

ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ
ΕΡΕΥΝΗΤΙΚΟ ΚΕΝΤΡΟ
ΑΘΗΡΟΘΡΟΜΒΩΣΗΣ

**THE PLATELET HYPORESPONSIVENESS
TO CLOPIDOGREL IN ACUTE CORONARY SYNDROME
PATIENTS TREATED WITH 75 MG/DAY CLOPIDOGREL
MAY BE OVERCOME WITHIN ONE MONTH OF TREATMENT**

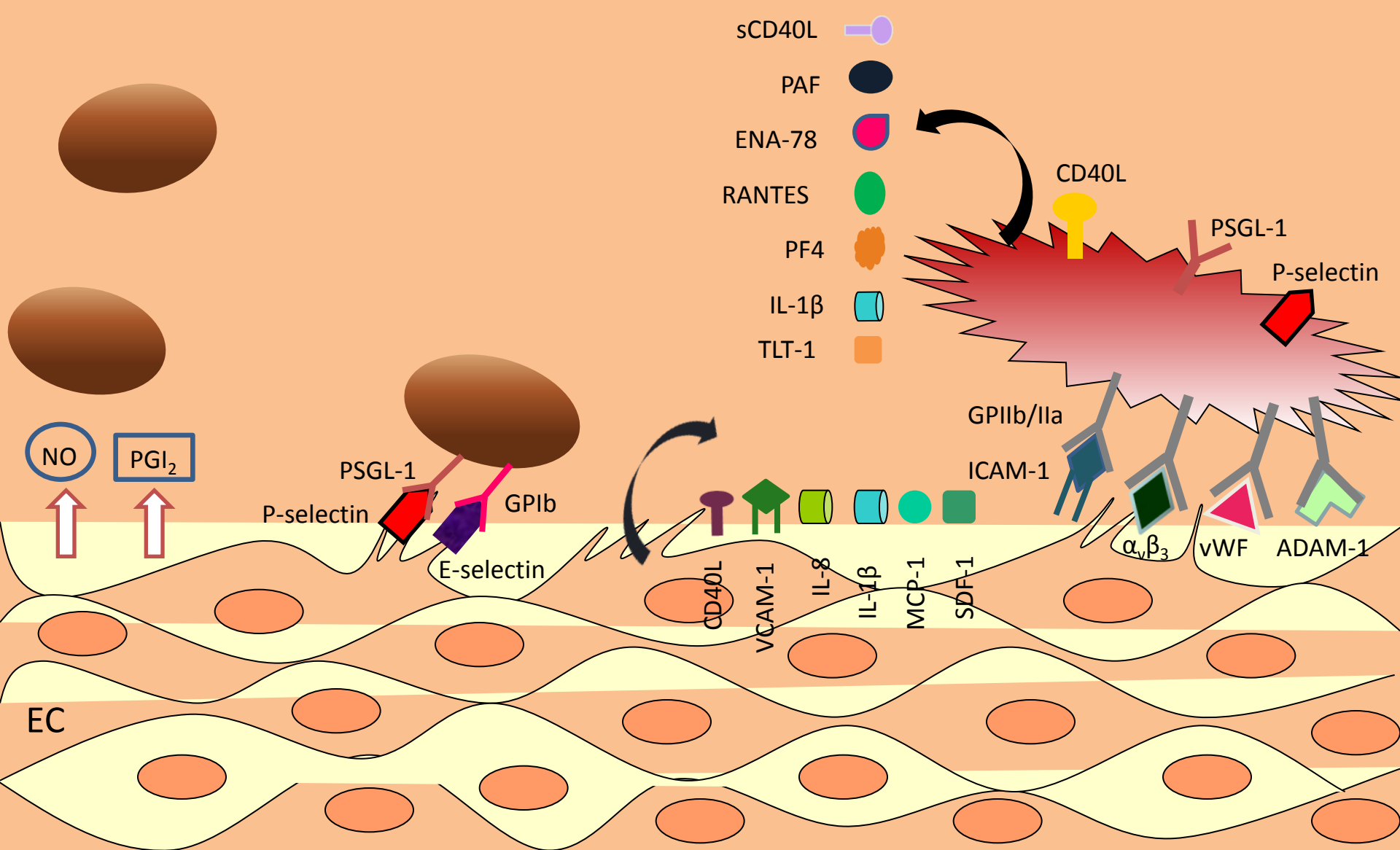
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A**B****C**

Resting Platelets

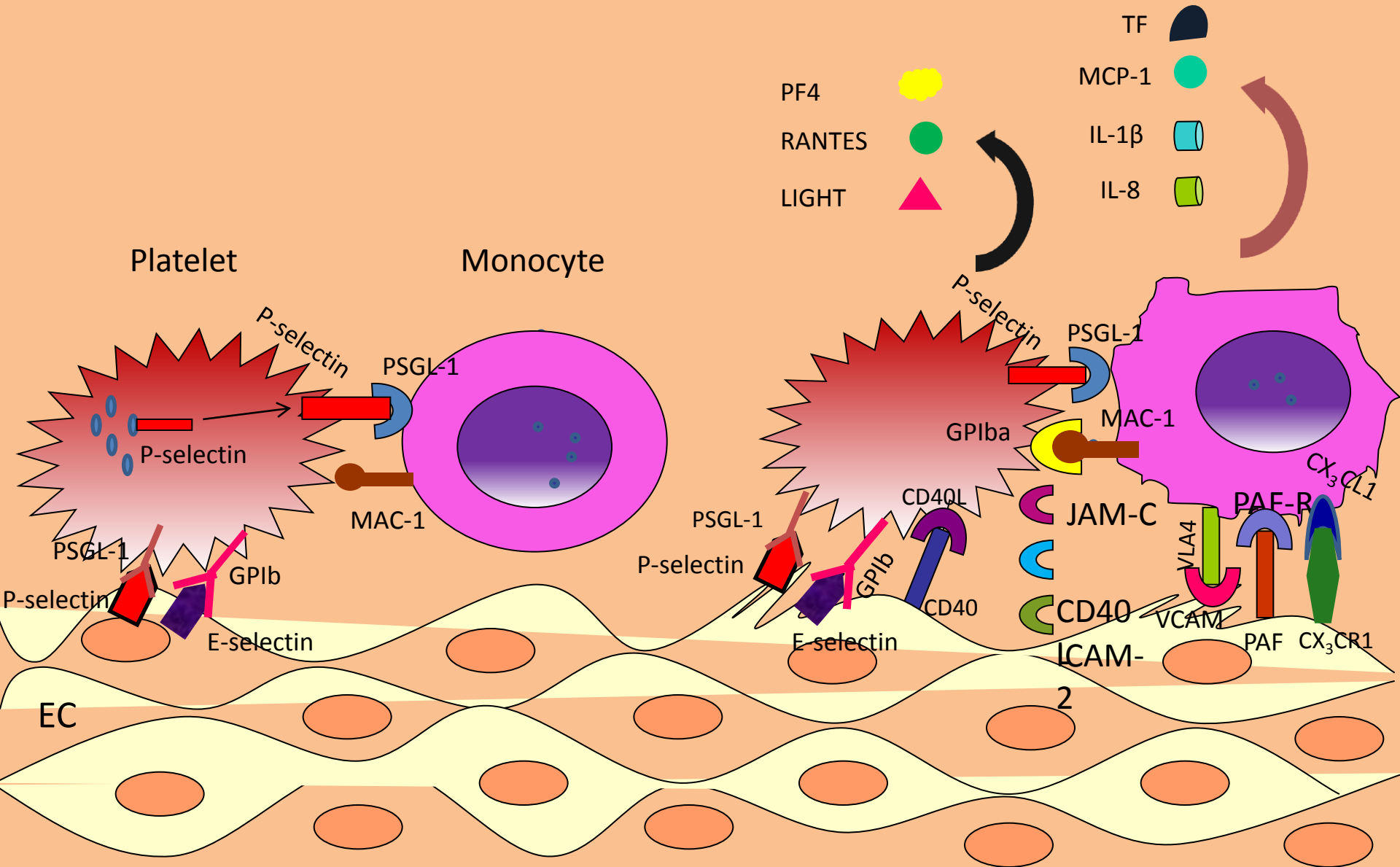
Tethering and Rolling

Firm adhesion



A. Tethering of leukocytes to activated platelets

B. Firm adhesion of ECs-platelets-leukocytes



Electron microscopy of circulating MPs from ACS patients

Boulanger et al. *Hypertension* 2006;48;180-186

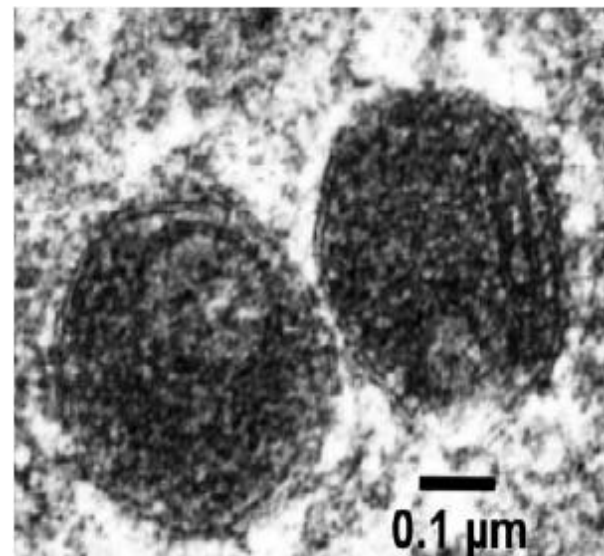
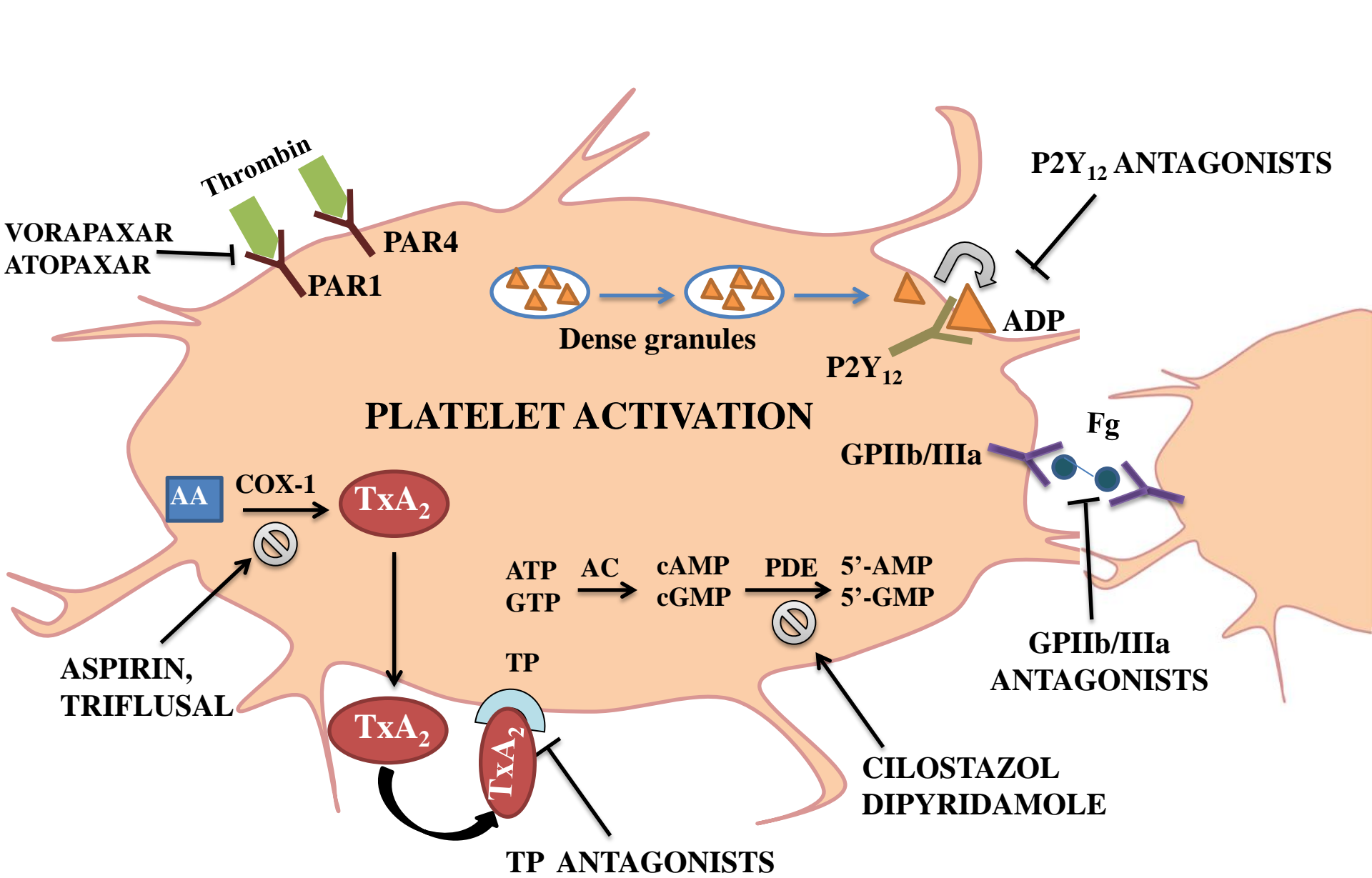


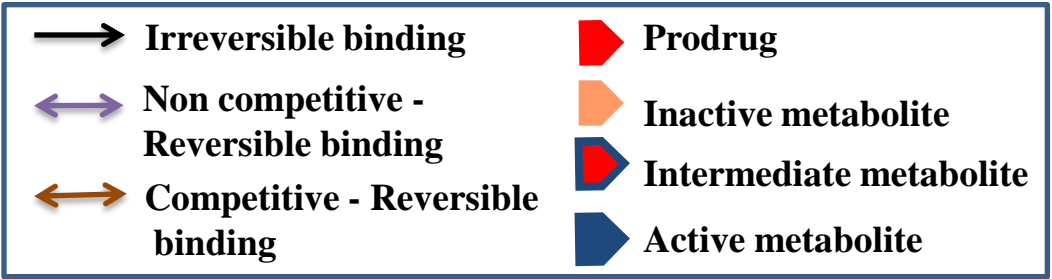
Table I: Some human studies of PMPs in atherosclerosis and associated conditions.

Authors	Reference	Findings	Number of patients
Lee et al	{4}	Patients with cerebrovascular disease have increased PMP levels.	71 patients
Zeiger F et al.	{6}	Patients with peripheral vascular disease have elevated PMP levels.	50 patients
Tan K et al.	{7}	Association between PMP levels and clinical severity of peripheral vascular disease	23 critical ischaemics, 36 intermittent claudicants
Bernal-Mizrachi L et al.	{8}	Patients with ACS have increased PMP levels.	64 patients
Tan K et al.	{9}	PMPs elevated in stable coronary heart disease	54 patients
Shouzu A et al.	{10}	PMPs increased in diabetic patients and decreased by administration of ticlopidine.	73 patients
Preston R et al.	{11}	Hypertension associated with elevated PMP levels	24 uncontrolled hypertension, 19 mildly hypertensives,

PMPs Platelet microparticles; ACS Acute Coronary Syndromes.

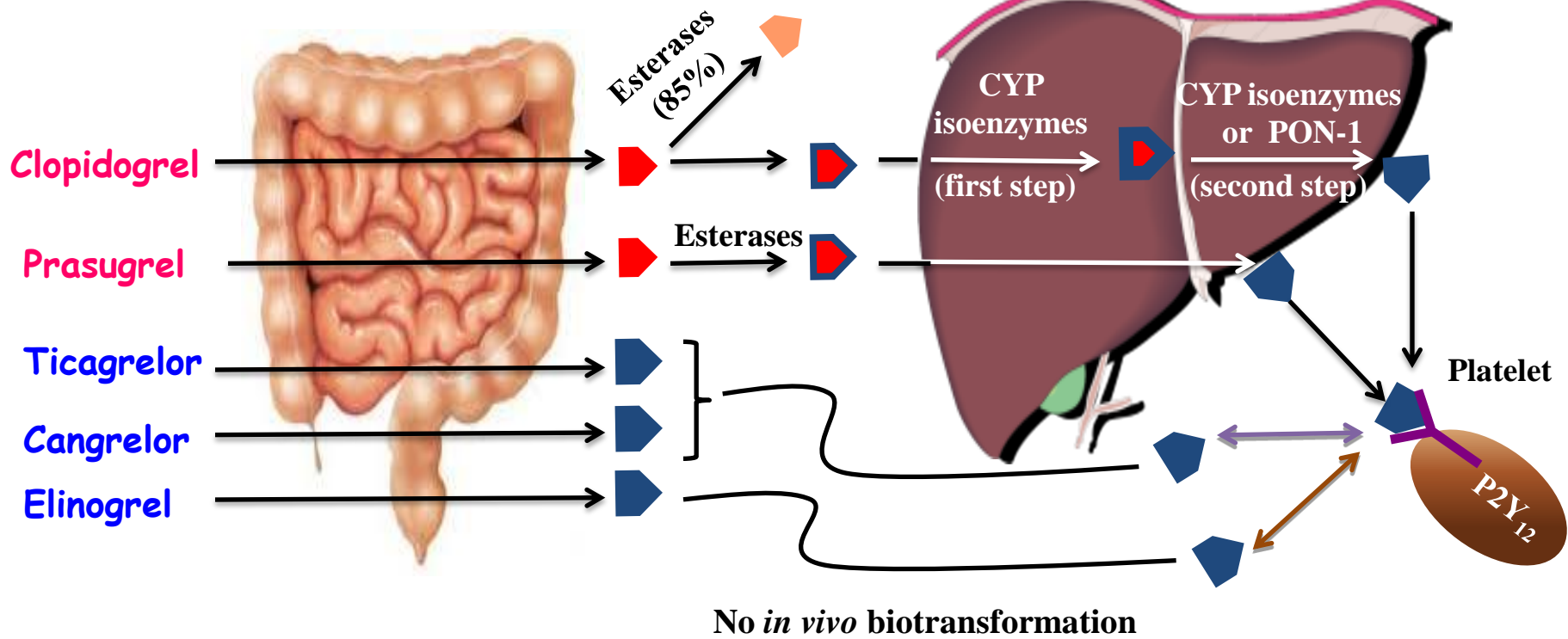
Kiat T. Tan K, et al. *Thromb Haemost* 2005;94:488–92





Intestinal Absorption

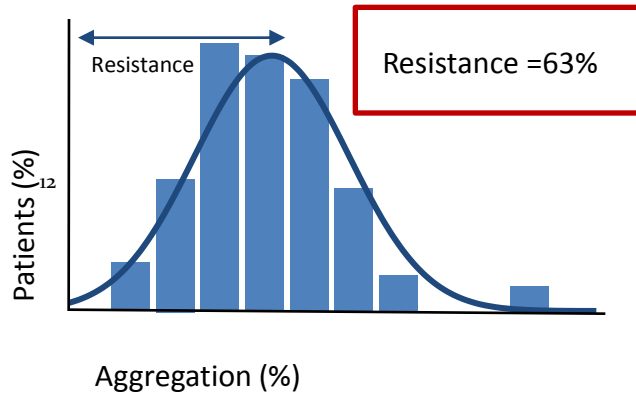
Liver



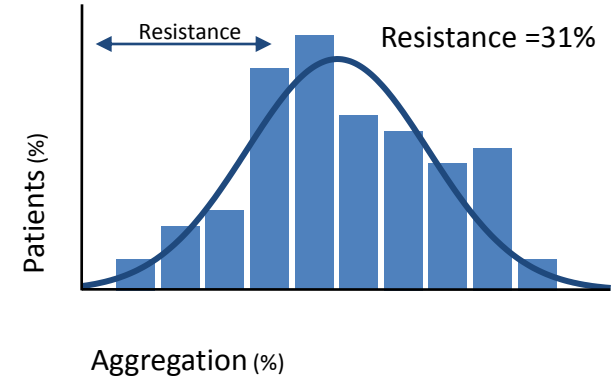
Clopidogrel resistance

The First Clopidogrel Resistance Study (300 mg)

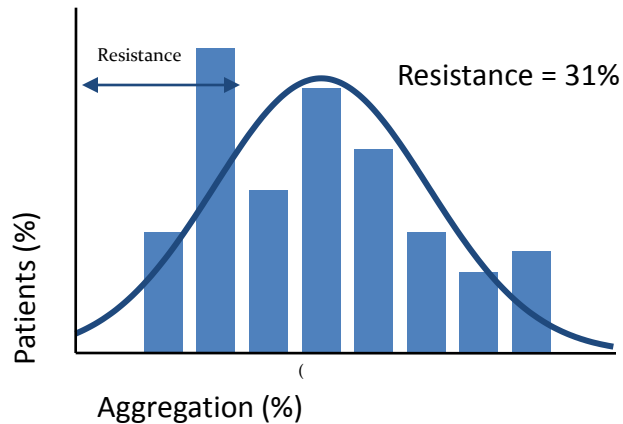
2 Hours



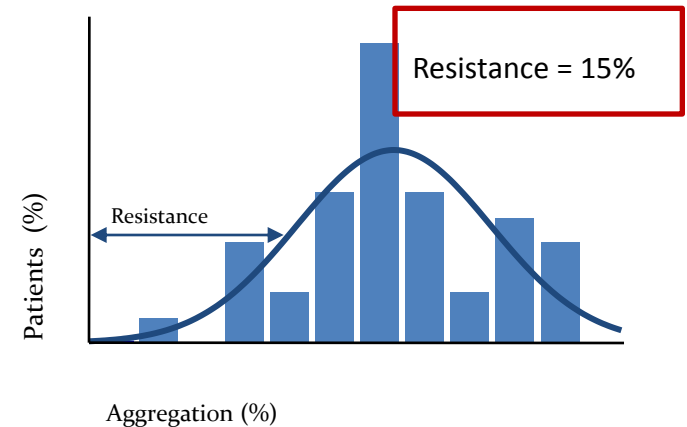
24 Hours



5 Days



30 Days

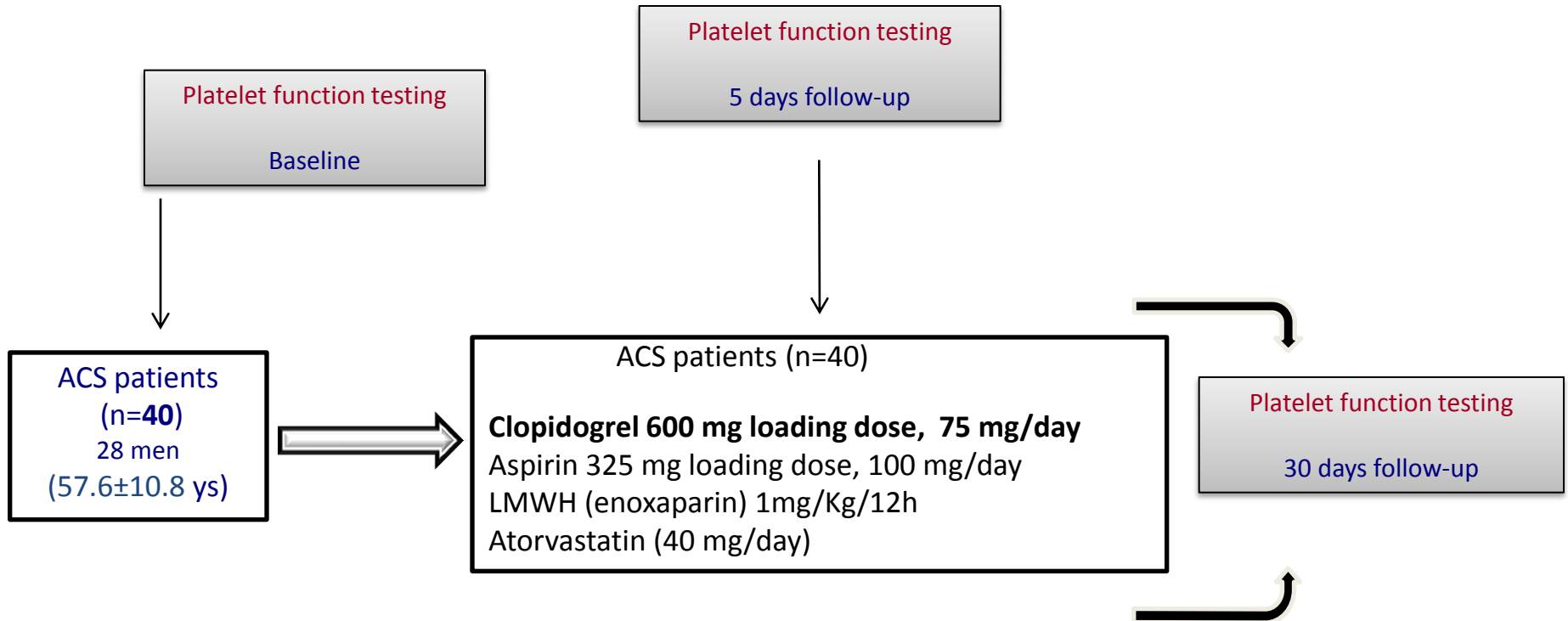


AIM

- We investigated various platelet-related prothrombotic and proinflammatory factors as well as the platelet aggregatory response in ACS patients receiving clopidogrel therapy as a function of the patient responsiveness to clopidogrel and the duration of treatment.

METHODS

DESIGN



Angiography was performed in all patients within 72 hours of hospital admission.

Patients were then underwent a percutaneous coronary intervention (PCI) (70%) within the first 4 days from clopidogrel loading or had a conservative management (30%)

Platelet function testing

- Platelet aggregation studies
 - Light transmittance aggregometry (LTA)
- Flow cytometry measurements
- VASP phosphorylation analysis (PRI)

RESULTS

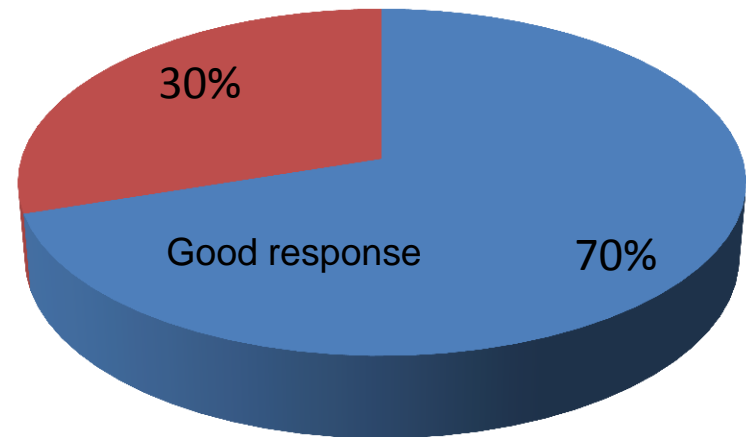
Table 1. Demographic, clinical and laboratory characteristics of the study population.

Variable	ACS patients (n=40)
Age (years)	57.6±10.8
Men/Women	28 / 12
Smoking, n (%)	23 (57.5)
Hypertension, n (%)	24 (60.0)
Hyperlipidemia, n (%)	16 (40.0)
Diabetes, n (%)	0 (0)
Family history of premature CAD, n (%)	27 (67.5)
Leucocytes (x 10 ⁹ /L)	11.07±3.01
Neutrophils (x 10 ⁹ /L)	7.53±2.32
Monocytes (x 10 ⁹ /L)	0.48±0.34
Platelets (x 10 ⁹ /L)	263.8±10.37
MPV (fl)	10.0±3.4
Total cholesterol (mg/dL)	193.2±42.5
HDL-cholesterol (mg/dL)	46.9±12.4
Triglycerides (mg/dL)	131±80.1
LDL-cholesterol (mg/dL)	130.1±42.1
hsCRP	3.39±1.82
Drug therapy	
<i>I. Antihypertensive treatment (%)</i>	23 (57.5)
Thiazide diuretics (%)	15 (37.5)
Beta blockers, n (%)	12 (30.0)
Calcium antagonists, n (%)	12 (30.0)
ACE inhibitors, n (%)	13 (32.5)
ARBs, n (%)	10 (25.0)
<i>II. Lipid lowering therapy</i>	
Statin prior to the event, n (%)	0 (0)
Statin after the event, n (%)	100 (100)

VASP phosphorylation analysis

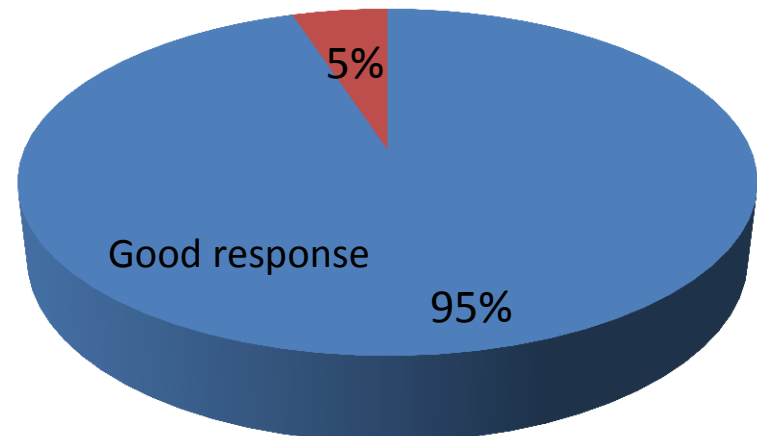
5 days

12 patients (30%) with PRI values \geq 50% (mean \pm SD, 62.3 \pm 12.1%)



30 days

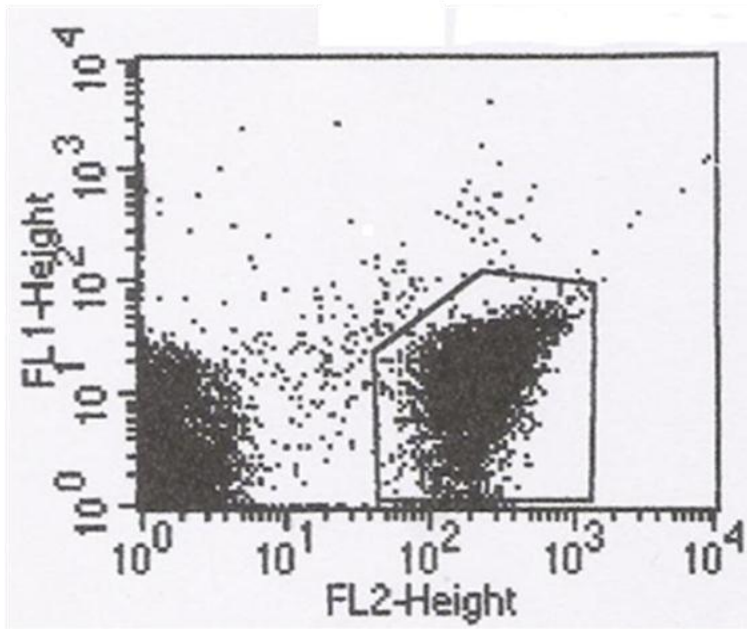
2 patients with PRI values \geq 50% (mean \pm SD, 44.2 \pm 15.4%)



VASP phosphorylation analysis-flow cytometry

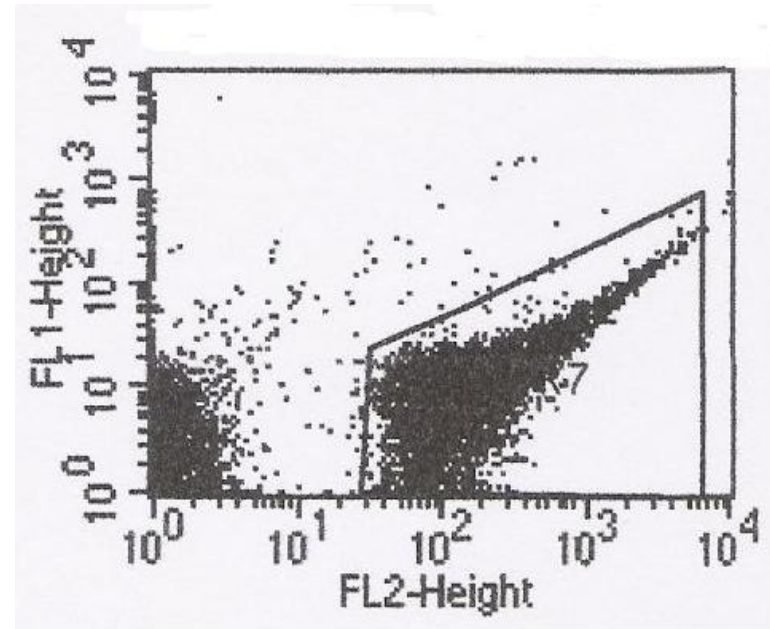
Clopidogrel responder

PRI= 26,1%



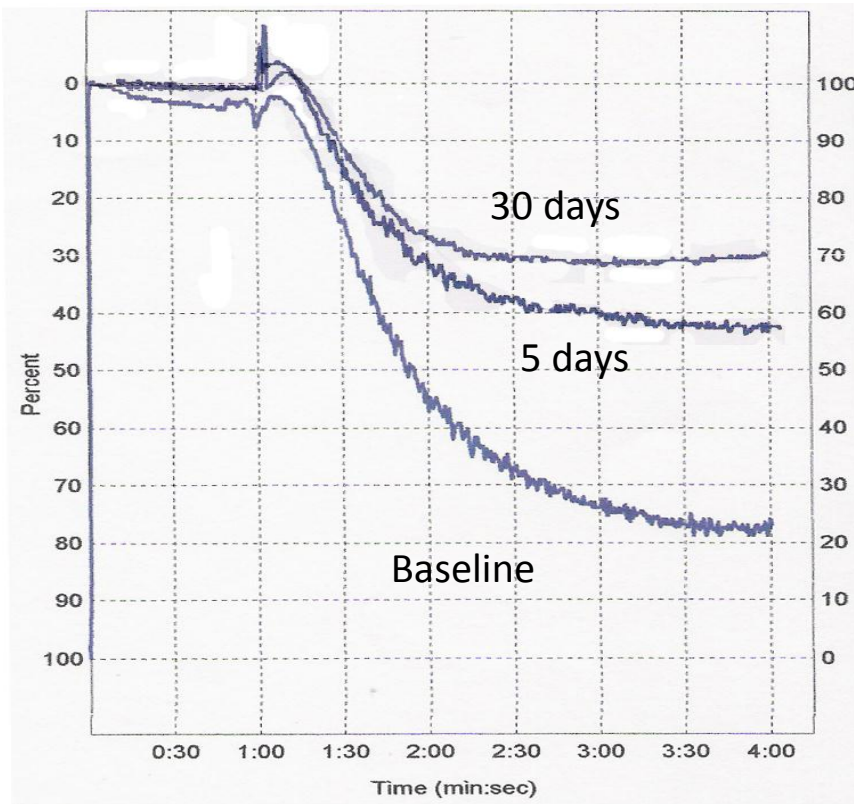
Clopidogrel nonresponder

PRI= 74,4%

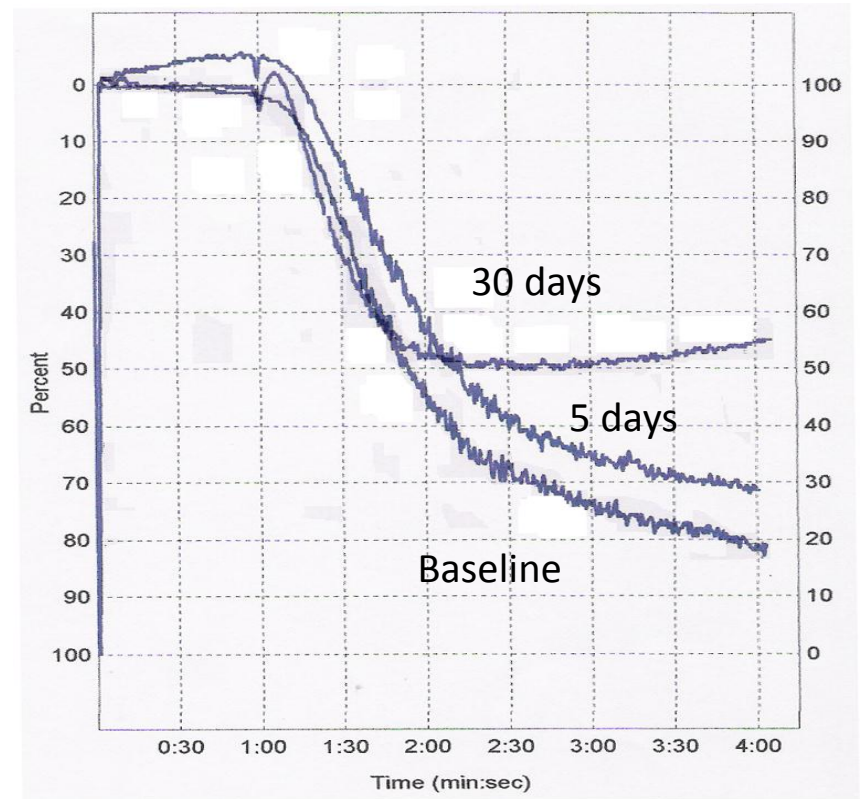


$$\text{PRI} = \left[\frac{\text{MFic PGE1} - \text{MFic (PGE1 + ADP)}}{\text{MFic PGE1}} \right] \times 100$$

Aggregometry



Clopidogrel responder



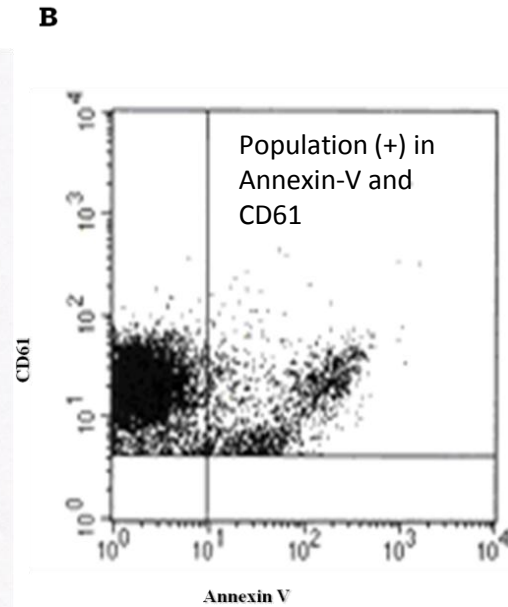
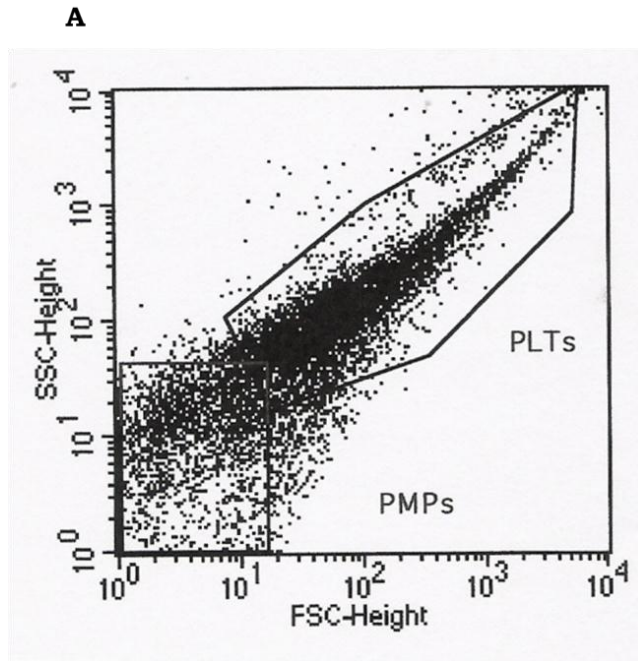
Clopidogrel nonresponder

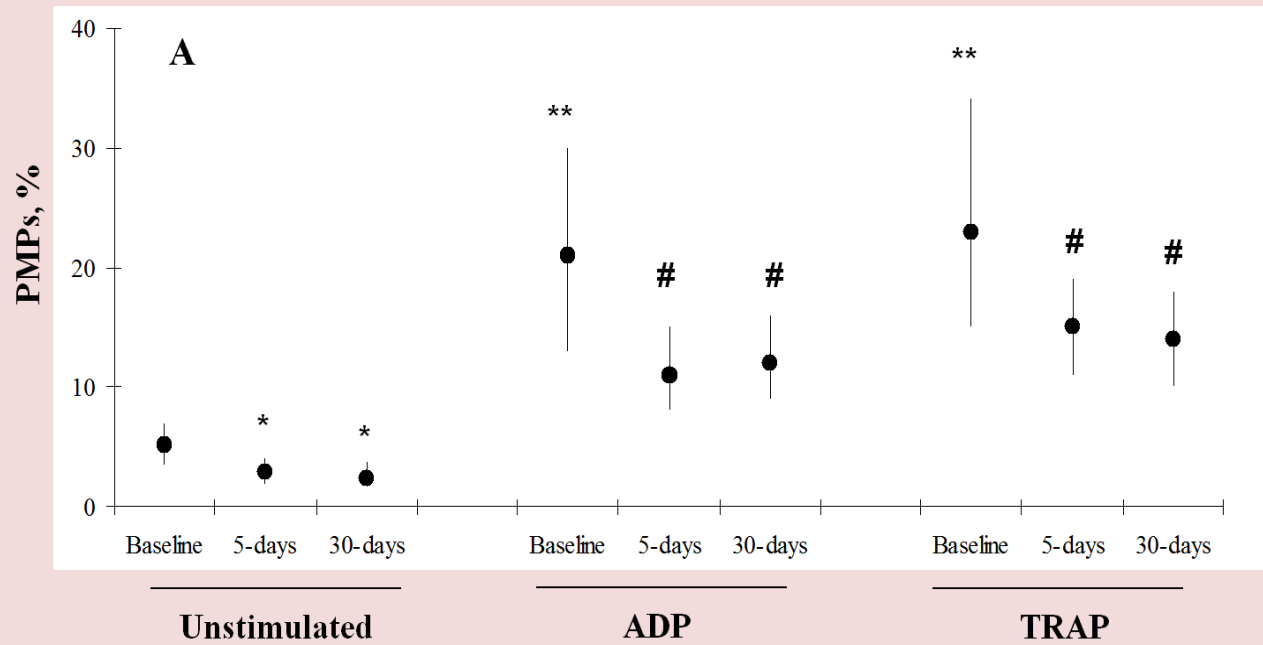
Platelet aggregatory response in ACS patients, at baseline and at 5 and 30 days postclopidogrel loading

Platelet Aggregation, %				
Clopidogrel treatment	ADP			TRAP
	2.5μM	5μM	10μM	10μM
Clopidogrel responders (n=28)				
Baseline	53 (24-64)	65 (42-75)	78 (69-89)	81 (70-90)
5-days	26 (15-40) ^a	35 (20-52) ^a	55 (35-70) ^a	62 (51-74) ^b
30-days	23 (12-35) ^a	32 (17-50) ^a	50 (31-65) ^a	59 (38-68) ^a
Clopidogrel nonresponders (n=12)				
Baseline	49 (23-60)	62 (41-70)	76 (67-81)	79 (65-86)
5-days	39 (17-50) ^{b,e}	54 (39-68) ^{b,e}	66 (58-75) ^{b,e}	72 (58-80) ^e
30-days	24 (10-30) ^{a,c}	37 (20-55) ^{a,c}	53 (33-62) ^{a,c}	62 (51-70) ^d

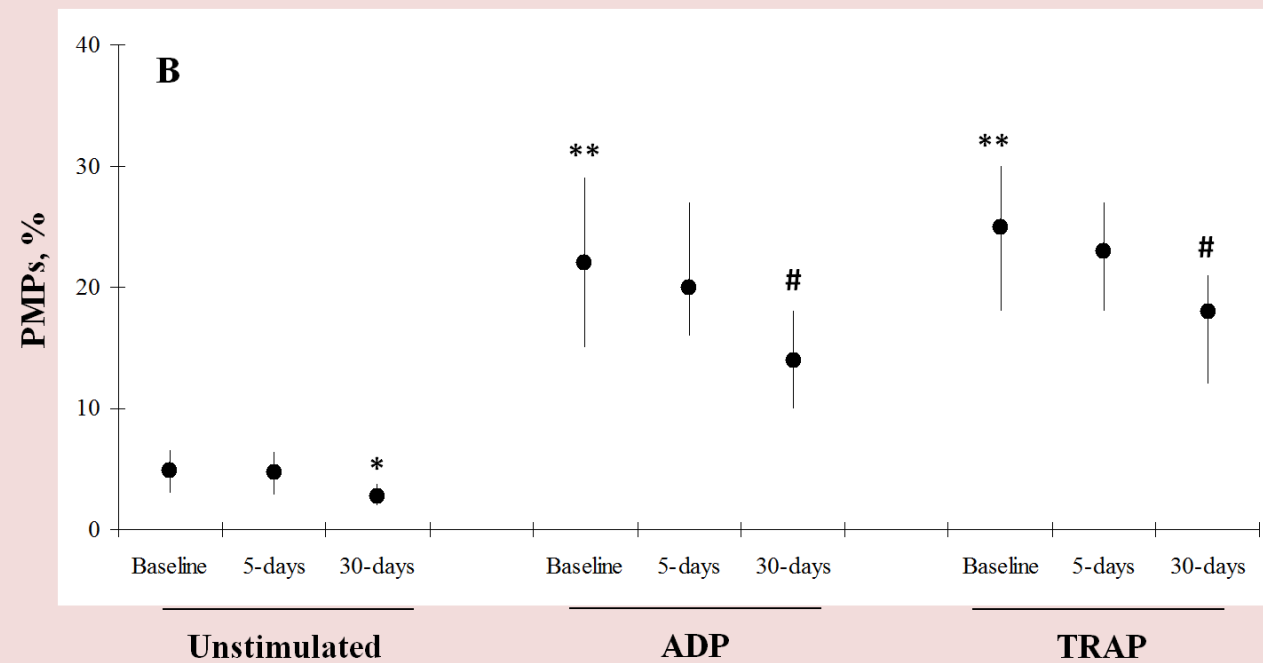
Data are expressed as median (range). ^a p<0.001 and ^b p<0.05 compared with corresponding baseline values. ^c p<0.01 compared with corresponding aggregation values at 5-days, ^d p<0.02 compared with corresponding aggregation values at baseline or at 5-days. ^e p<0.01 compared with corresponding values at 5-days of clopidogrel responders.

PMPs shedding



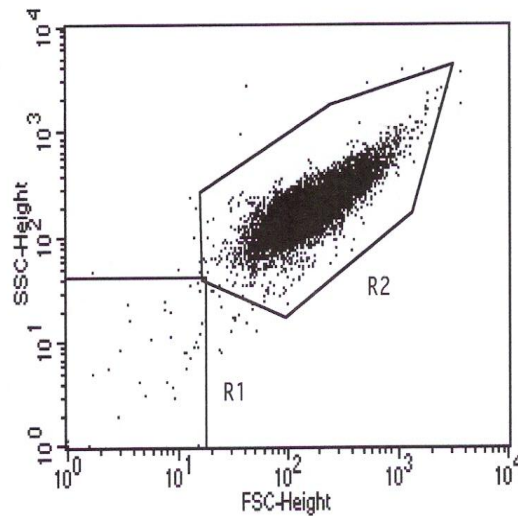


Good responders

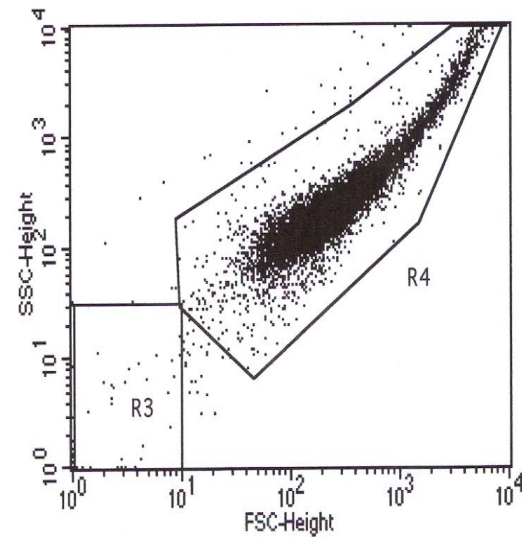


Bad responders

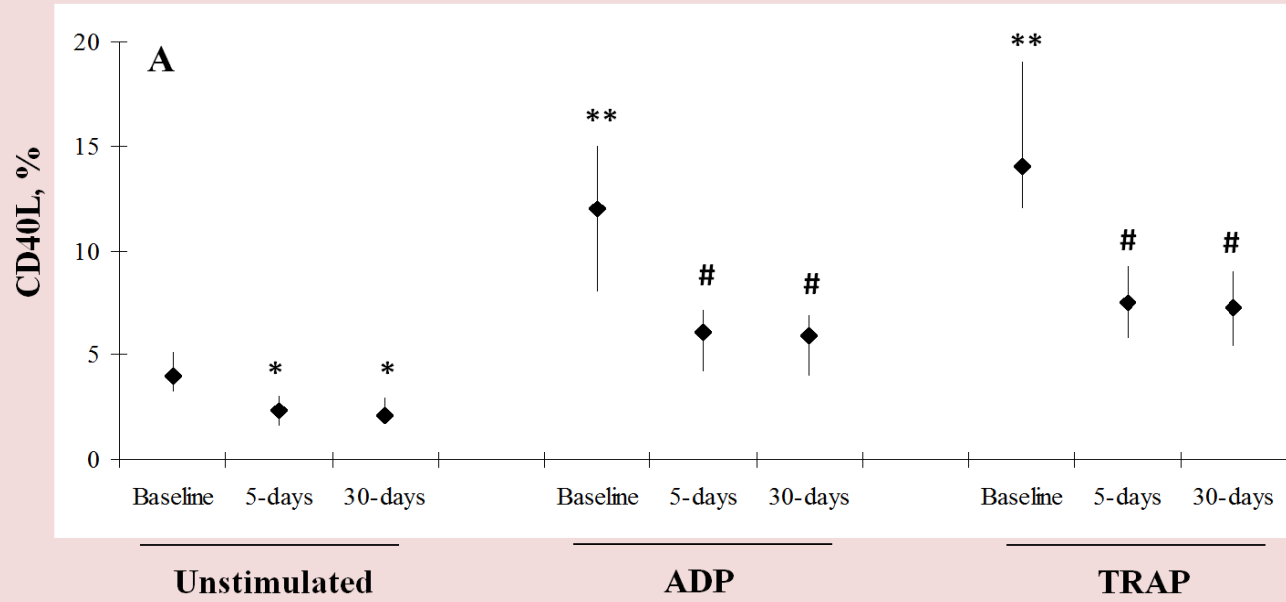
CD40L expression



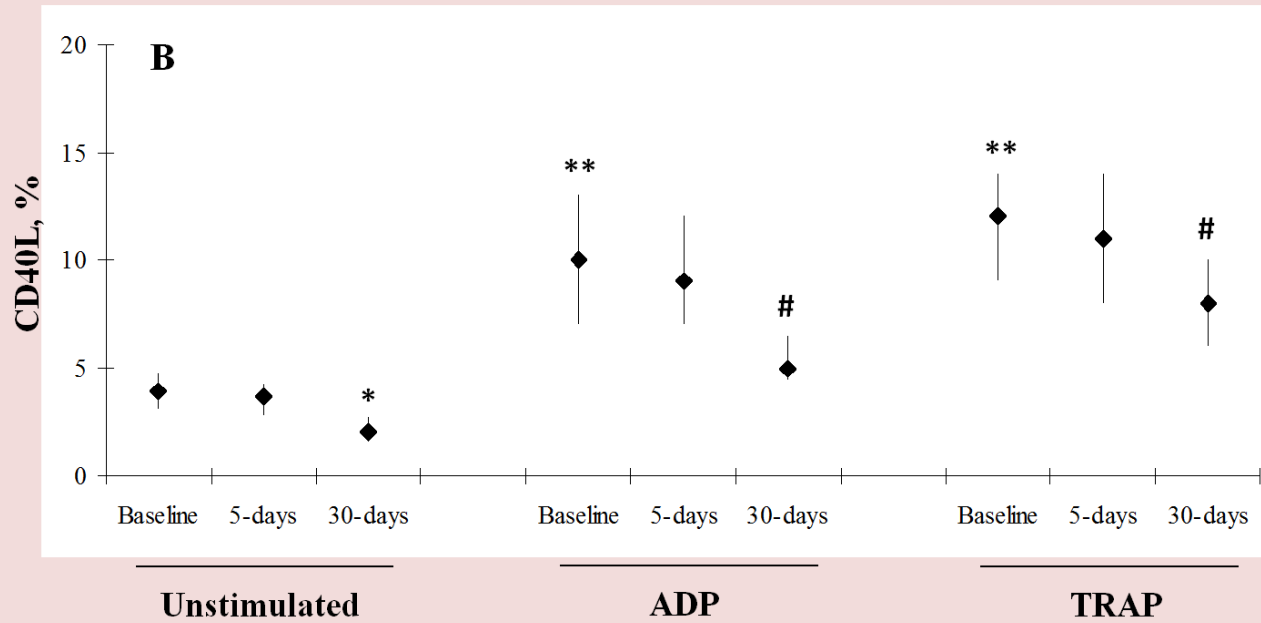
Unstimulated platelets



Stimulated platelets

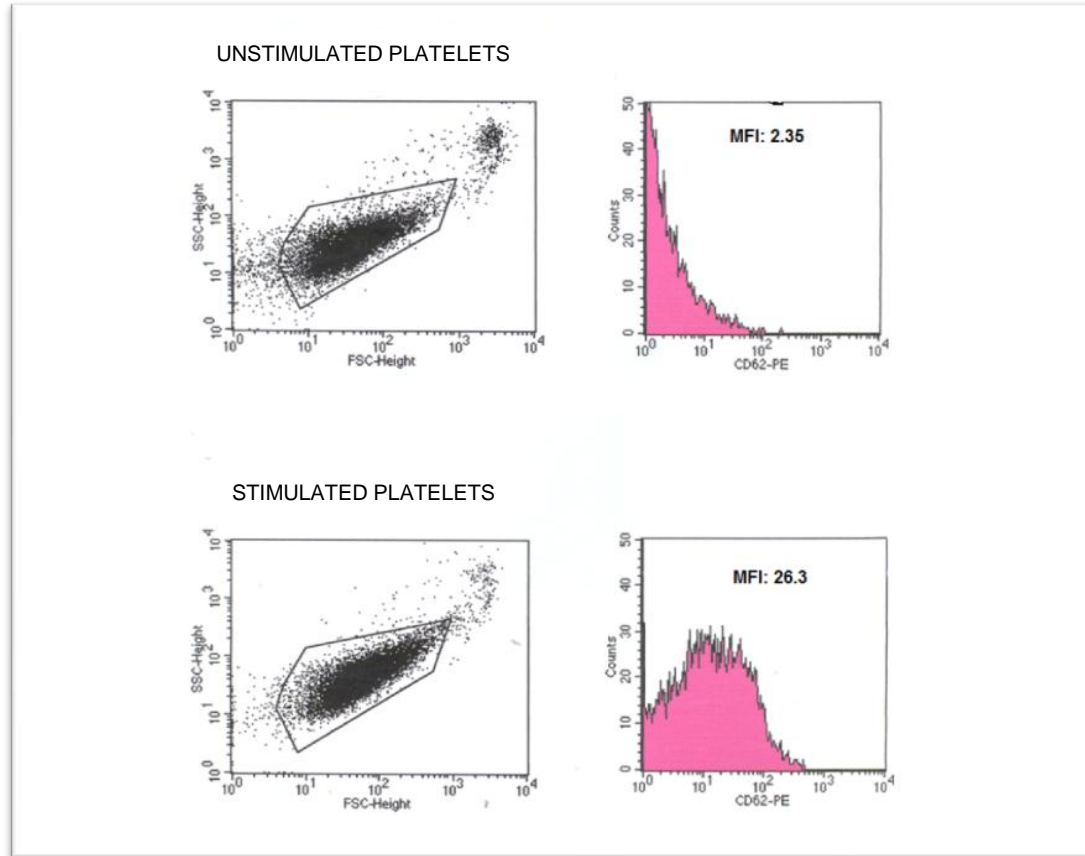


Good responders

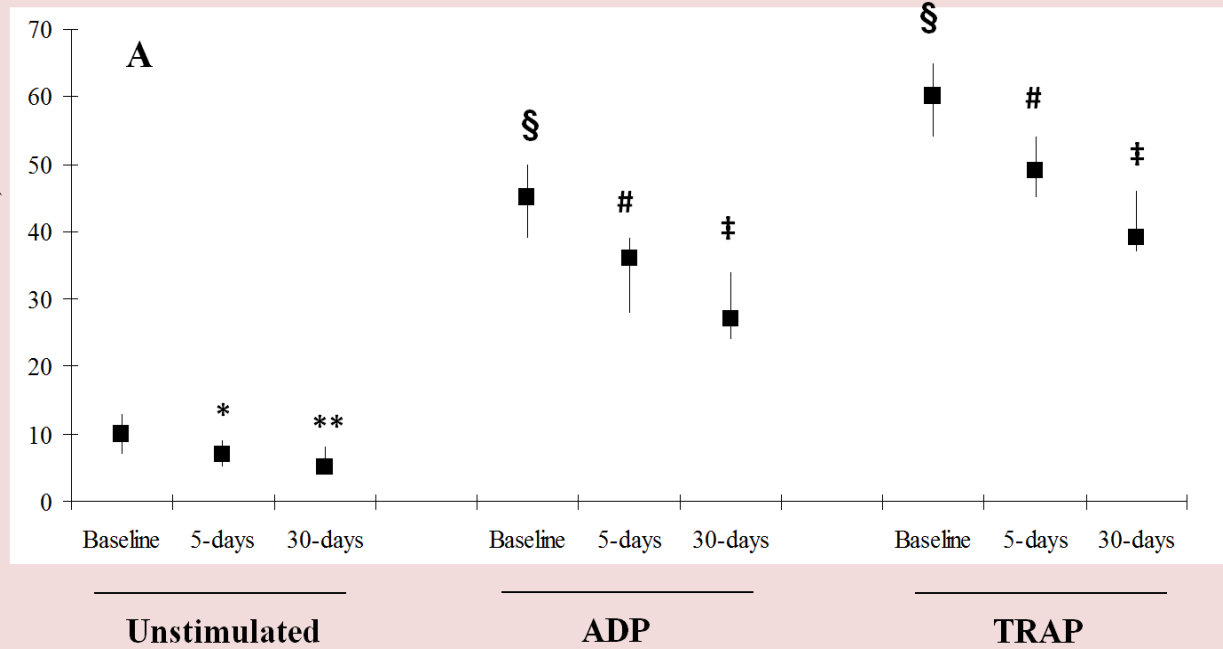


Bad responders

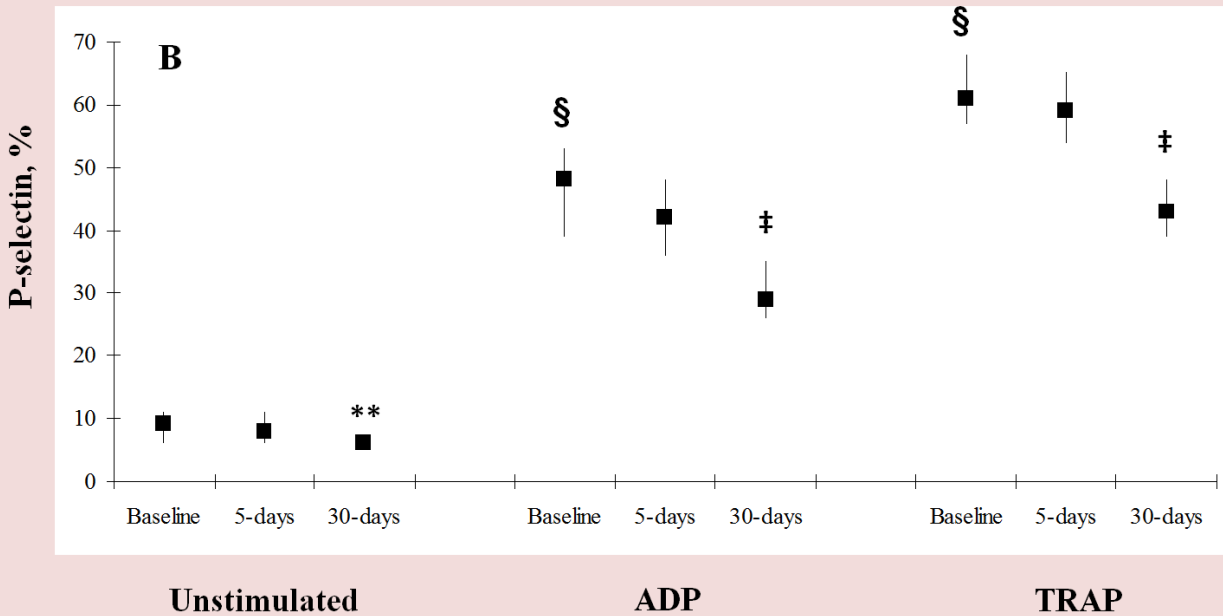
P-selectin expression



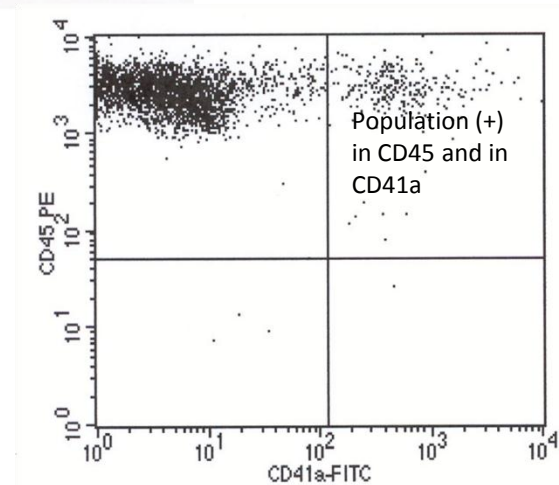
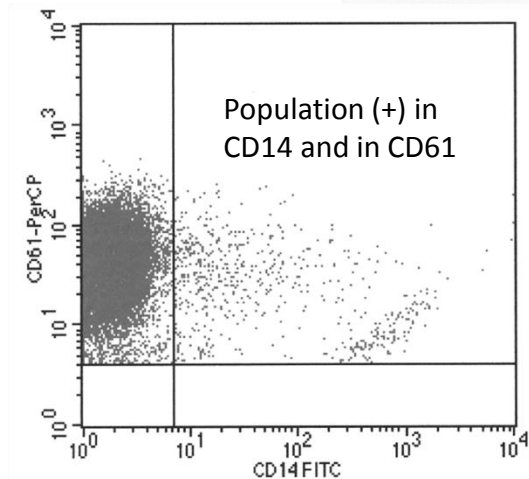
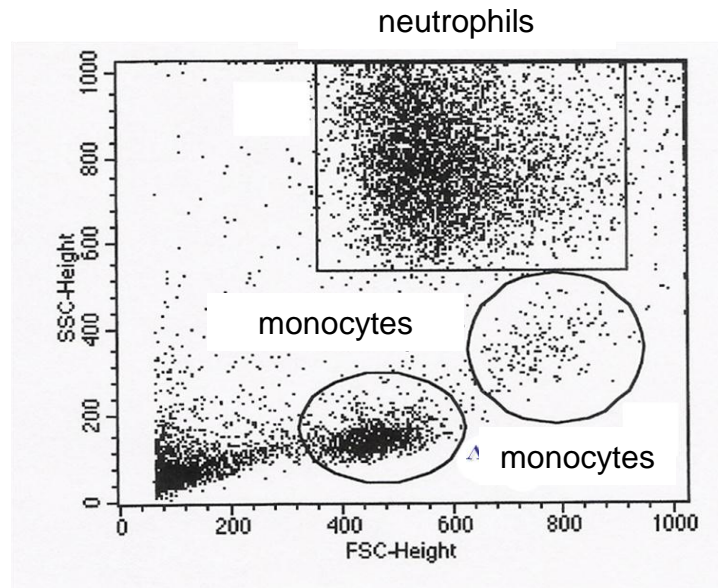
Good responders

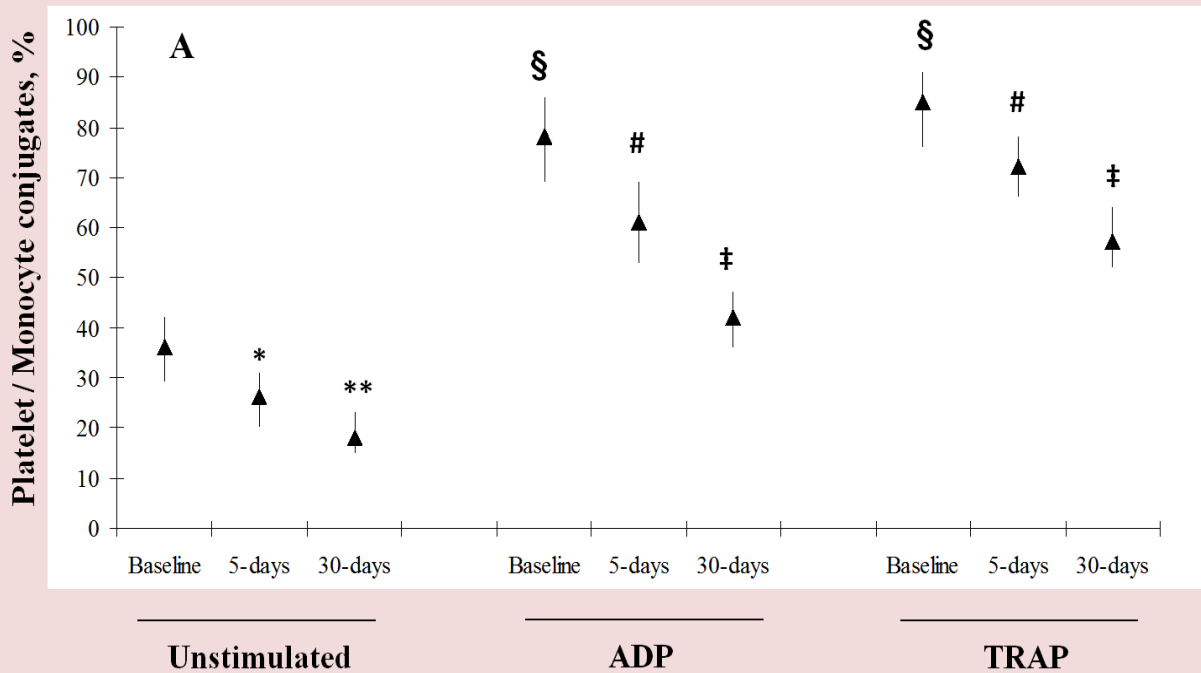


Bad responders

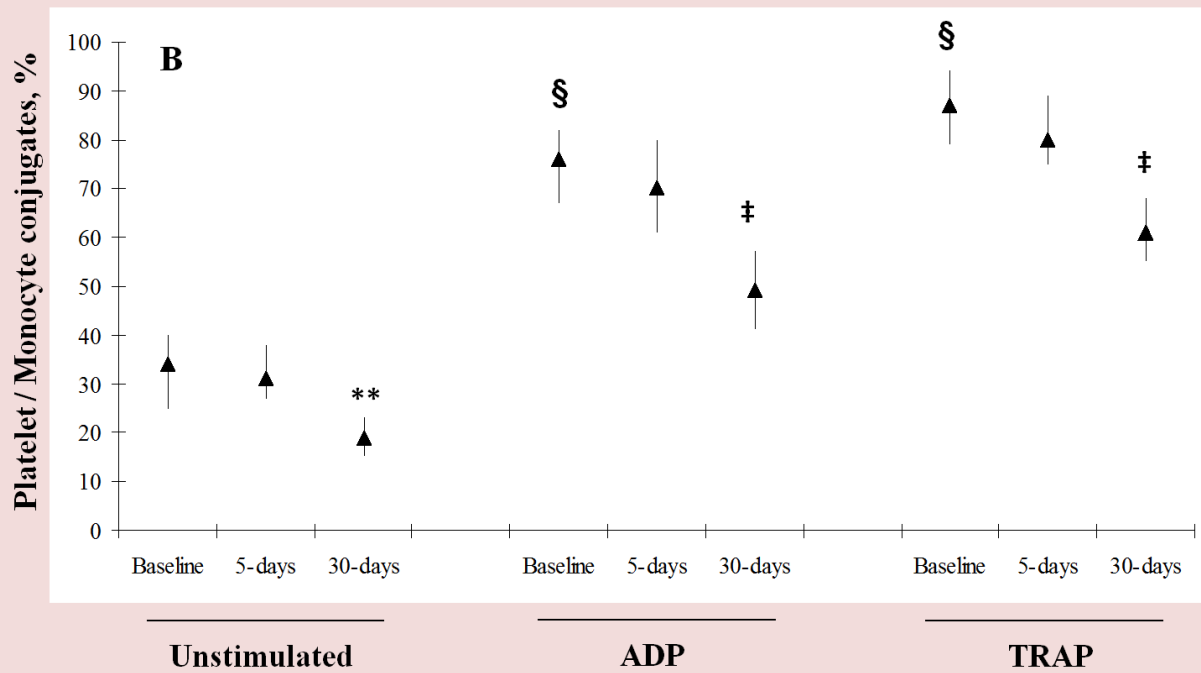


Platelet-leukocyte interaction



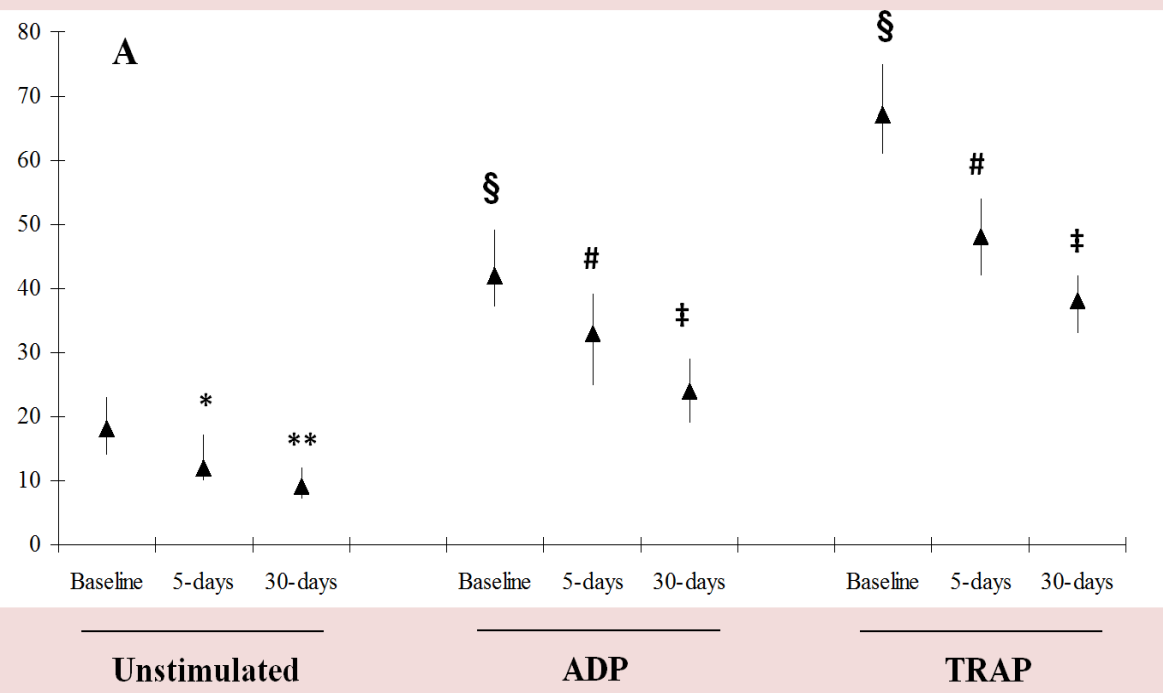


Good responders



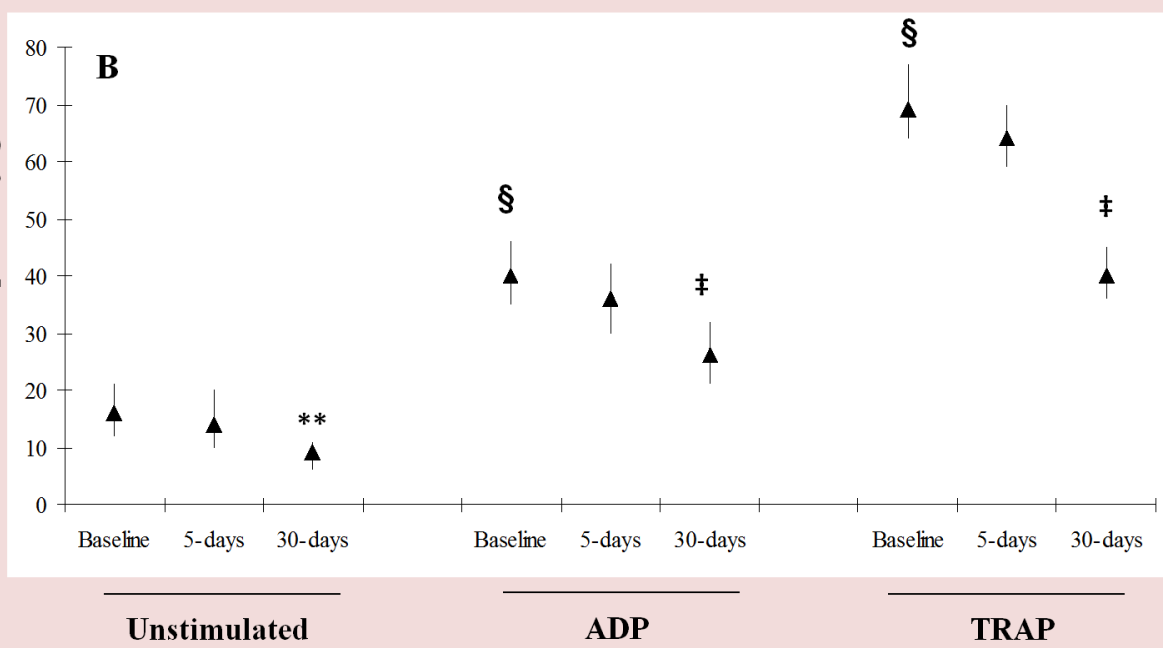
Bad responders

Platelet / Neutrophil conjugates, %



Good responders

Platelet / Neutrophil conjugates, %



Bad responders

Conclusion

- A maintenance dose of 75mg clopidogrel differentially affects platelet aggregation and platelet-derived prothrombotic and proinflammatory mediators in ACS patients within the first month of the treatment, a phenomenon that is highly influenced by the drug response variability.
- Importantly, the VASP phosphorylation index and the drug antiplatelet effects are significantly improved in clopidogrel nonresponders within 30 days of therapy.