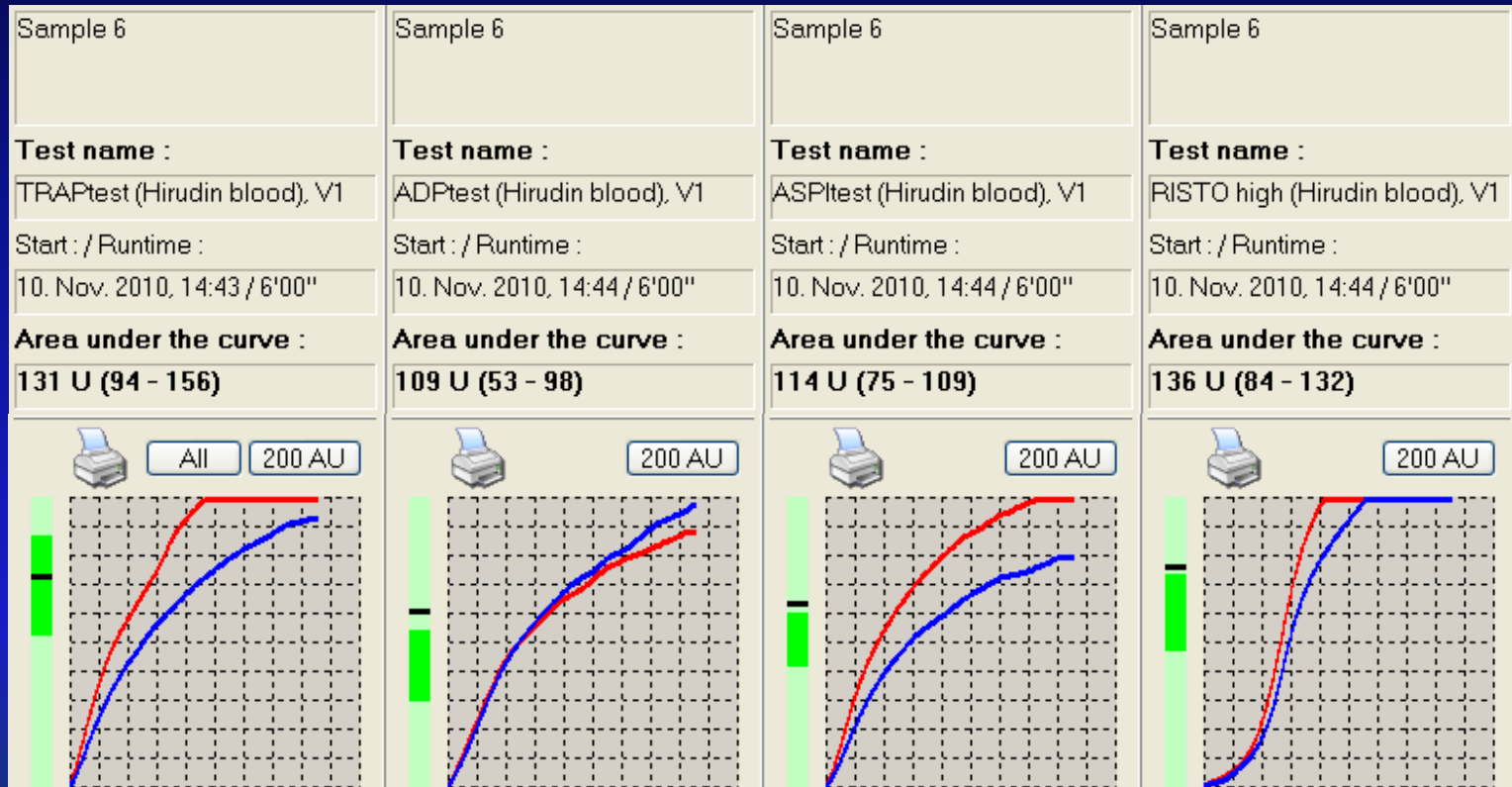
“On treatment platelet reactivity” is a better marker



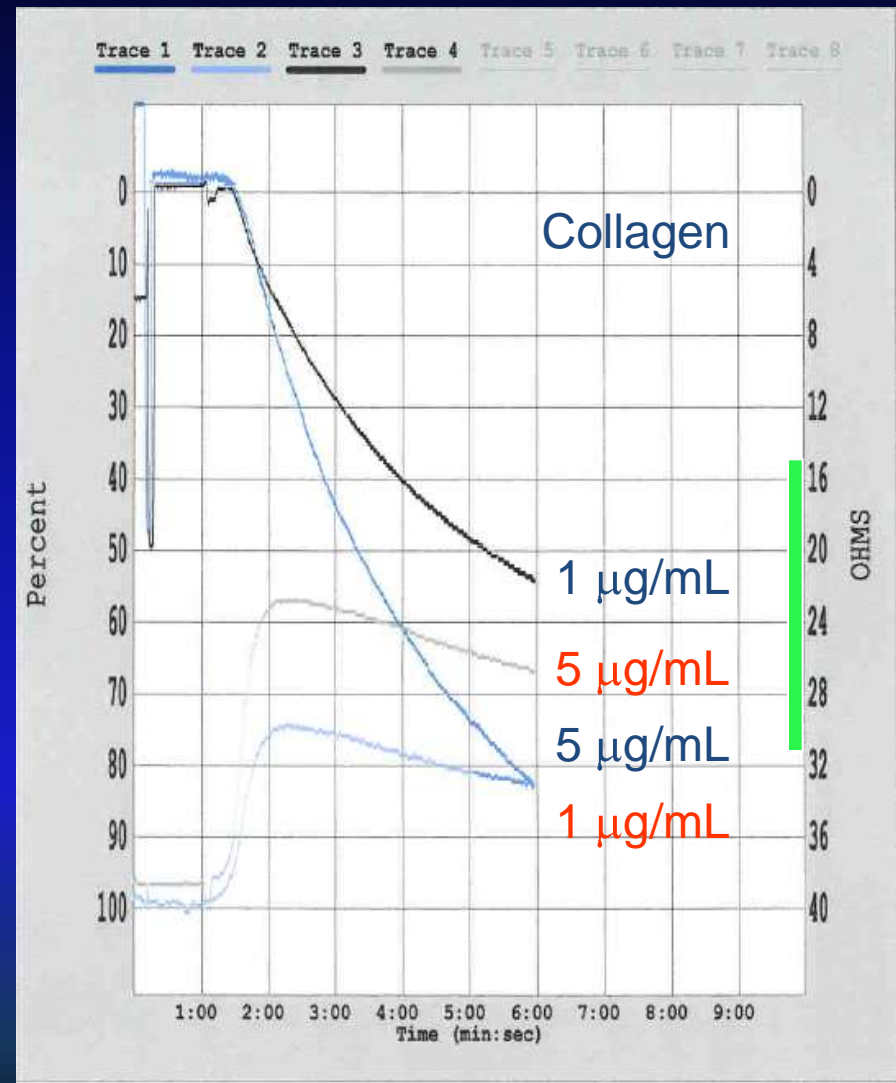
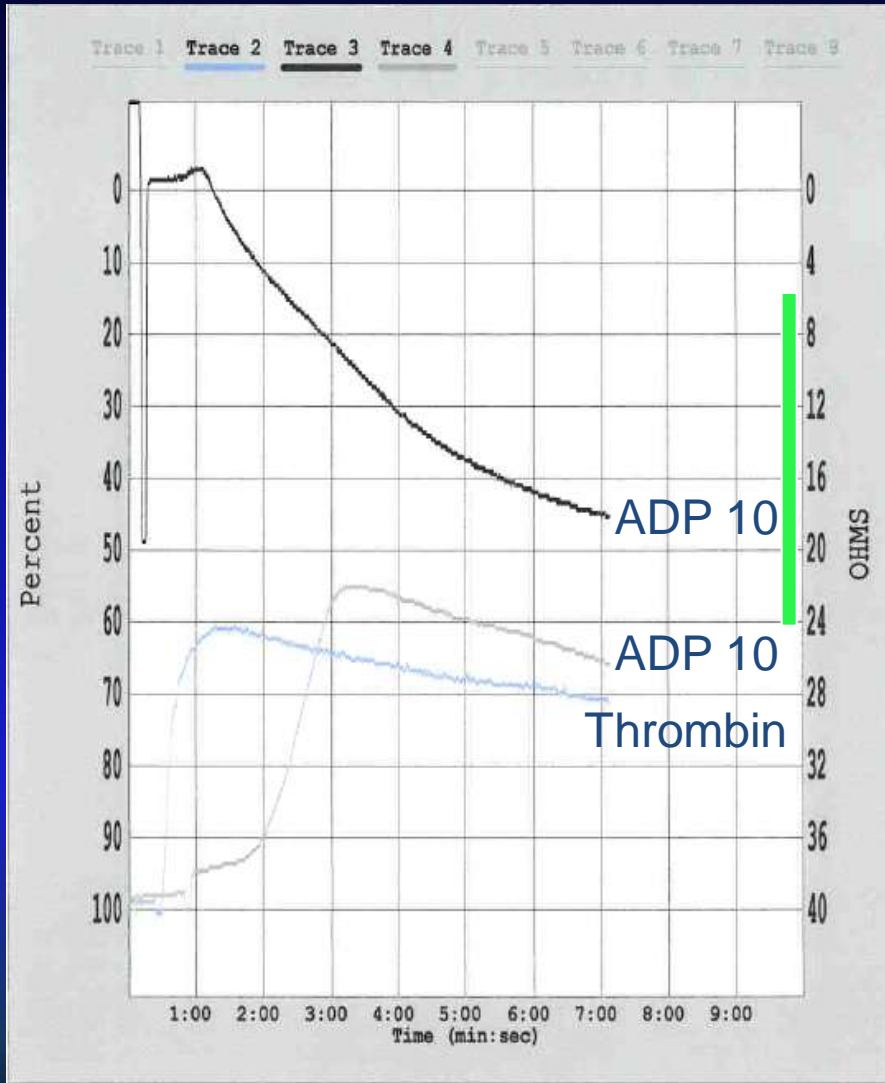
# Case 6

## Hemostatic screening for kidney biopsy

No abnormalities expected



# Case 6



# Case 6

## PFA-100:

	group 1	group 2	group 3	reference
COL/ADP	140	125	126	71 – 118 sec
COL/EPI	149	168	188	85 – 165 sec
P2Y*	116	89	98	< 106 sec

## VerifyNow

	group 1	group 2	group 3	reference
VN-ASA	664	662	neg	< 550 ARU (on ASA)
VN-P2Y	255	334	neg	< 235 PRU (on clopidogrel)
BASE	310	333		

Conclusion: No abnormalities (or mild uremic thrombopathy)

# Cases 1 and 2

## Sisters, Von Willebrand Disease type 2A

Case 1: female, 33 yr. Severe menstruations causing anemia, since she stopped oral contraception 2 years ago. She also had severe bleeding after biting her tongue .

Bleeding time 10 min

Hb 6,4 mmol/L; Ht 0,34 L/L; Trombocytes 404 G/L

VWF:Ag 0,41 IU/mL; VWF:RCof < 0,20 IU/mL; FVIII:C 0,66 IU/mL

Multimers: absence of HMW multimers, increase in proteolytic bands triplet

# Cases 1 and 2

## Sisters, Von Willebrand Disease type 2A

Case 2: female, 43 yr. Obtains advice because of eyelid correction. A coagulation anomaly was established in the past. With reference to three pregnancies and giving birth precautions were taken.

Bleeding time > 10 min

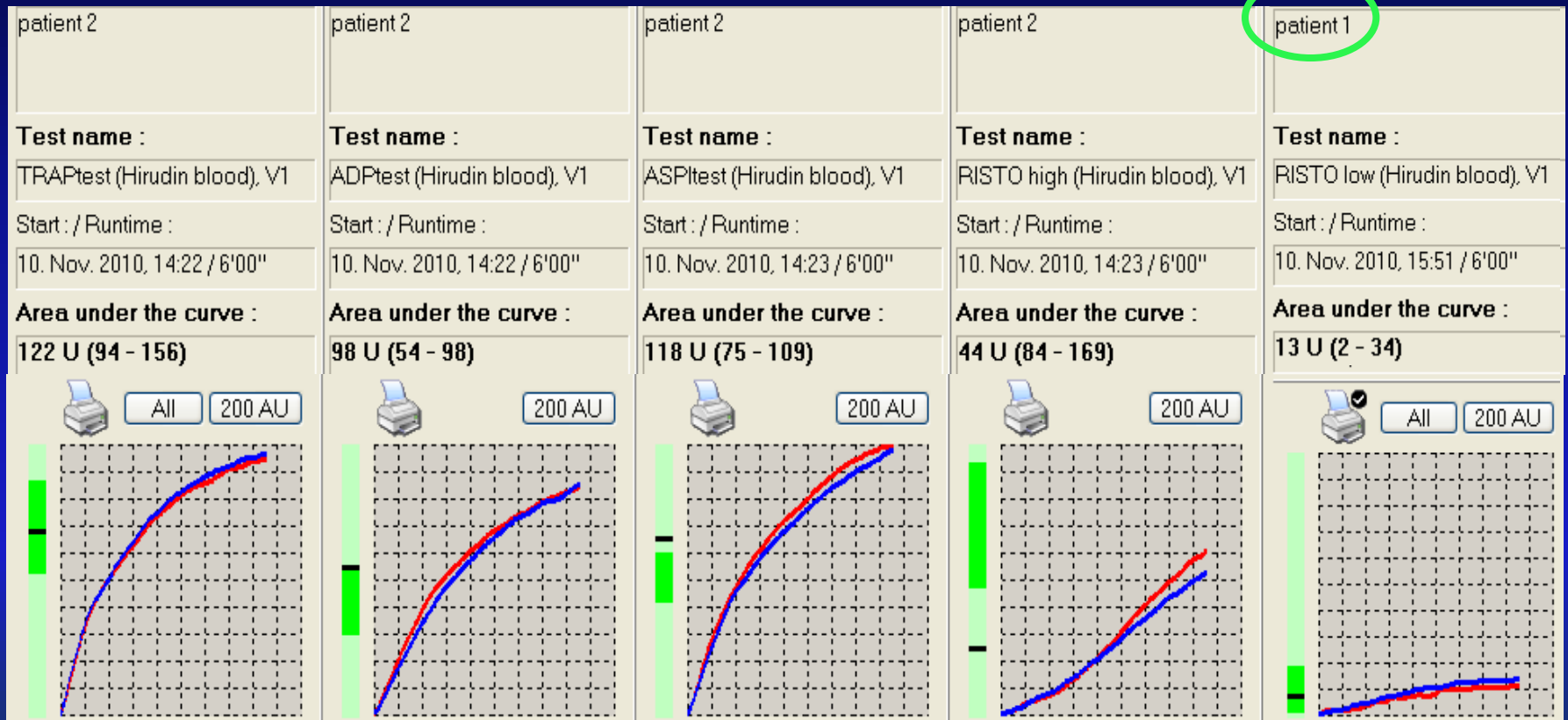
Hb 8,5 mmol/L; Ht 0.41 L/L; Trombo 313 G/L

VWF:Ag 0,34 IU/mL; VWF:RCof < 0,20 IU/mL; FVIII:C 0,42 IU/mL

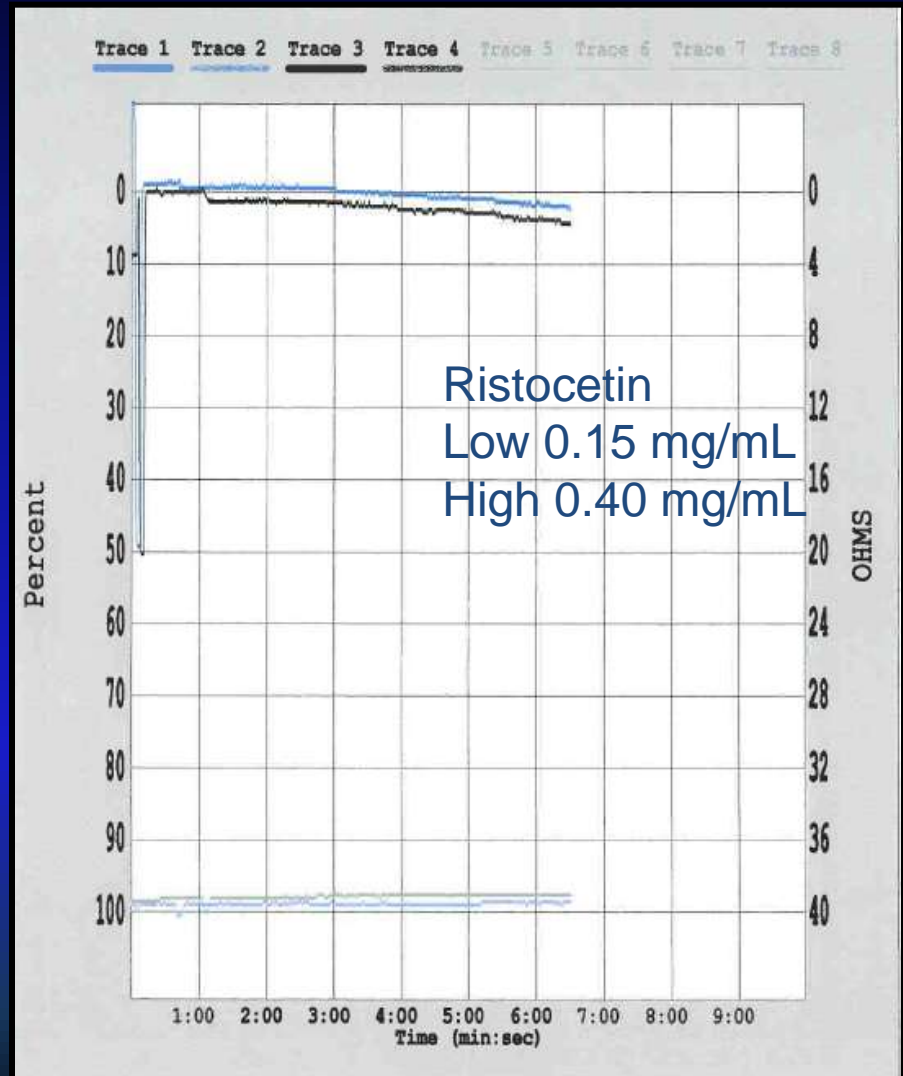
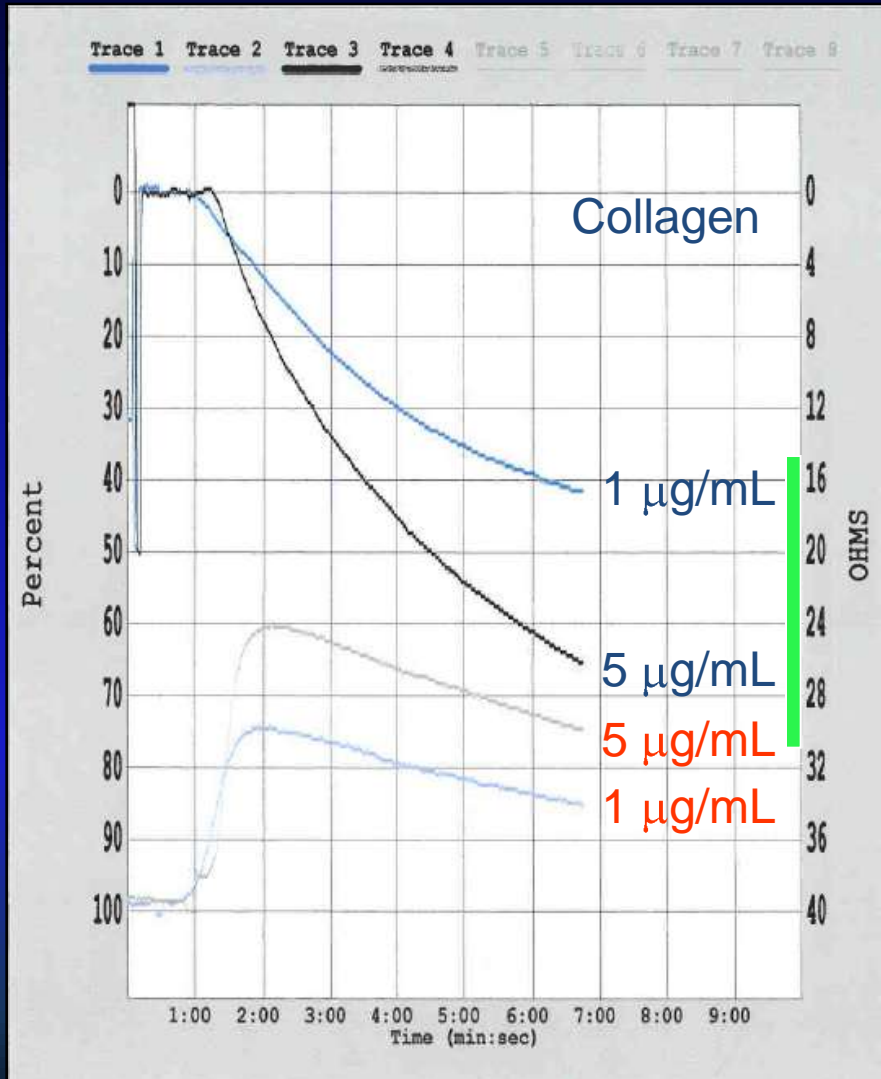
Multimers: ND, diagnosis on basis of family history VWD 2A

# Cases 1 and 2

## Sisters, Von Willebrand Disease type 2A



# Cases 1 and 2



# Cases 1 and 2

## PFA-100:

	group 3	group 4	group 5	reference
COL/ADP	> 283	> 283	> 250	71 – 118 sec
COL/EPI	> 276	> 276	> 278	85 – 165 sec
P2Y*	> 300	> 300	> 300	< 106 sec

## VerifyNow

	group 3	group 4	group 5	reference
VN-ASA	666	667	666	< 550 ARU (on ASA)
VN-P2Y	ND	340	335	< 235 PRU (on clopidogrel)
BASE				

Conclusion: Two siblings with von Willebrand Disease (not 2B; the subtype should be derived from vWF measurement and multimers)



# Case 3

## Von Willebrand Disease type 2B

Female, 63 yr. Frequently hospitalized in childhood for a “blood disorder”. No results after long-term prednisolon treatment, after which splenectomy at age 6 yr. During her life frequent bleeding: ovulation bleedings (oophorectomie), menstrual bleedings (hysterectomy), chronic blood loss from the bowel.

VWD 2B, mutation V1316M

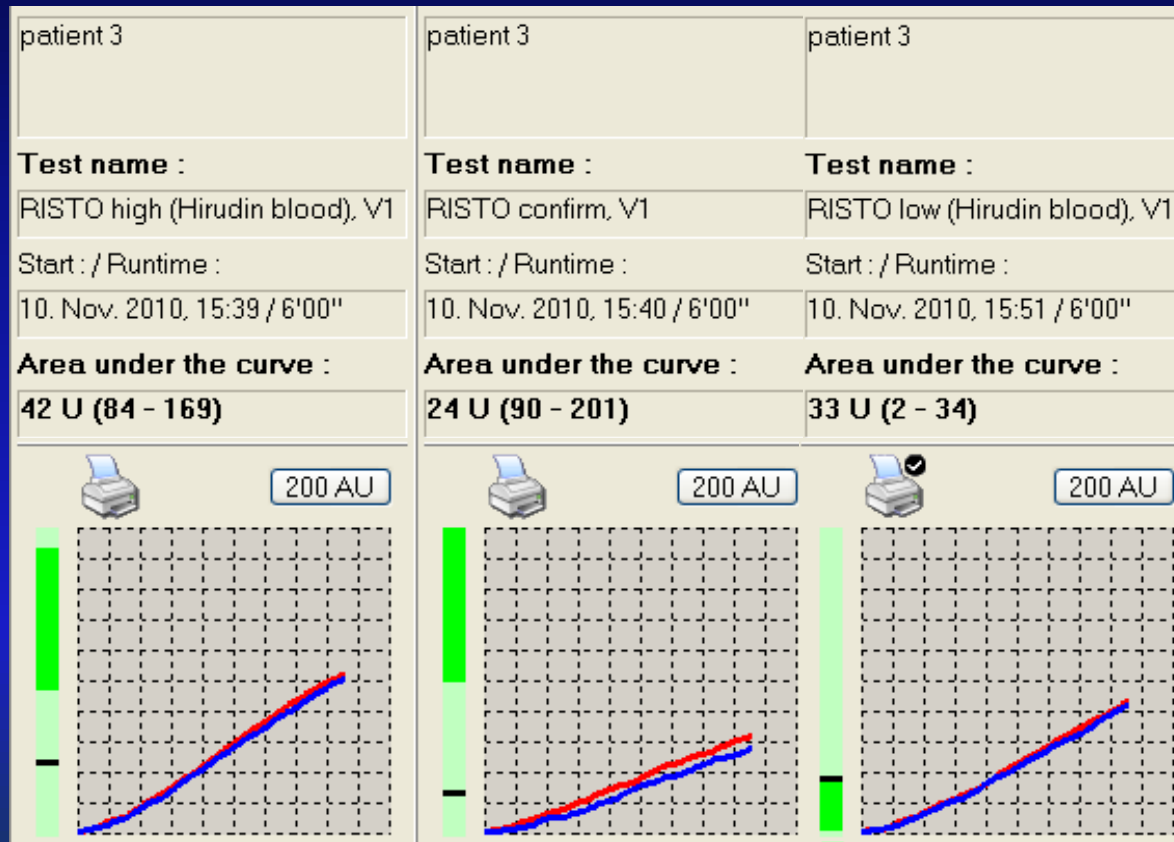
Hb 8,5 mmol/L; Ht 0.42 L/L; Trombocytes 23 G/L (!)

VWF:Ag 0,73 IU/mL; VWF:RCof 0,35 IU/mL; FVIII:C 1,00 IU/mL

Multimers: absence of HMW multimers, increase in proteolytic bands triplet

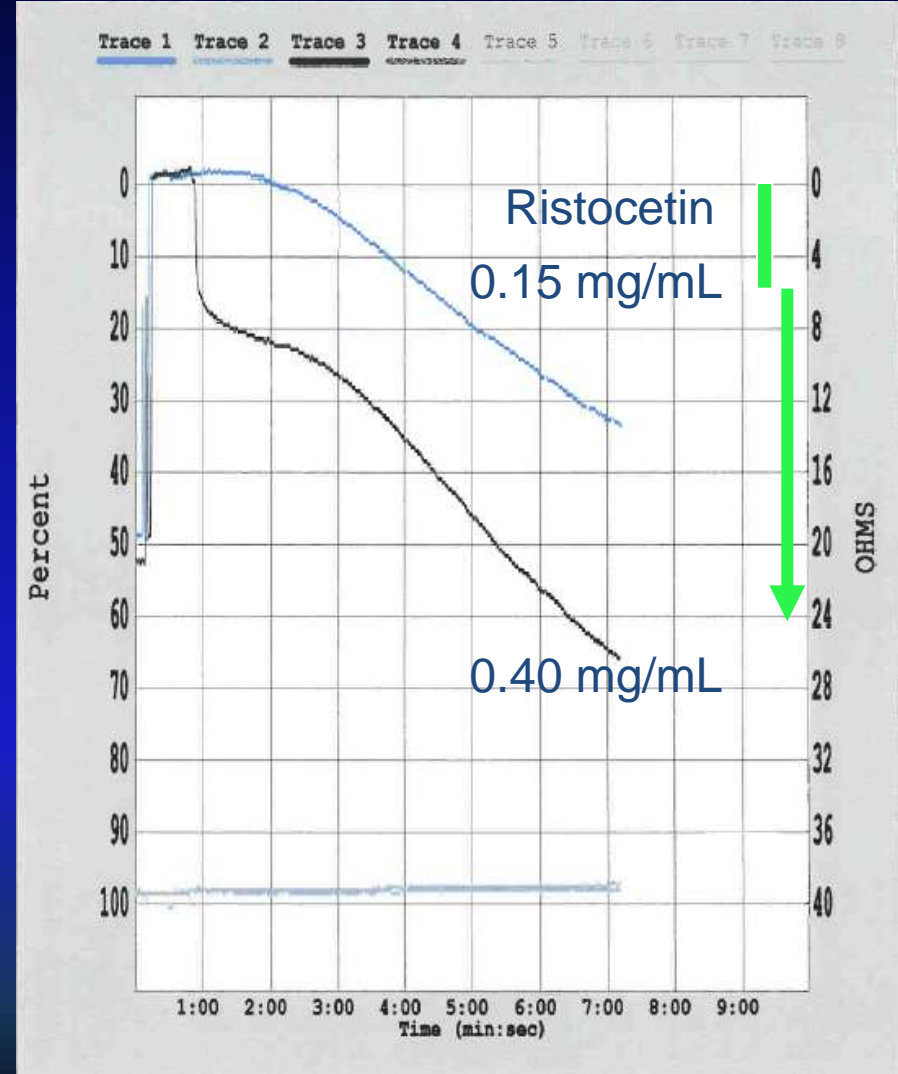
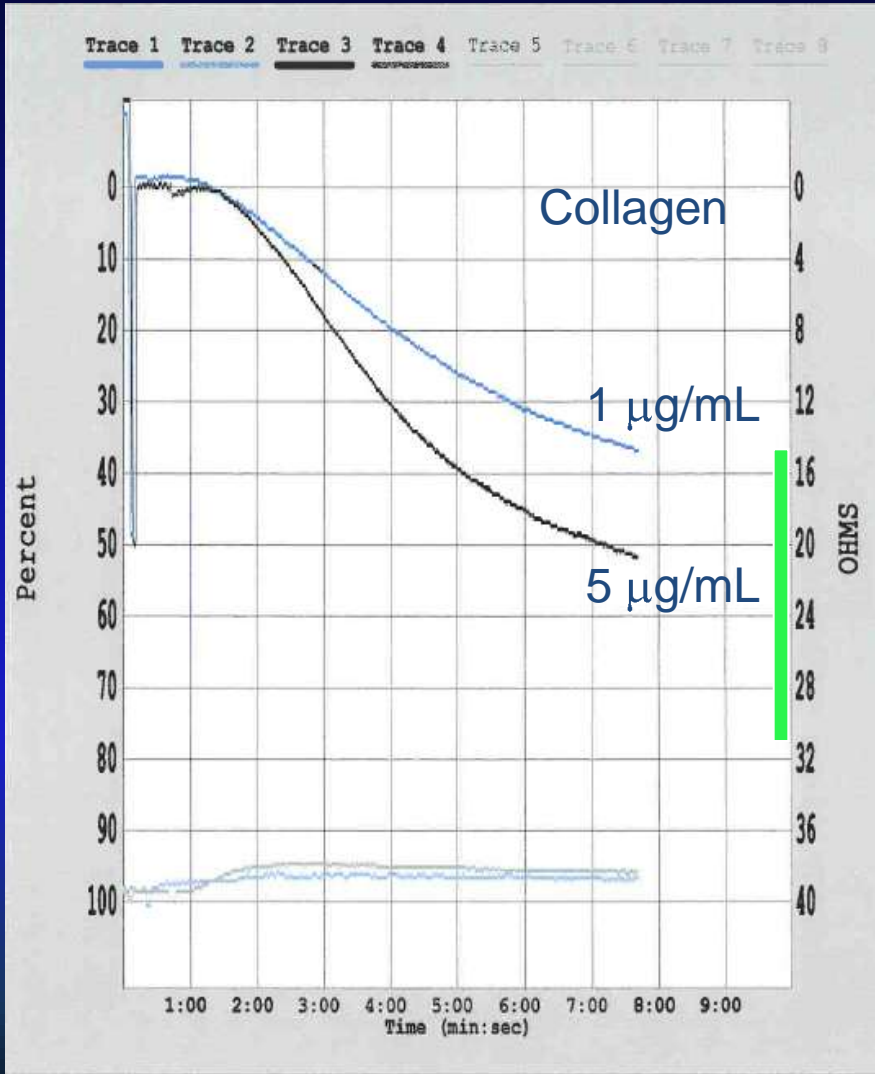
# Case 3

## Von Willebrand Disease type 2B



Lower vWF Confirm due to competition ?

# Case 3



# Case 3

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## PFA-100:

Closure times > 300 due to low platelet count, defective vWF (?)

## VerifyNow

Error flag or inconsistent measurement due to low platelet count

Conclusion: von Willebrand Disease 2B

# Case 4

## Glanzmann's thrombastenia type 2

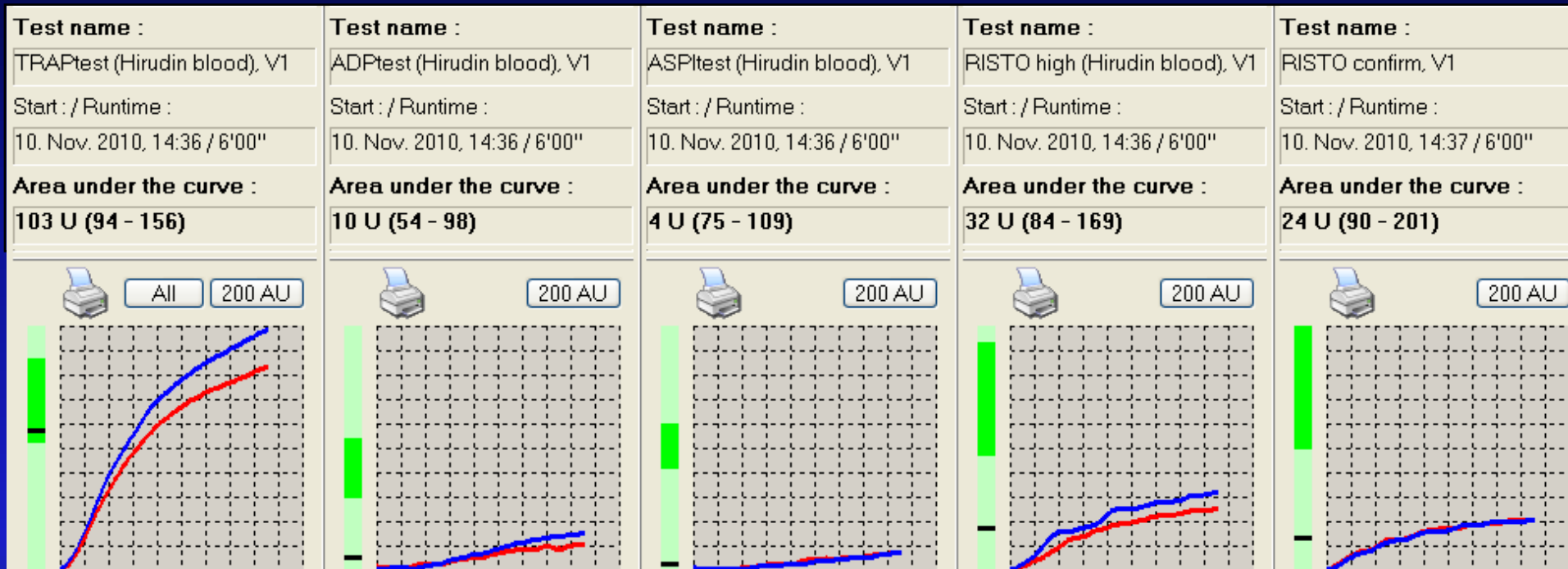
Female, 22 yr. Frequent hematomas and epistaxis, specifically when cold. Always severe menstruations. She has a sister with the same anomaly.

Hb 6,0 mmol/L; Ht 0,29 L/L; Trombo 239 G/L

Light Transmittance Aggregometry pattern specific for Glanzmann (ADP, arachidonic acid, collagen aggregation absent; ristocetin induced agglutination present); No quantitative abnormalities in GP IIB/IIIA expression found. The same anomaly was found in probands sister

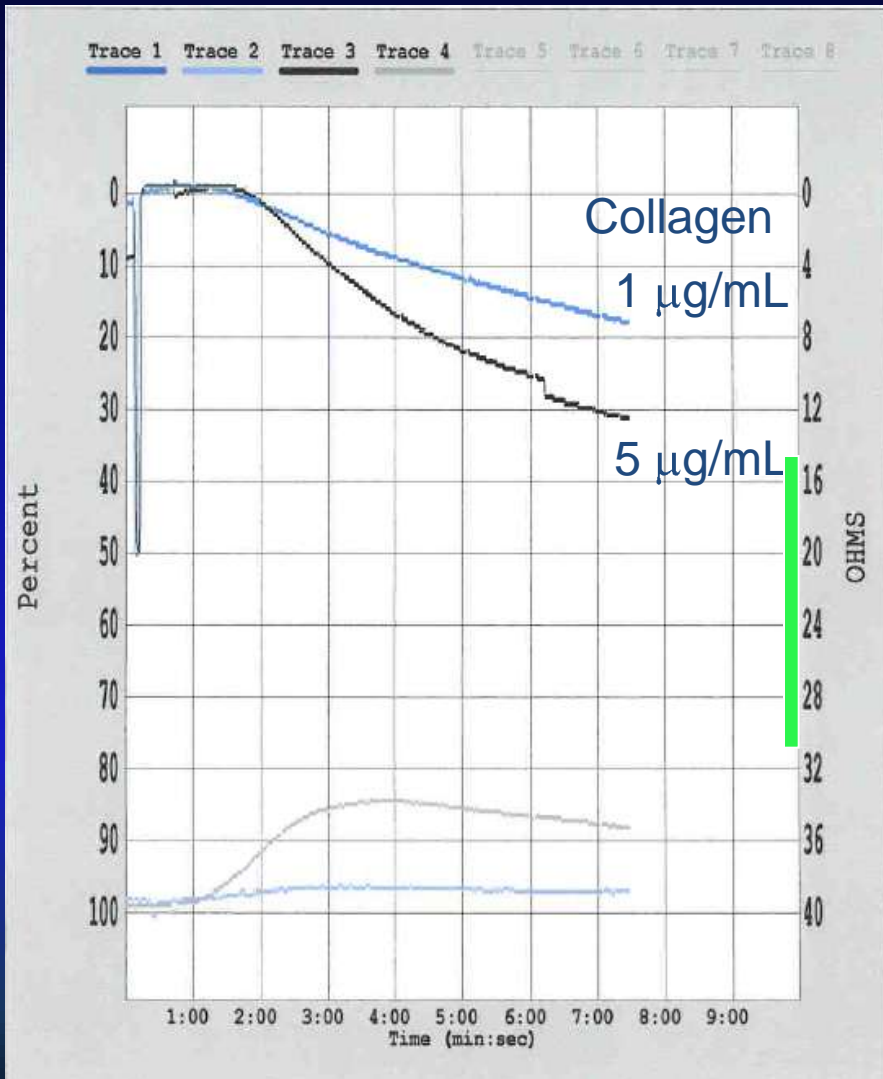
# Case 4

## Glanzmann's Thrombastenia type 2



Not a typical Glanzman patient according to Multiplate

# Case 4



ADP response absent  
Low response to collagen  
Ristocetin response absent

No response to ristocetin because  
IIb/IIIa mediated deposition on  
electrodes.

# Case 4

## PFA-100:

No closure for all tests due to defective GP IIb/IIIa

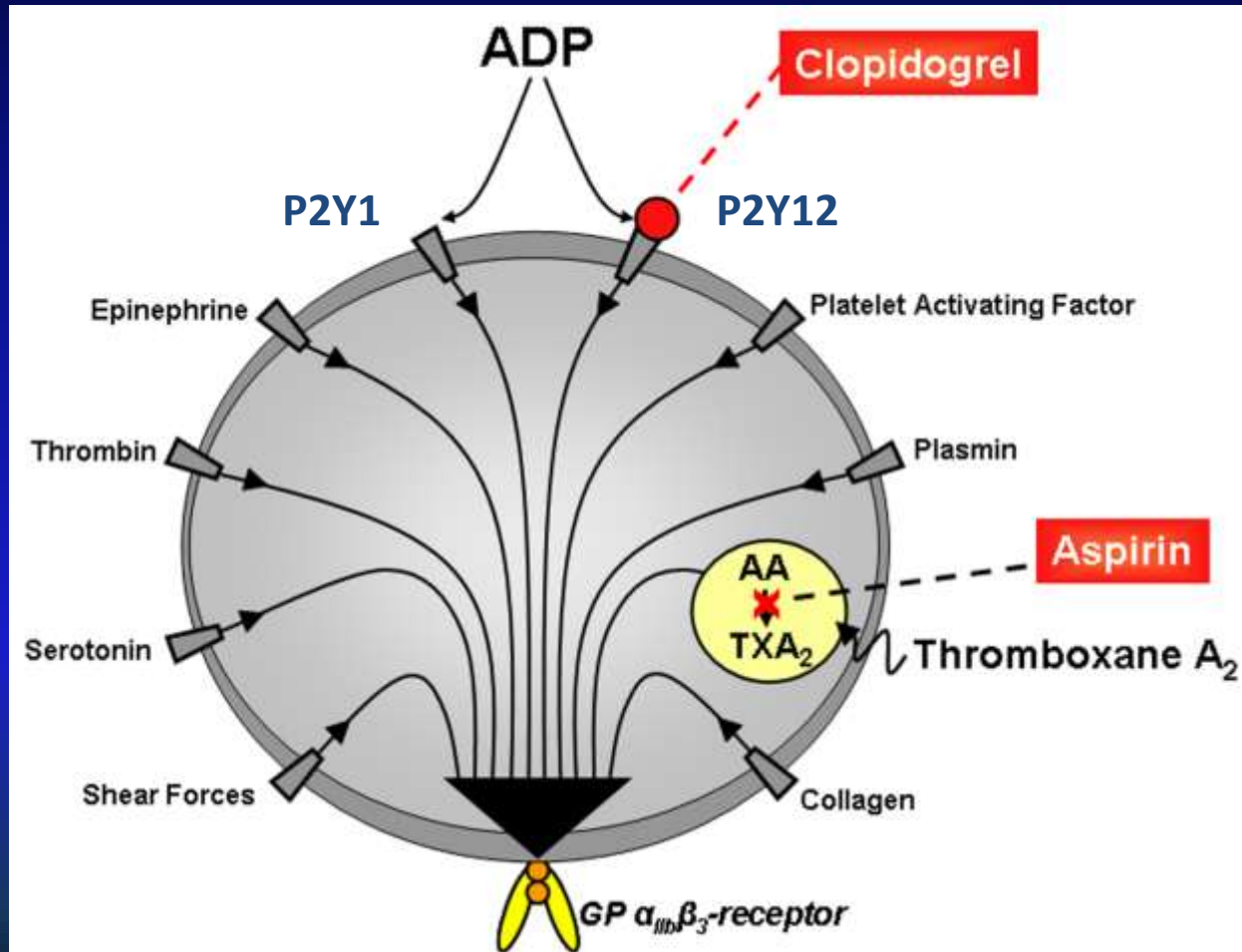
## VerifyNow

	group 2	group 4	group 5	reference
VN-ASA	432	435	408	< 550 ARU (on ASA)
VN-P2Y	172	143	ND	< 235 PRU (on clopidogrel)
BASE	390	343		

Conclusion: Glanzmann's Thrombastenia, with some residual GP IIb/IIIa activity



# Clopidogrel (Plavix®)



# Case 5

## Polycythemia Vera with Thrombocytosis (prophylactic aspirin use)

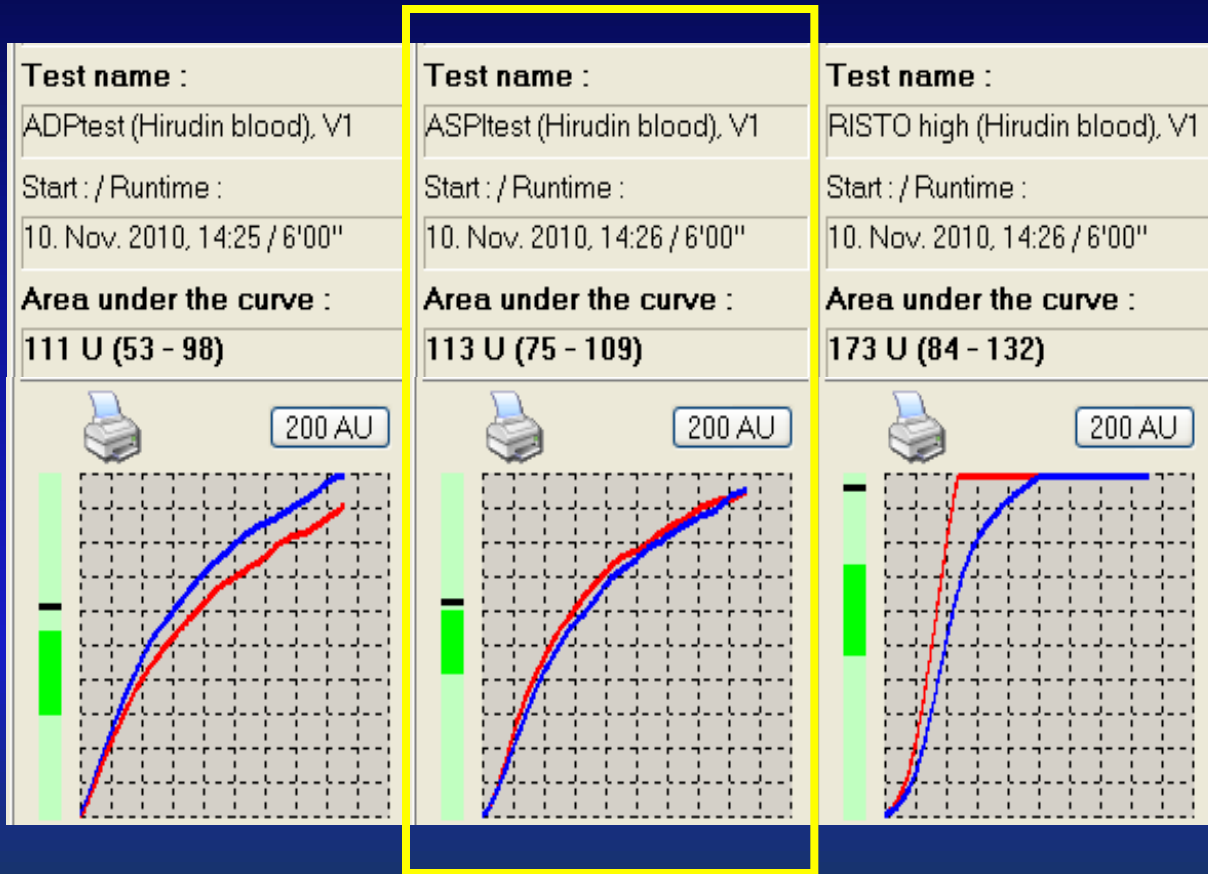
Male, 54 yr. Polycythemia Vera with thrombocytosis. Suffered hemiparesis 13 years ago, from which he completely recovered. Thrombocytes decreased due to interferon treatment. For his condition he regularly has a phlebotomy

Hb 8,4 mmol/L; Ht 0,45 L/L; Trombocytes 497 G/L

Higher thrombocyt counts in history. JAK-2 mutation status not known.

# Case 5

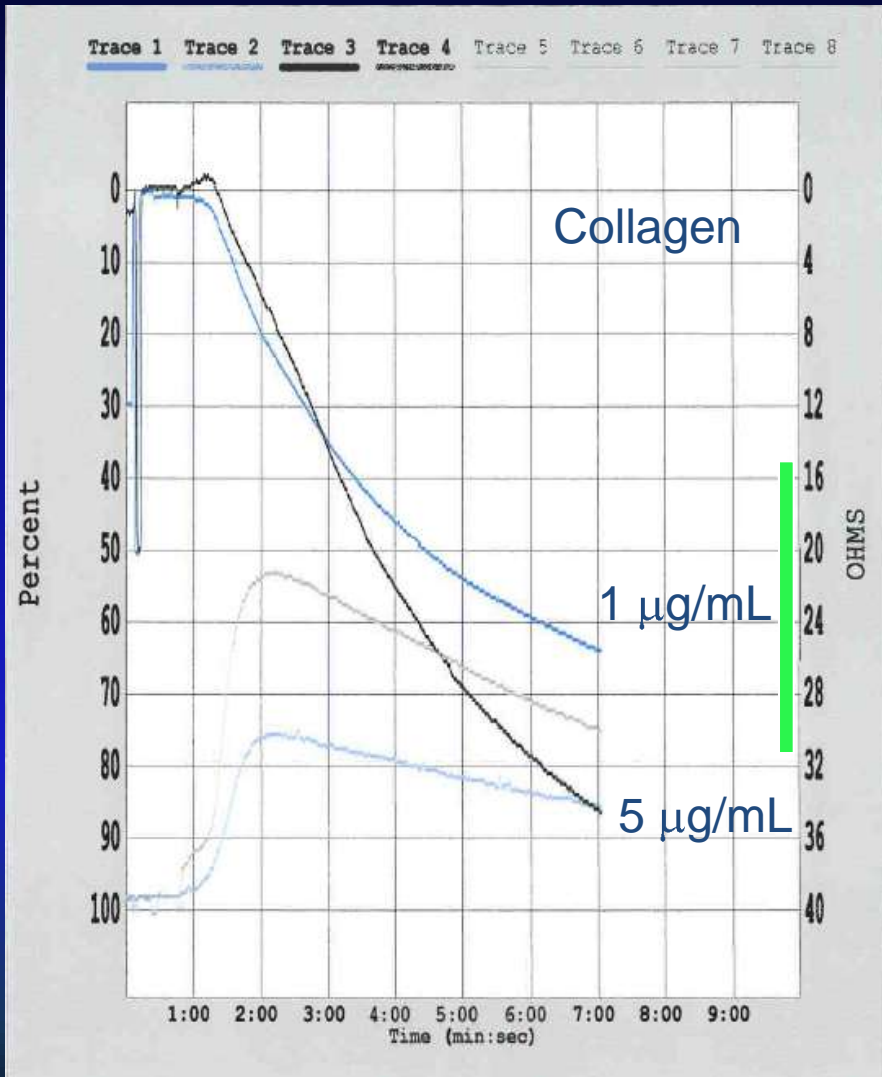
## Thrombocytosis with aspirin



JAK2 mutation (V617F)  
positive thrombocytosis  
might be thrombocytopathic

Thrombocytosis might react  
poorly on aspirin

# Case 5



High normal to increased response to collagen.

In aspirin treated subjects, the chronolog impedance signal to 1 µg/mL should be  $< 15 \Omega$  and  $< 50\%$  of collagen 5 µg/mL

# Case 5

## PFA-100:

	group 1	group 5	reference
COL/ADP	138	145	71 – 118 sec
COL/EPI	> 300	257	85 – 165 sec
P2Y*	88	71	< 106 sec

## VerifyNow

	group 1	group 5	reference
VN-ASA	640	650	< 550 ARU (on ASA)
VN-P2Y	289	279	< 235 PRU (on clopidogrel)
BASE	249	280	

Conclusion: Aspirin non-responsive according to Multiplate, Chronolog and VerifyNow. Aspirin responsive according to PFA-100. Tx-measurements?

# Case 7

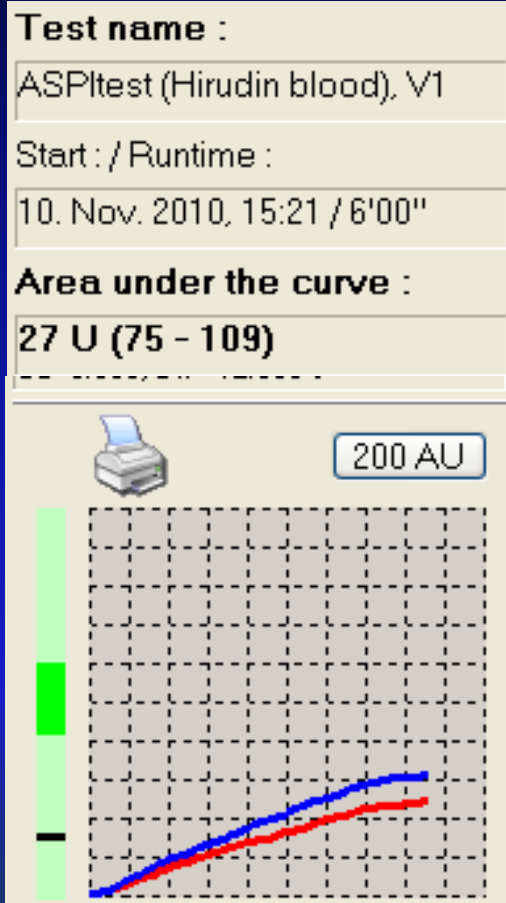
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## Patient using aspirin to prevent back pain

Male, 46 yr. Consults hematologist for easy bruising. He has no bleeding history and uses aspirin 500 mg for his hernia.

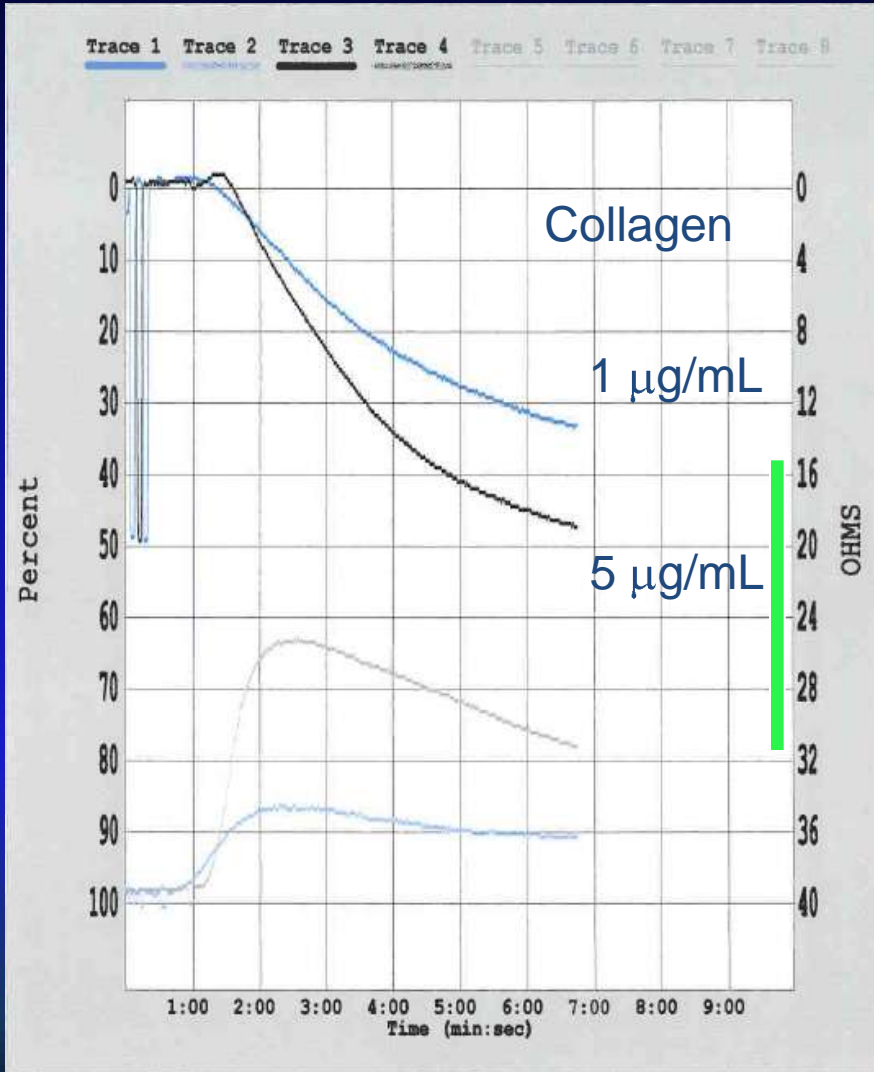
# Case 7

## Patient on aspirin



Normal respons to agonists except  
Arachidonic Acid.

# Case 7



In aspirin treated subjects, the chronolog impedance signal to 1  $\mu\text{g/mL}$  should be  $< 15 \Omega$  (in this patient 13  $\Omega$  ;

and  $< 50 \%$  of collagen 5  $\mu\text{g/mL}$  (in this patient 20  $\Omega$

No clear conclusion of aspirin responsiveness: other than “partially working”



# Case 7

## PFA-100:

	group 3	group 4	group 5	reference
COL/ADP	93	93	93	71 – 118 sec
COL/EPI	> 300	> 300	> 300	85 – 165 sec
P2Y*	93	81	93	< 300

## VerifyNow

	group 3	group 4	group 5	reference
VN-ASA	438	456	438	< 550 ARU (on ASA)
VN-P2Y	ND	333	304	< 235 PRU (on clopidogrel)
BASE		316		

Conclusion: Good Aspirin response

# Case 8

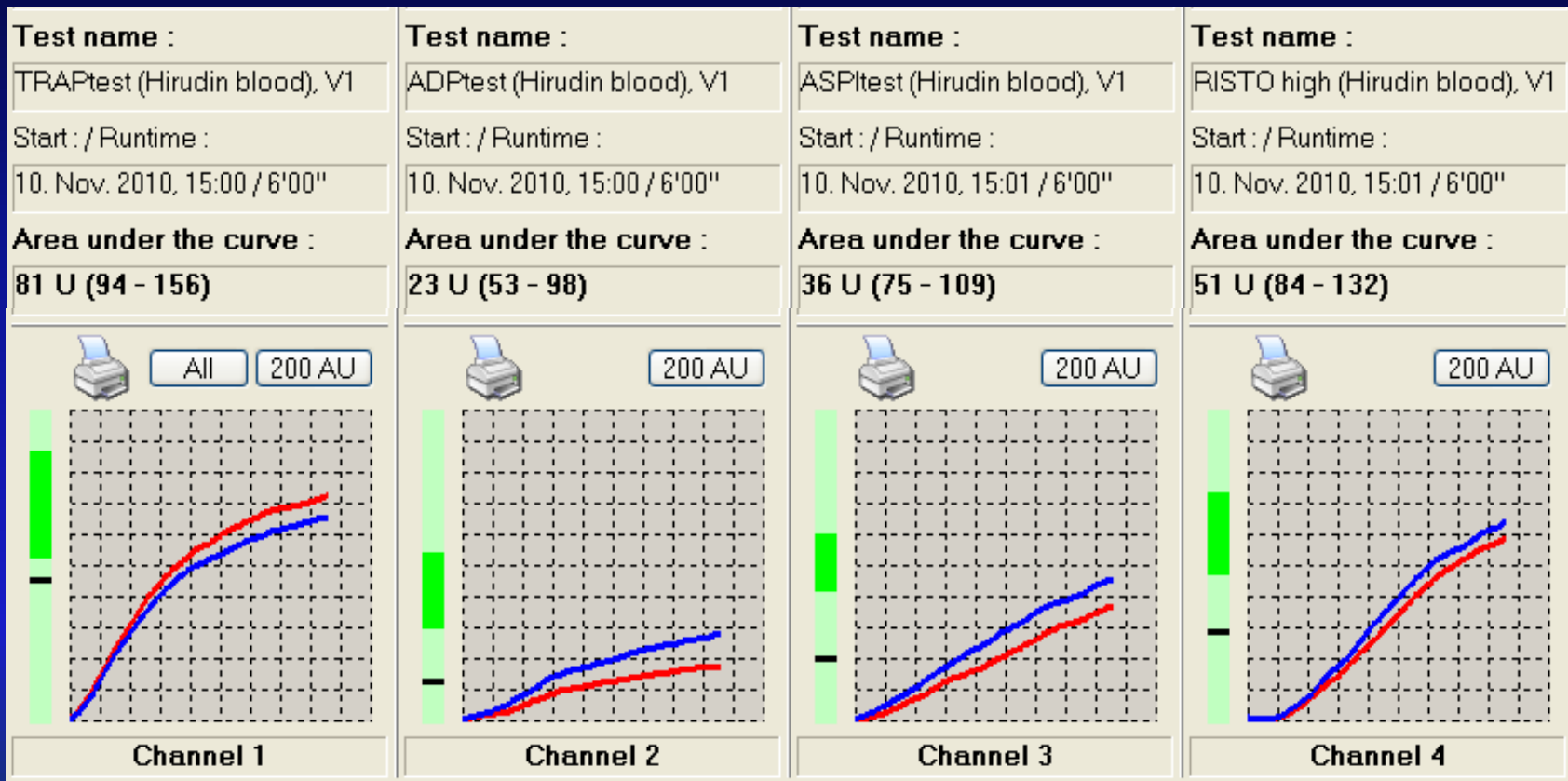
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Normal control spiked with sub-optimal concentration of Cangrelor

Male, 43 yr, visits the cardiologist 6 weeks after a Percutaneous Coronary Intervention (PCI) with stent placement.

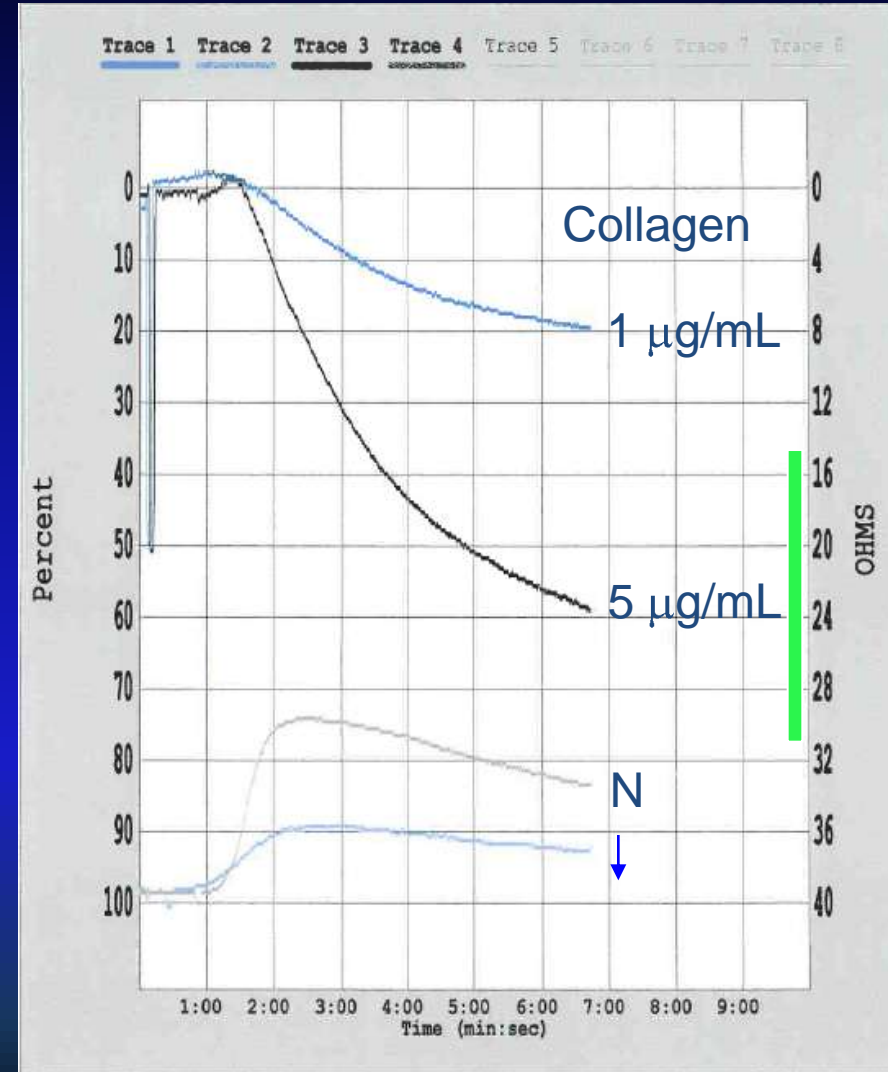
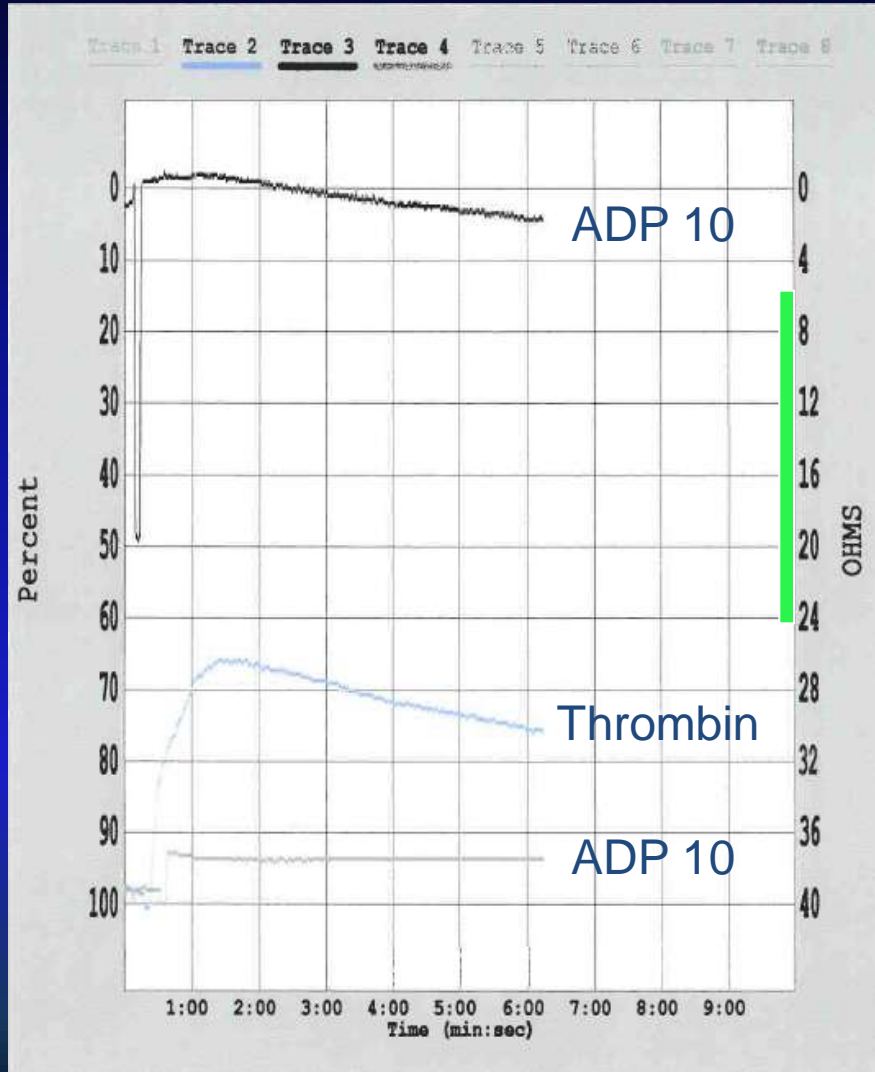
# Case 8

## Patient on cangrelor



Low ASPI test due to positive feedback loops. Ristocetin low ?

# Case 8



# Case 8

## PFA-100:

	group 1	group 2	group 4	reference
COL/ADP	169	107	128	71 – 118 sec
COL/EPI	123	111	101	85 – 165 sec
P2Y*	> 300	> 300	> 300	< 300

## VerifyNow

	group 1	group 2	group 4	reference
VN-Aspirin	648	644	641	< 550 ARU (on ASA)
VN-P2Y12	55	75 % inh	94	< 235 PRU (on clopidogrel)
BASE	366		408	

Conclusion: Conflicting results with impedance aggregometry, both methods point to aspirin use. Clear-cut results from bedside tests

<u>Patient</u>	<u>Group 1</u>	<u>Group 2</u>	<u>Group 3</u>	<u>Group 4</u>	<u>Group 5</u>
1 VWD 2A	X	VWD (I or II)	VWD		
2 VWD 2A			VWD	VWD 2A?	VWD 2A
3 VWD2B	X	ITP		Platelet defect/low	
4 Glanzmann		Glanzmann		Platelet defect	Glanzmann
5 ET/aspirin	ET / PV		X		High platelet
6 Control	No thrombopathy	Hyper-aggregability	Platelet defect-ureamia		
7 Aspirin			Aspirin	Aspirin like defect	ASA
8 Cangrelor	P2Y12 inhibitor	Clopidogrel / ASA resist.		Clopidogrel / ASA resist.	

# In conclusion

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- No big surprises on diagnosis
- One real “mismatch” in a patient on aspirin
- PFA is a useful screeningsassay and picks up IIb/IIIa blockade
- VerifyNow recognises antiplatelet therapy
- Multiplate and Chronolog equiped to specific diagnoses
- Both impedance aggregometers pointed to aspirin use in a non-user
- Multiplate more user friendly, lags luminescence
- Chronolog has a possibility for luminescence

# Thanks to:



**kordia**  
scienceforlife

Chronolog  
VerifyNow (Accumetrics)

**SIEMENS**

Siemens Healthcare  
Diagnostics

PFA-100

Dr. J.C.J Eikenboom  
Dr. H.W. Verbruggen  
Dr. P. Meijer

**NODIA**

Multiplate

ZIEKENHUIS

**ST ANTONIUS**

**St. Antonius Center for  
Platelet Function Research**

