



ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΙΑΤΡΙΚΗ ΣΧΟΛΗ
ΜΕΤΑΠΤΥΧΙΑΚΟ ΠΡΟΓΡΑΜΜΑ ΣΠΟΥΔΩΝ «ΛΟΙΜΩΞΙΟΛΟΓΙΑ»
Διευθυντής: Καθηγητής Ε. Ι. Γιαμαρέλλος-Μπουρμπούλης

Αιμοπετάλια (& αντι-αιμοπεταλιακα) και μη ειδική (& ειδική) ανοσία

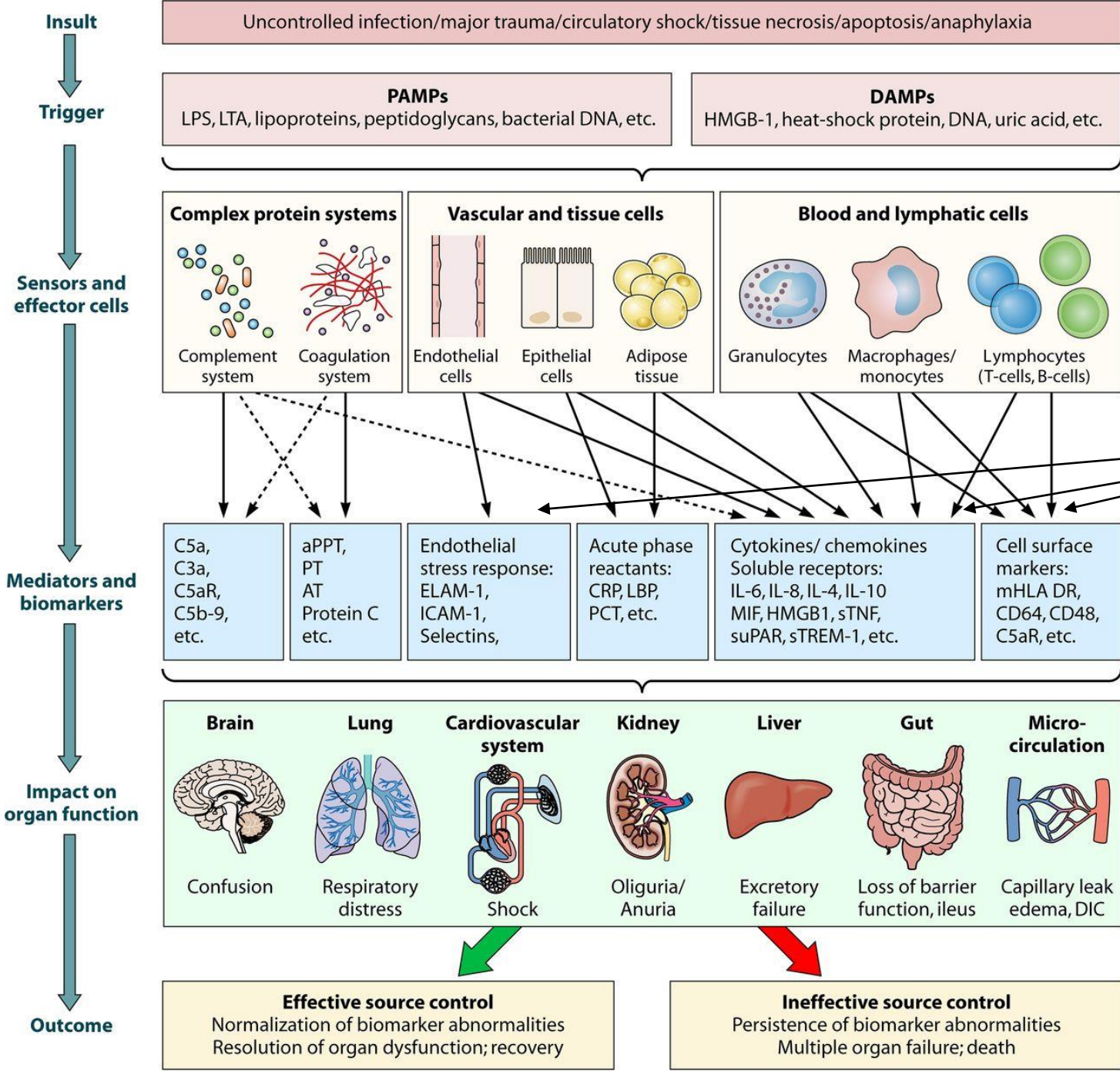
Καρολίνα Ακινόσογλου

Παθολόγος – Λοιμωξιολογος

Επικουρη Καθηγητρια Παθολογιας

Πανεπιστημίου Πατρών

The inflammatory response

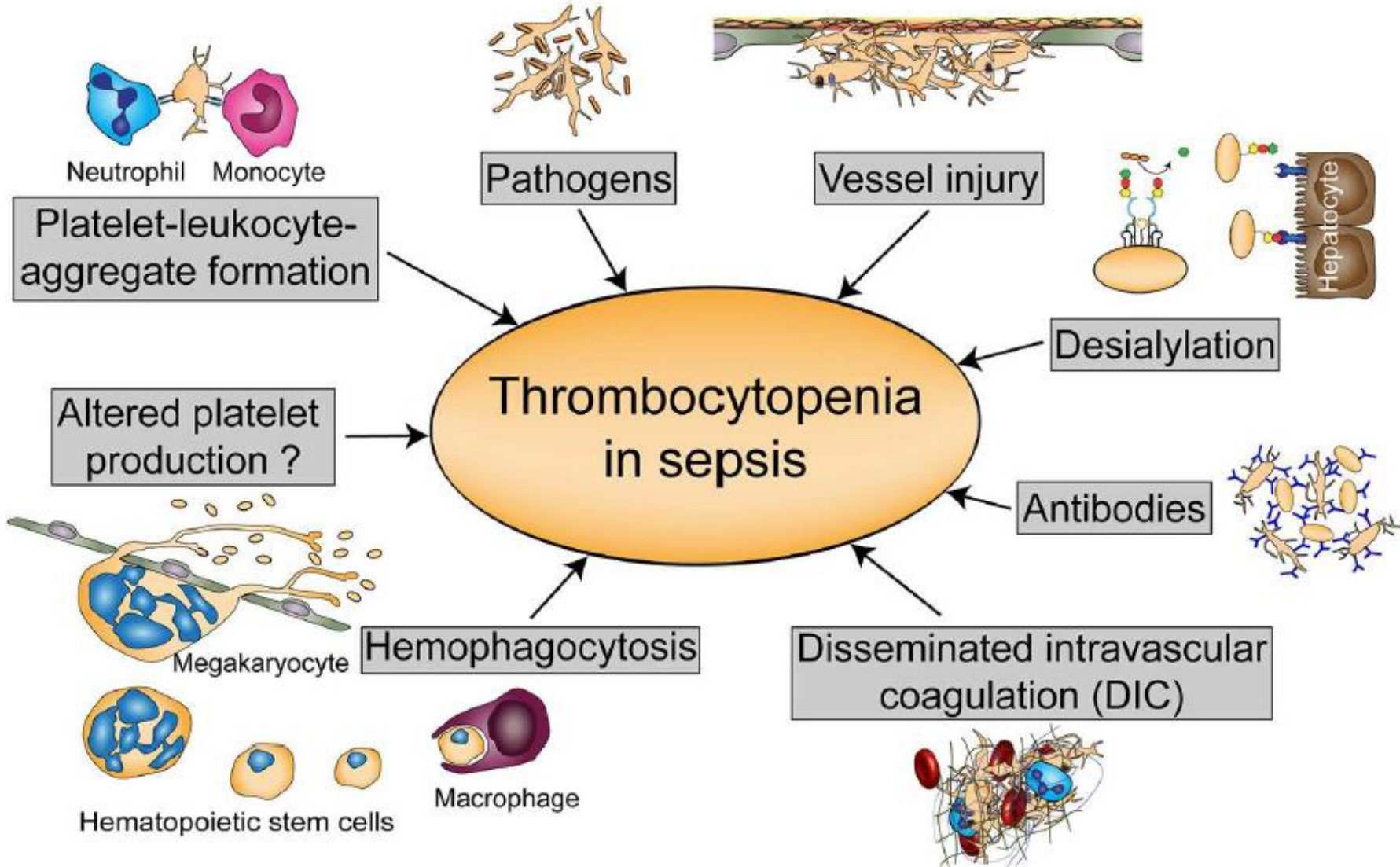


Platelets

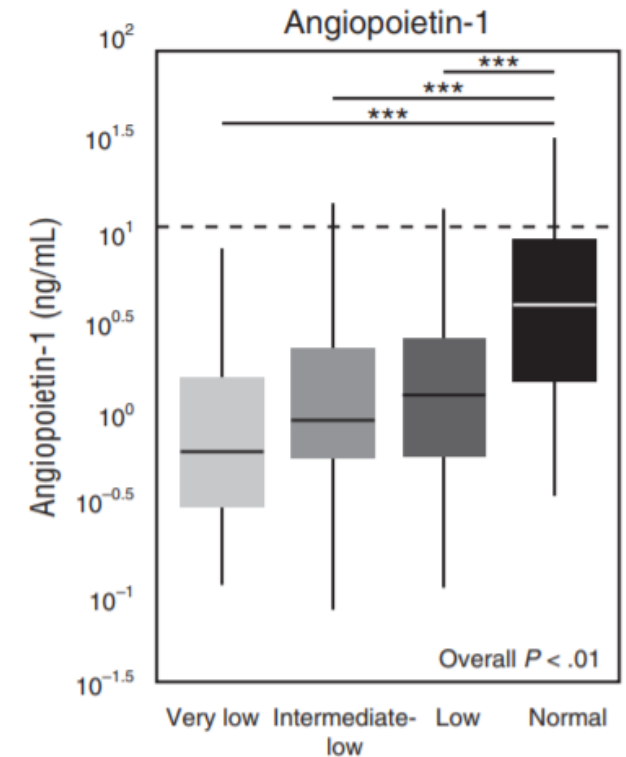
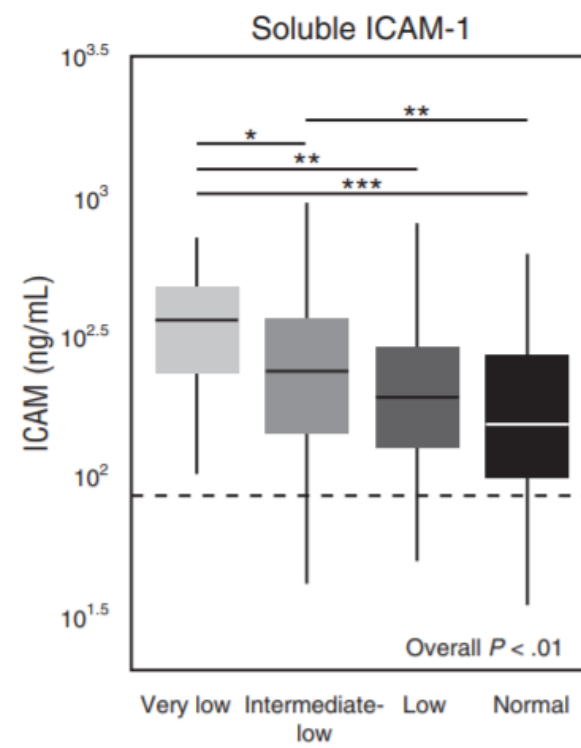
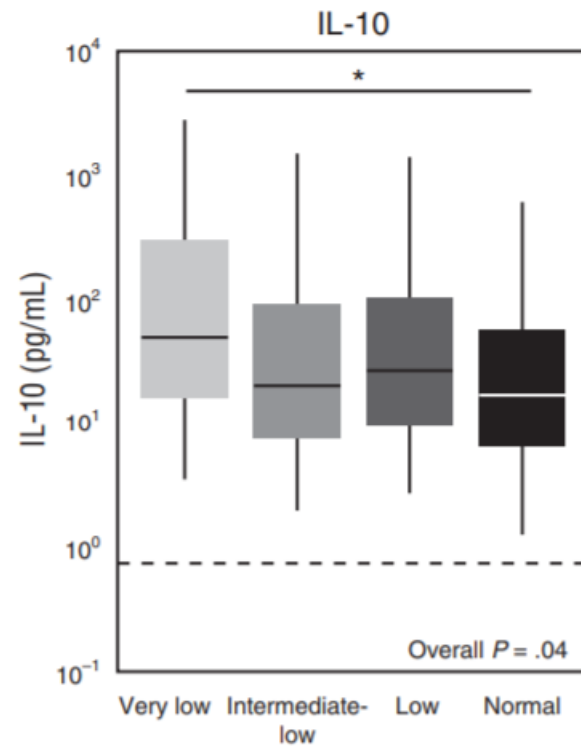
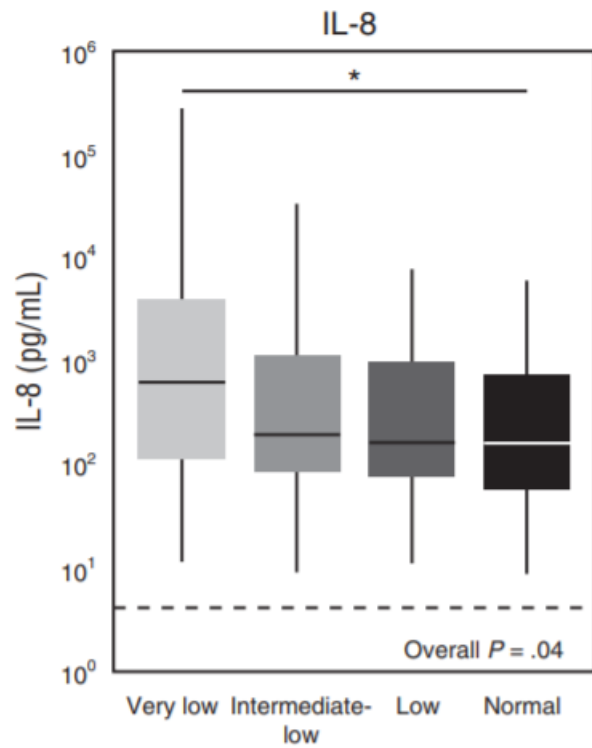
- Vascular tissue damage and repair
- Haemostasis
- Implication in immune response
- Thrombocytopenia

1. Quantity : Thrombocytopenia...

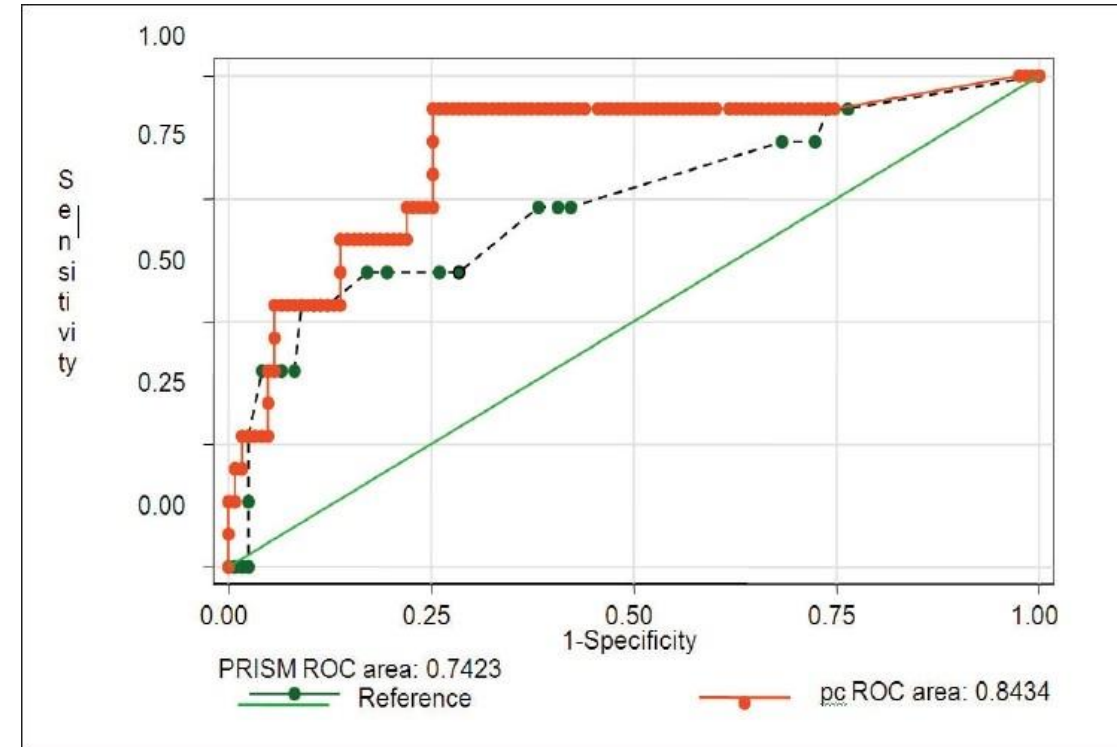
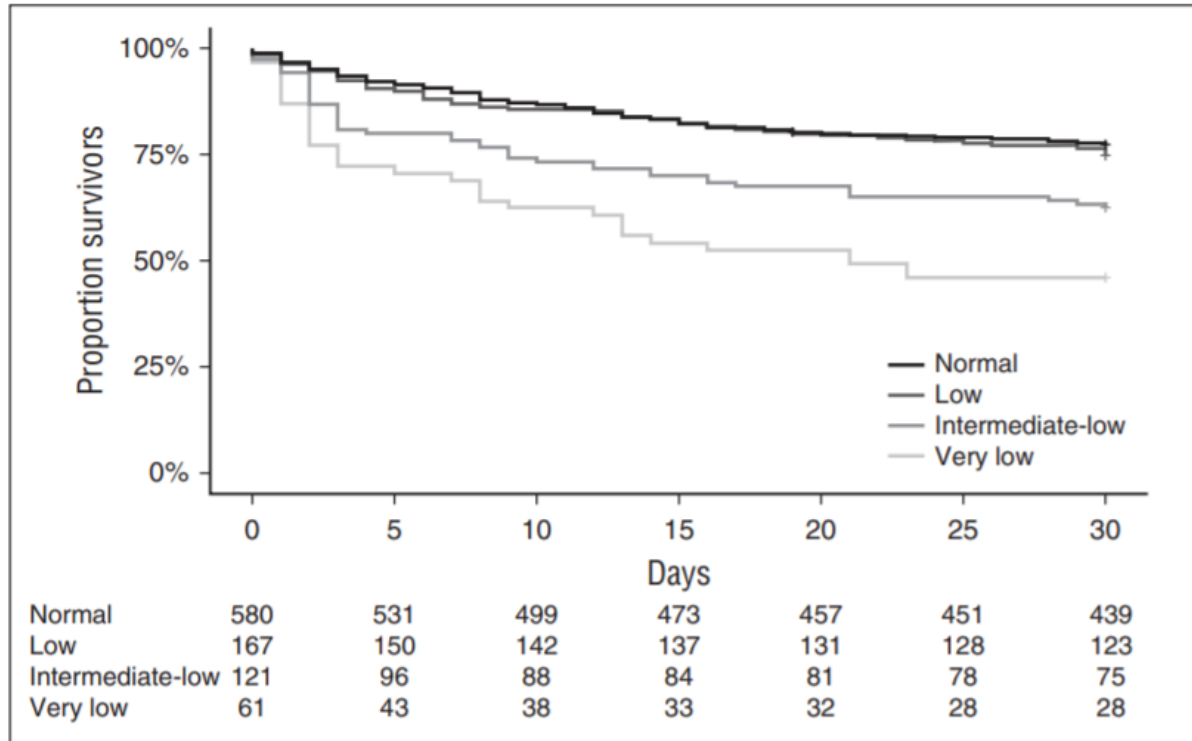
Possible causes of thrombocytopenia in sepsis



Severe thrombocytopenia increased cytokine levels and enhanced endothelial cell activation

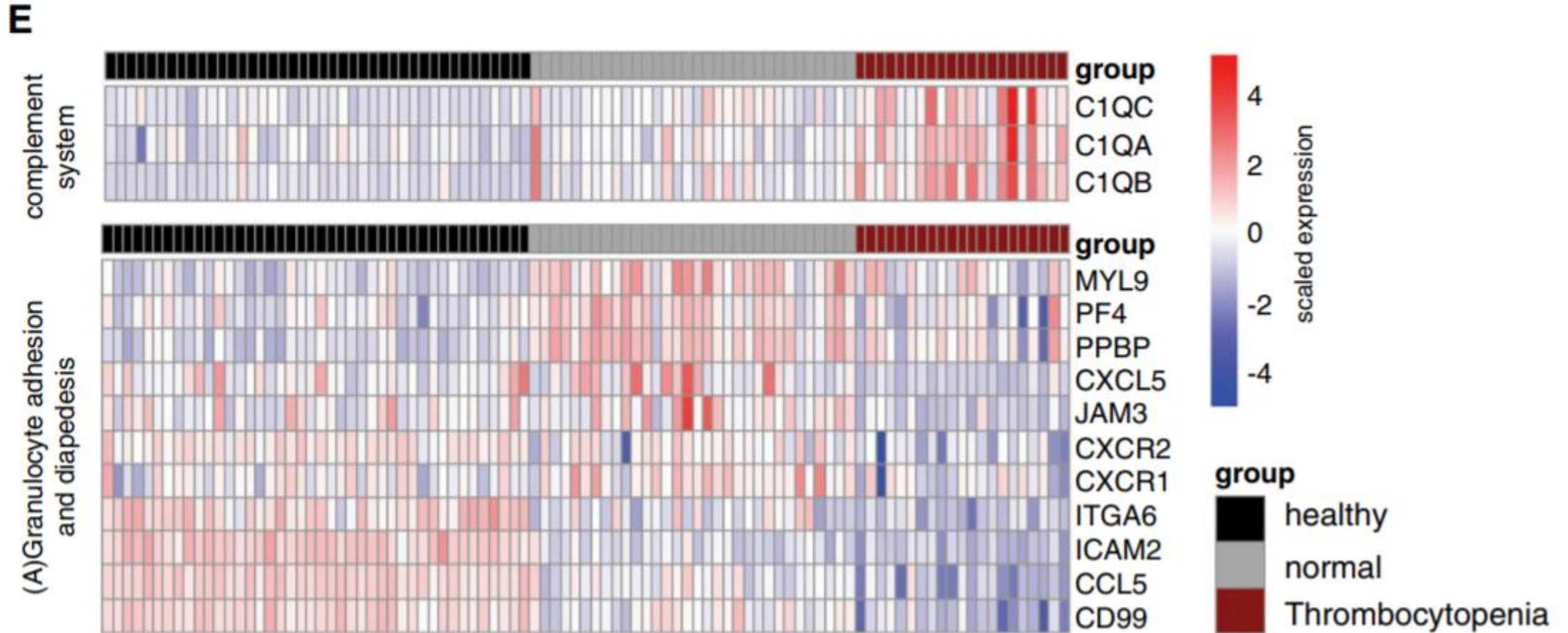


Admission thrombocytopenia is associated with enhanced mortality



In children, the magnitude of the drop in platelet count rather than thrombocytopenia per se and its non – resolution are strong predictors of mortality

Severe thrombocytopenia is associated with reduced signaling in leukocyte adhesion and diapedesis and increased complement signaling



2. Quality : Cells, Mediators and Interactions...

Platelet-Derived Mediators Linking Thrombosis, Infection, and Immunity

Category of Platelet Mediator	Platelet Receptor/ Protein/Molecule	Vascular or Circulating Cell Interaction	Setting	Functions
Integrins	GP1b (Cd42)	vWF; endothelial cells; leukocytes; bacteria	High shear stress/ infection	Unfolded vWF deposited on collagen; endothelial P-selectin; binds thrombin limiting leukocyte recruitment; binds complement C3 on bacteria to enhance adaptive immunity
	GP1a/2a	Subendothelial collagen	Low shear stress	Collagen
	GP2b/3a (CD41)	Other platelets through Fgn; T cells; bacteria	Hemostasis; infection; immunity	Platelet aggregation; endothelial ICAM-1 or $\alpha V\beta 3$ leading to firm adhesion; aggregation with activated T-cytolytic and T-helper cells; aggregation around bacteria; increase after H1N1 infection
α -granule proteins	PF4 (CXCL4)	T cells; monocytes; RBCs; bacteria	Infection; atherosclerosis	Limit Th17 expansion and differentiation; monocyte recruitment (heterodimer with RANTES); inhibits TGF- β signaling; binds Gram-negative bacteria increasing opsonization; kill plasmodium in RBCs
	RANTES (CCL5)	immunity; atherogenesis	infection; chronic inflammation	T-cell activation and differentiation; monocyte/macrophage adhesion and recruitment;
	TGF- β	Tumor cells (TGF- β R)	Metastasis	Reduce NK antitumor activity; contribute to induction of invasive epithelial-mesenchymal transition to metastasis
	β -defensin	Neutrophils	<i>Staphylococcus aureus</i> α -toxin	Netosis
δ -granule molecules	Serotonin (5HT)	Endothelial cells; T cells	Hemostasis/ thrombosis; adaptive immunity	Constricts injured blood vessels; enhances platelet aggregation to minimize blood loss; T-cell activation and differentiation; endothelial cell proliferation
	ADP	Platelet P2Y12; P2Y1	Hemostasis	Platelet recruitment, activation, and aggregation during clot formation; exposure of P-selectin (P2Y12, P2Y1) and PS and thrombin generation (P2Y12)
Surface protein expression	P-selectin	Leukocyte PSGL1 (neutrophils, monocytes, DC); endothelial PSGL1; metastatic cells PSGL1	Infection; other platelets	Platelet-neutrophil and platelet-monocyte HAGs; interactions of leukocytes with the thrombi; platelet-DC interactions; increase as a result of TLR7 stimulation; metastatic PSGL1 adhesion
	PSGL1	Endothelial P-selectin	High shear stress	Platelet-endothelial interactions for thrombus formation in small venules
	CD40	Leukocyte CD154	Inflammation/ infection/immunity	Surface expression as a result of TLR7 platelet-neutrophil tethering to the endothelium; platelet-DC leading to T-cell antigen presentation
	CD154	Endothelial CD40	Inflammation/infection	Increase in endothelial expression of E-selectin, VCAM-1, and ICAM-1, as well as secretion of MCP-1 and IL-8
Synthesized/secreted	IL-1 β	Endothelial IL-1R associated with $\alpha V\beta 3$ in the presence of Fgn	Infection; inflammation	Increases endothelial permeability by secreting NO

Immune cells

Endothelial cells

Inflammatory signals/ Infection (TLRs)

direct interaction

granule content release

↑ activation, differentiation, IgG-production

↑ expression of E-selectin, VCAM1, ICAM1
↑ recruitment of leukocytes

↑ permeability

PSGL1—P-selectin
CD40—CD154

CD154—CD40
P-selectin—PSGL1

TGFβ; PF4; RANTES; 5HT

IL-1β

(TLR2,4,7)

(Dengue, CMV)
(TLR4)



Endothelial Cell

Neutrophil

Monocyte

Dendritic cell

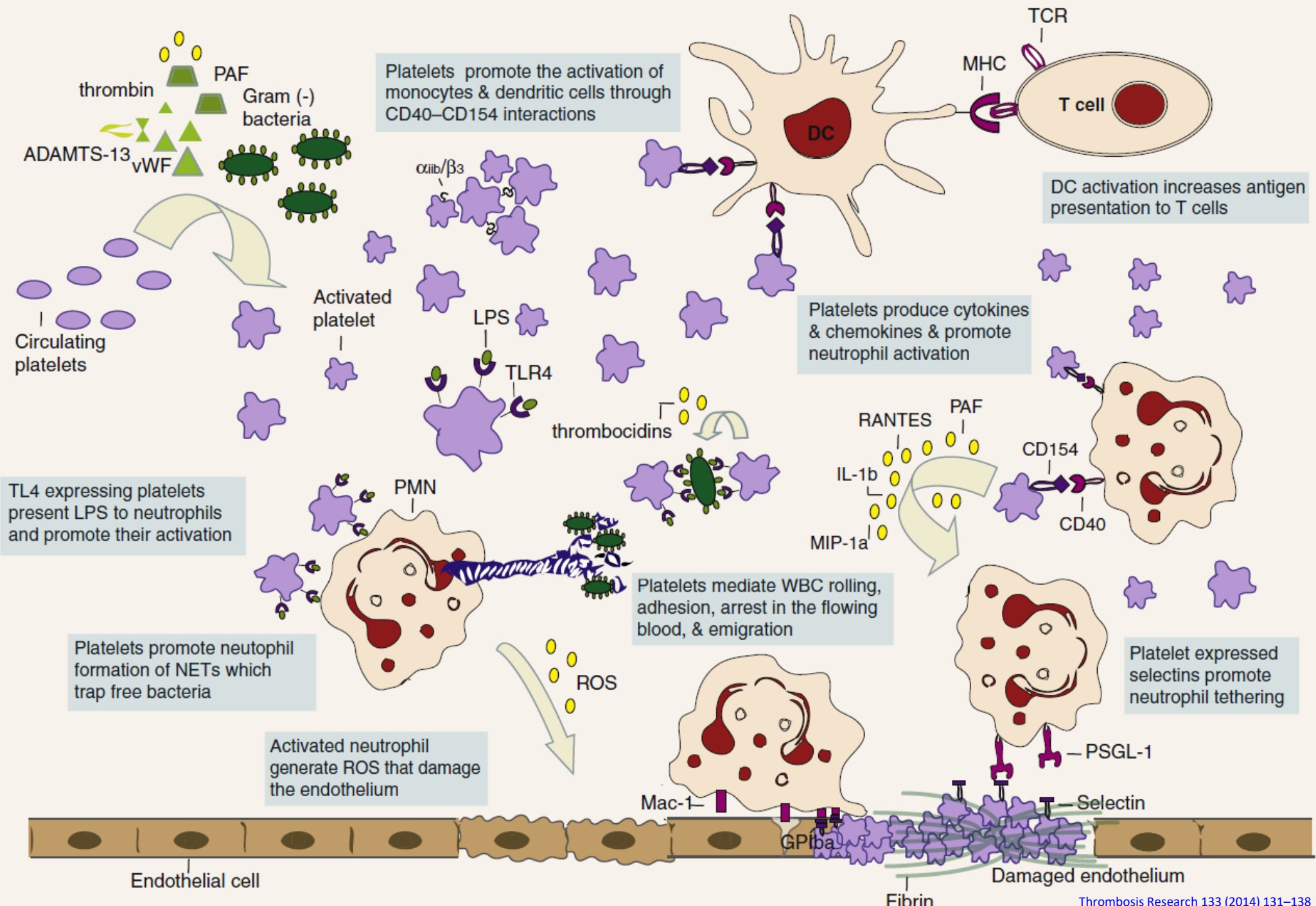
Natural killer

T-cells

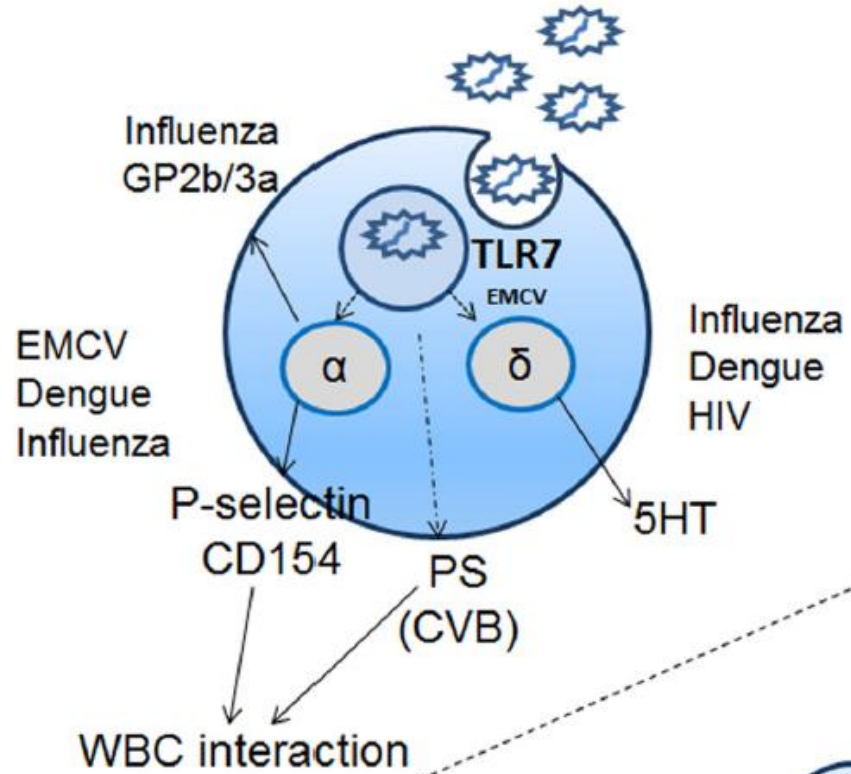
B-cells

Platelet

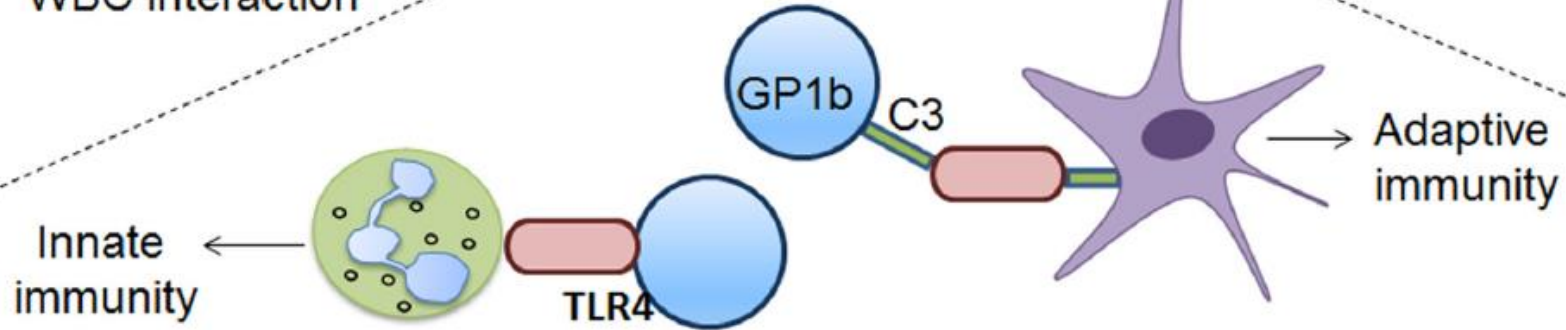
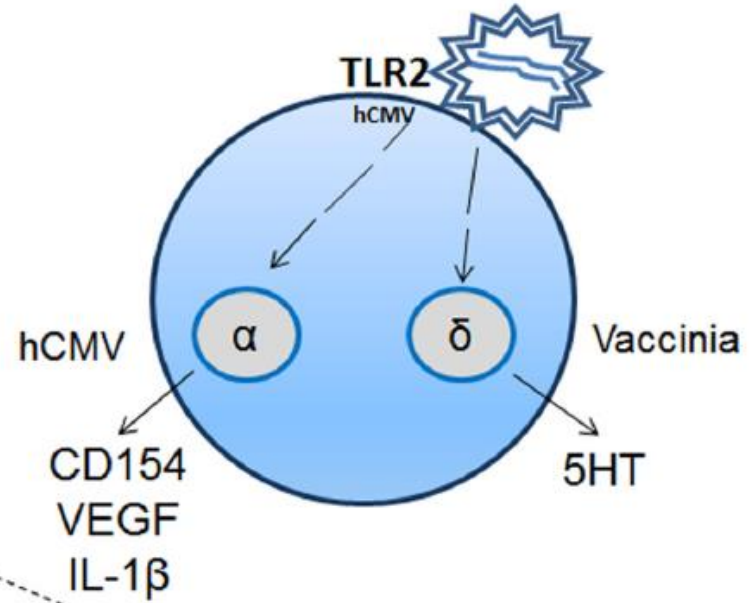
Eosinophil



RNA viruses

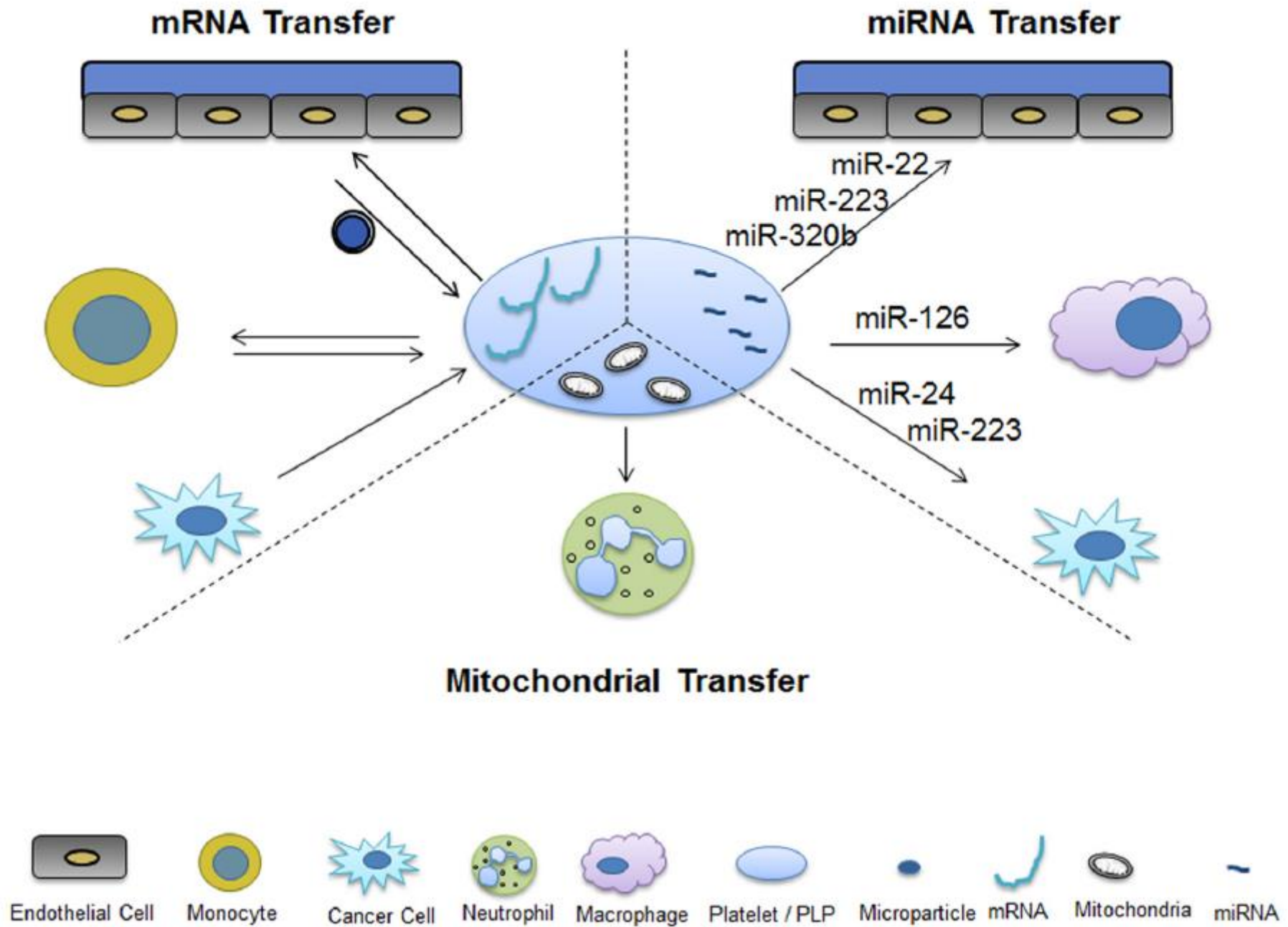


DNA viruses



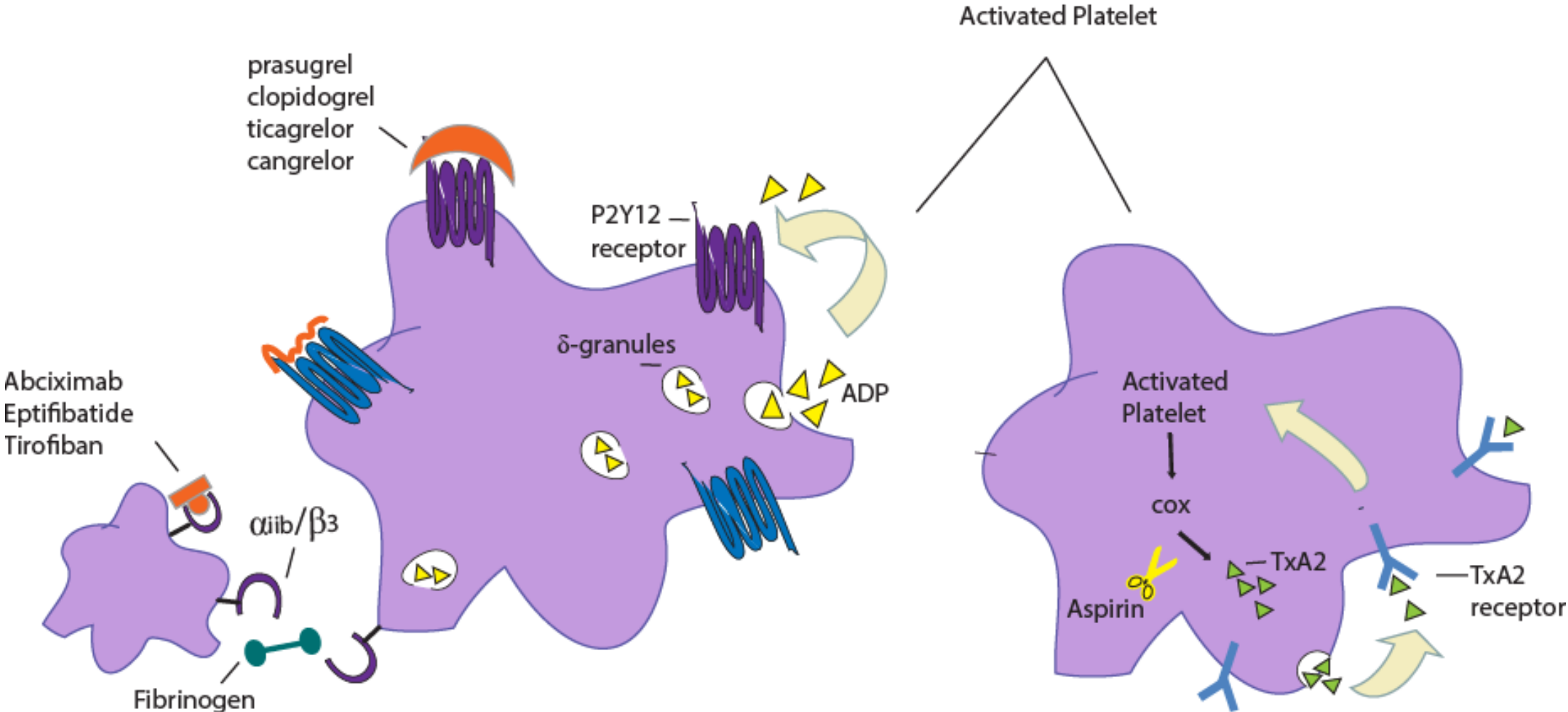
Bacteria





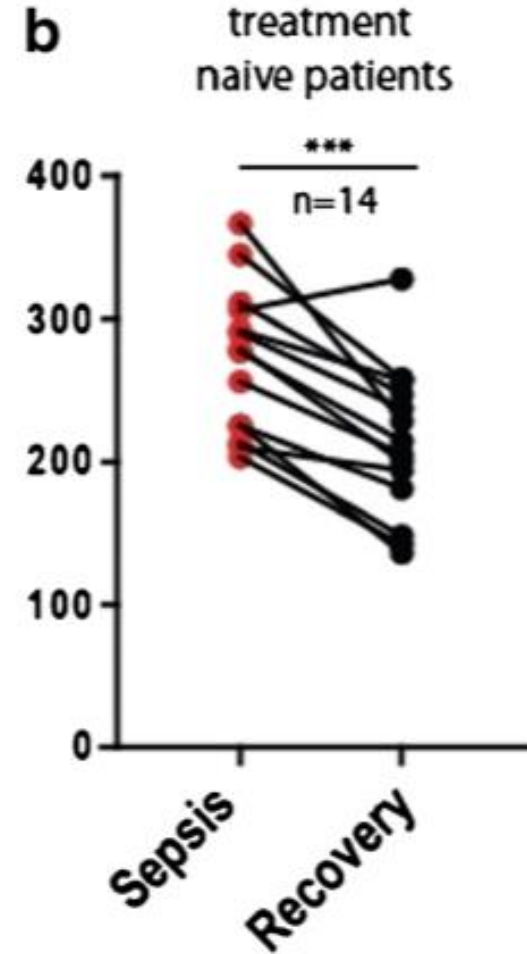
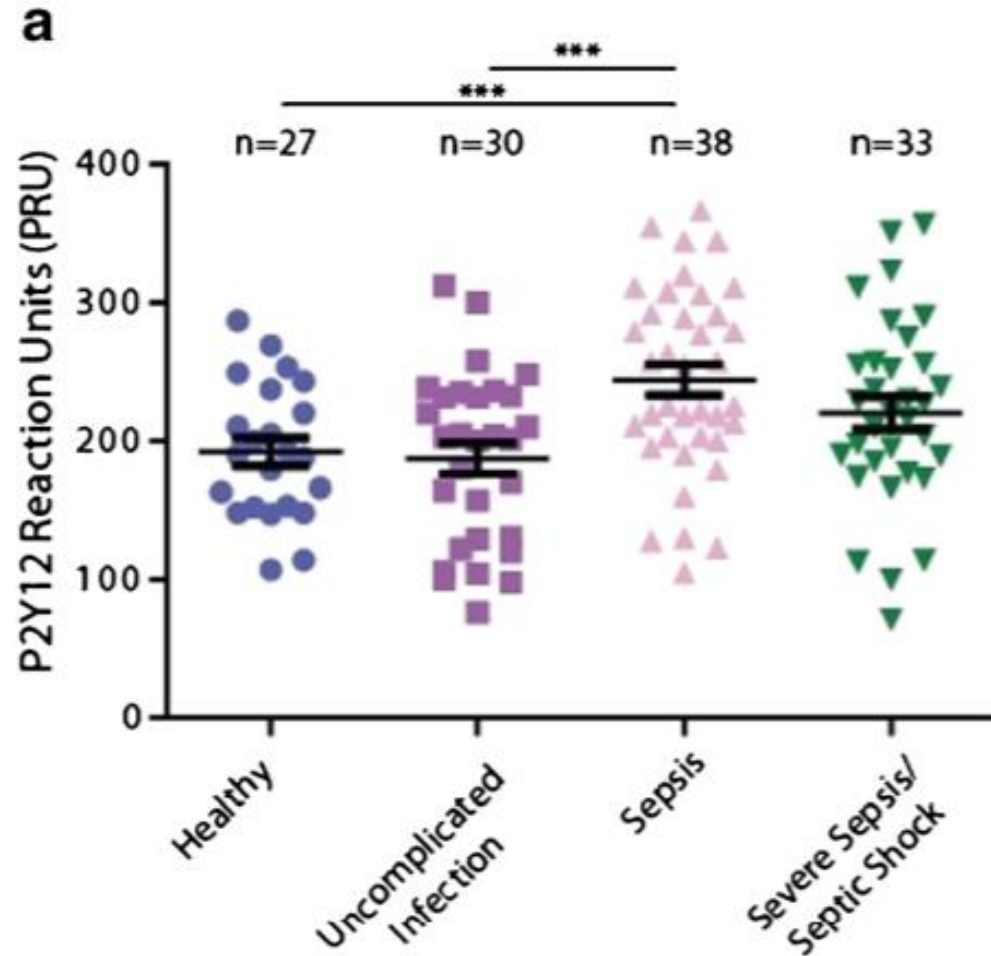
3. Clinical significance...

A quick reminder on platelets and platelet receptors

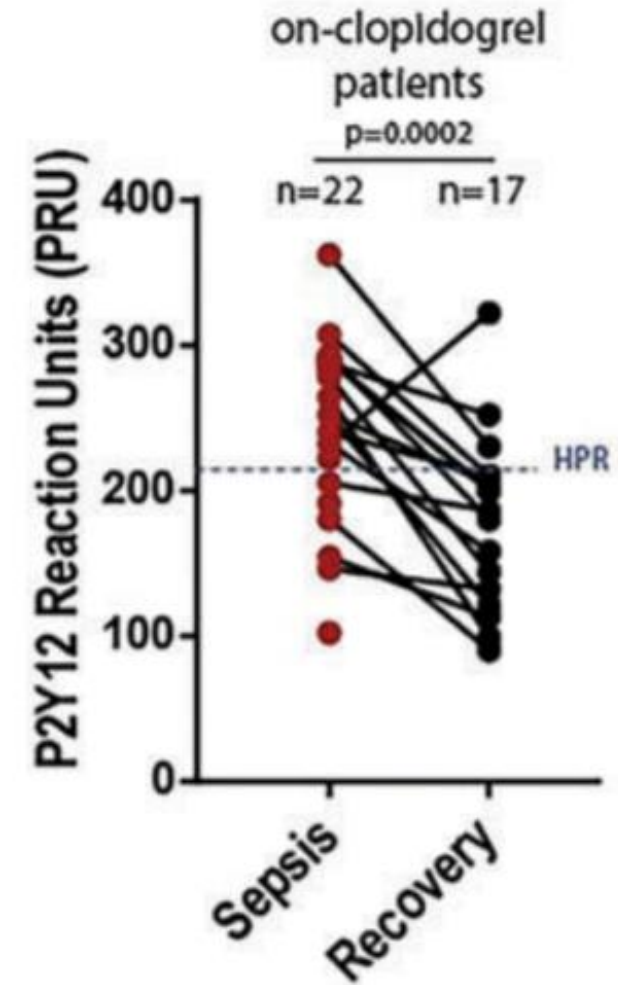
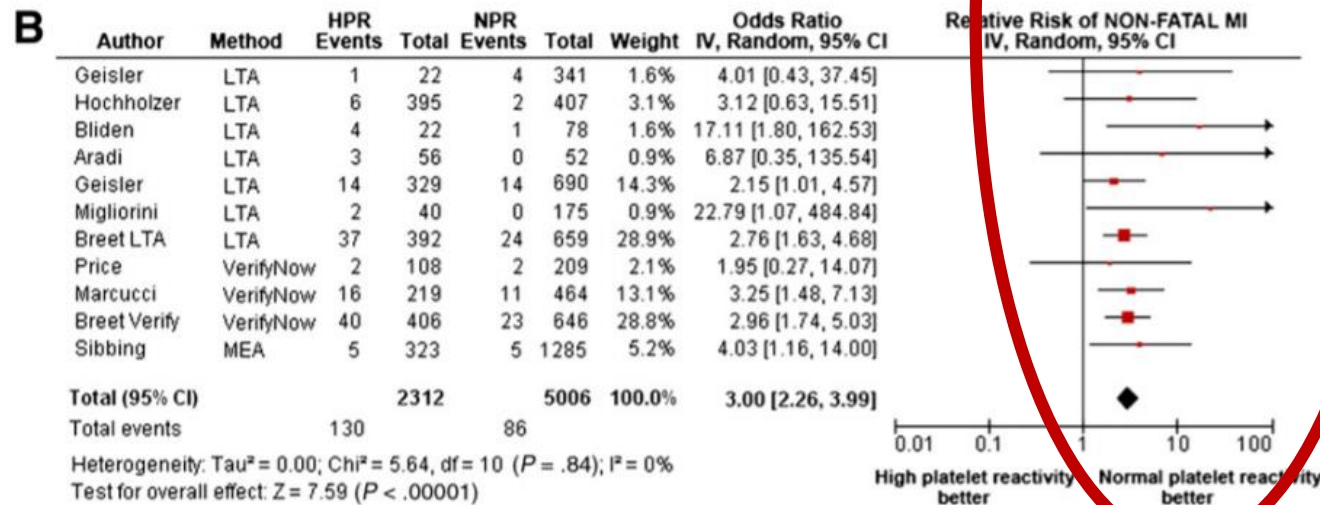
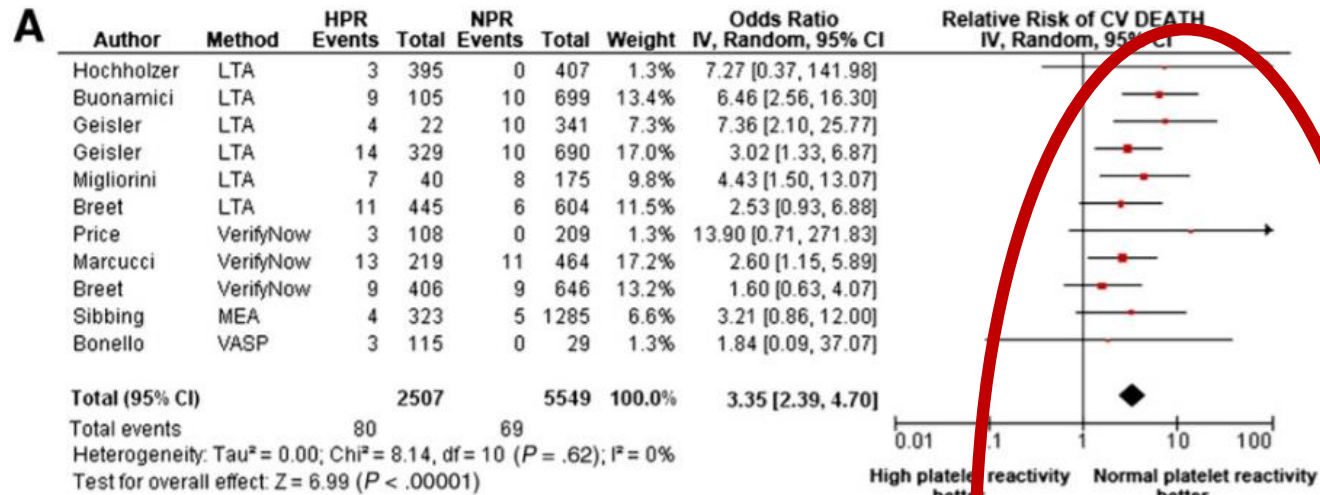


ADP and TxA2 stimulates amplification of platelet activation response

P2Y12 mediated platelet reactivity significantly and reversibly increases during sepsis



High on clopidogrel platelet reactivity increased risk of CV death and MI



Sepsis favors high-on-clopidogrel platelet reactivity

Sepsis stage uniquely accounts for 15.5% of the variance of platelet reactivity

Variable	B (95% CI)	Standard error	Beta	p-value	% of variance uniquely explained by each predictor
Constant	191.692 (129.515 to 253.868)	31.403		<0.000	
Male gender	-22.963 (-42.999 to -2.927)	10.119	-0.174	0.025	2.8%
WBC count (K/ μ l)	-1.929 (-3.353 to -0.506)	0.719	-0.237	0.008	4.0%
Hb (g/dL)	-4.385 (-6.932 to -1.838)	1.286	-0.261	0.001	6.4%
SAPS II	-0.694 (-1.373 to -0.014)	0.343	-0.180	0.046	2.3%
TNF- α (pg/ml)	-0.020 (-0.038 to -0.002)	0.009	-0.167	0.030	2.7%
Sepsis stage	41.277(25.892 to 56.663)	7.771	0.507	0.000	15.5%
PLT (per 50,000 K/ μ l)	7.195(1.315 to 13.074)	2.969	0.198	0.017	3.24%

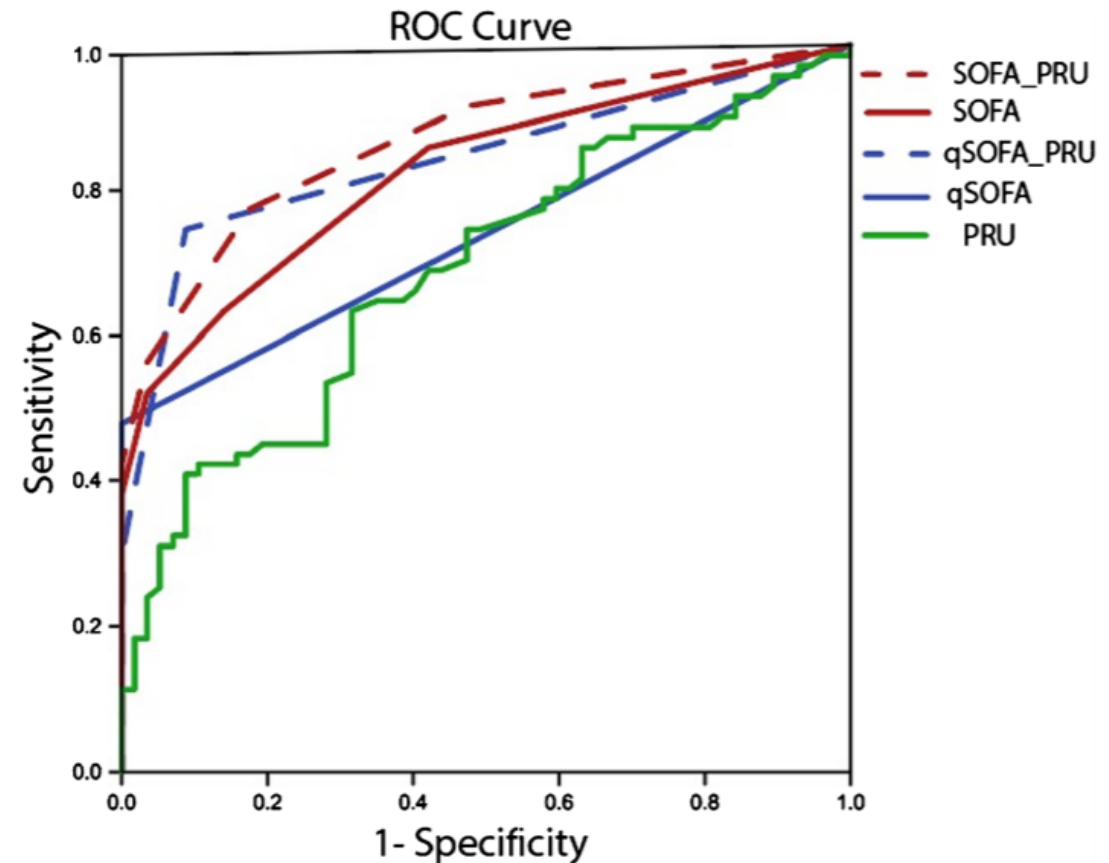
Hb hemoglobin, *TNF* tumor necrosis factor, *SAPS* simplified acute physiology score, *WBC* white blood cells, *PLT* platelets, *CI* confidence interval

Platelet reactivity as diagnostic marker for sepsis

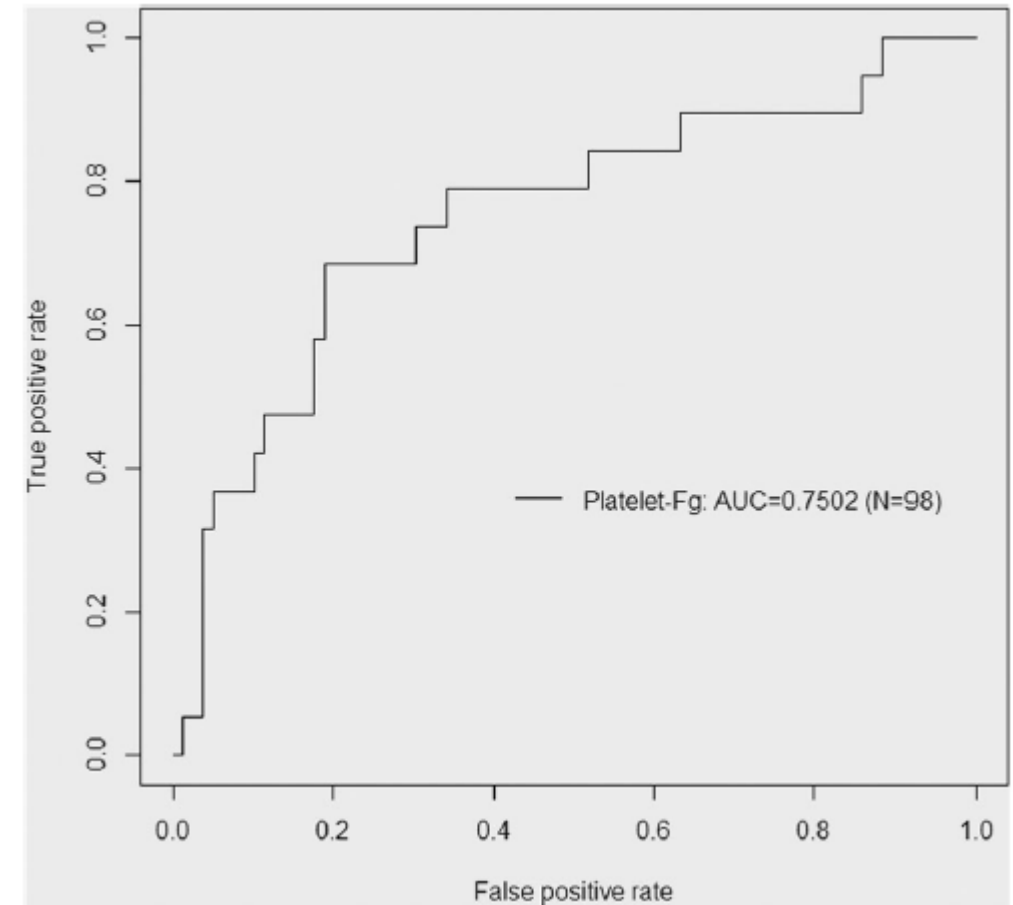
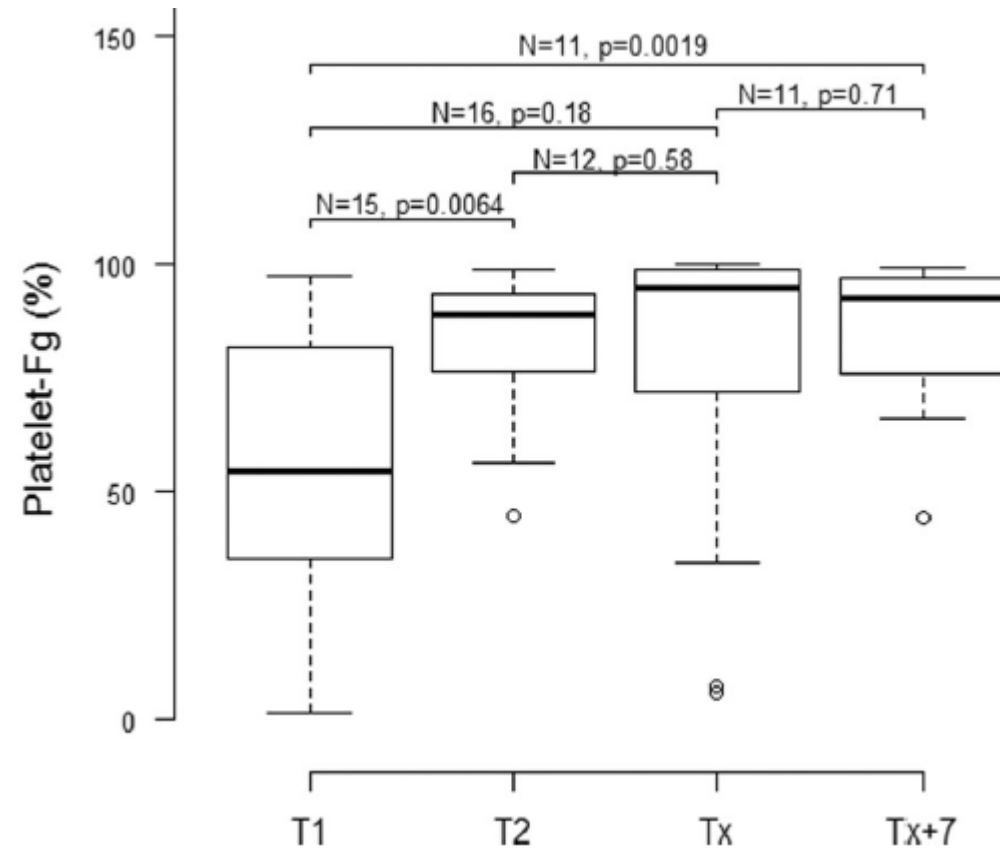
Variable	AUC (95% CIs)	Cut-off	Sensitivity (%)	Specificity (%)
PRU	0.683 (0.595–0.762)	>253	40.8	91.2
IL-6	0.784 (0.703–0.852)	>24.9	77.5	70.2
WBC	0.794 (0.713–0.860)	>9	80.3	70.2
CRP	0.861 (0.789–0.916)	>4	81.7	77.2
APACHE II	0.879 (0.810–0.930)	>6	74.6	87.7
SAPS II	0.840 (0.765–0.899)	>13	85.9	66.7
SOFA	0.824 (0.747–0.886)	>1	63.4	86.0
SOFA-PRU ^a	0.867 (0.796–0.921)	>1	77.5	82.5
qSOFA	0.739 (0.654–0.813)	>0	47.9	100
qSOFA-PRU ^{**}	0.842 (0.767–0.901)	>0	74.6	91.2

^a One point added to SOFA score if PRU > 253

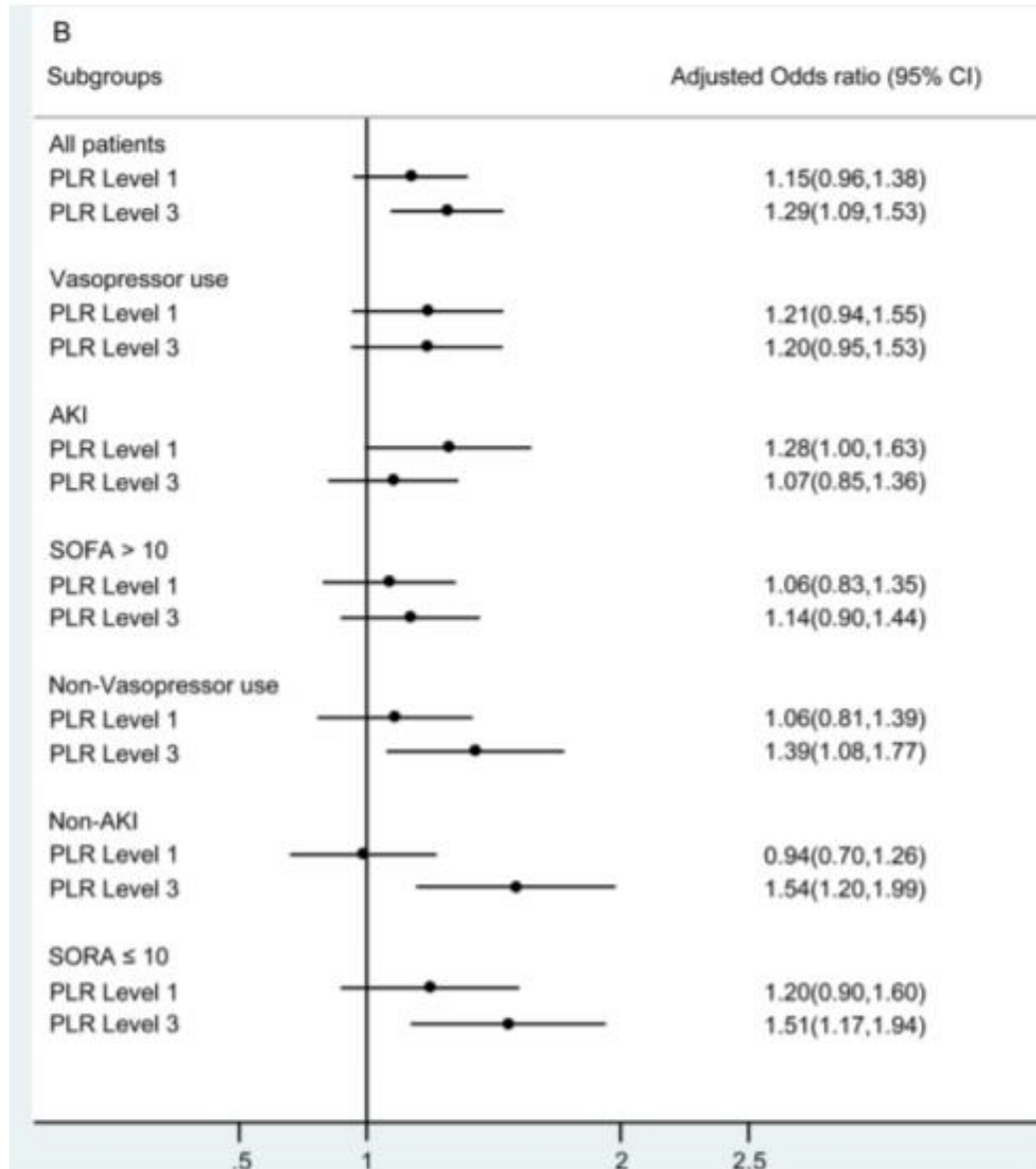
^b One point added to qSOFA score if PRU > 253



Platelet-bound fibrinogen levels help identify critically ill patients at risk of developing sepsis



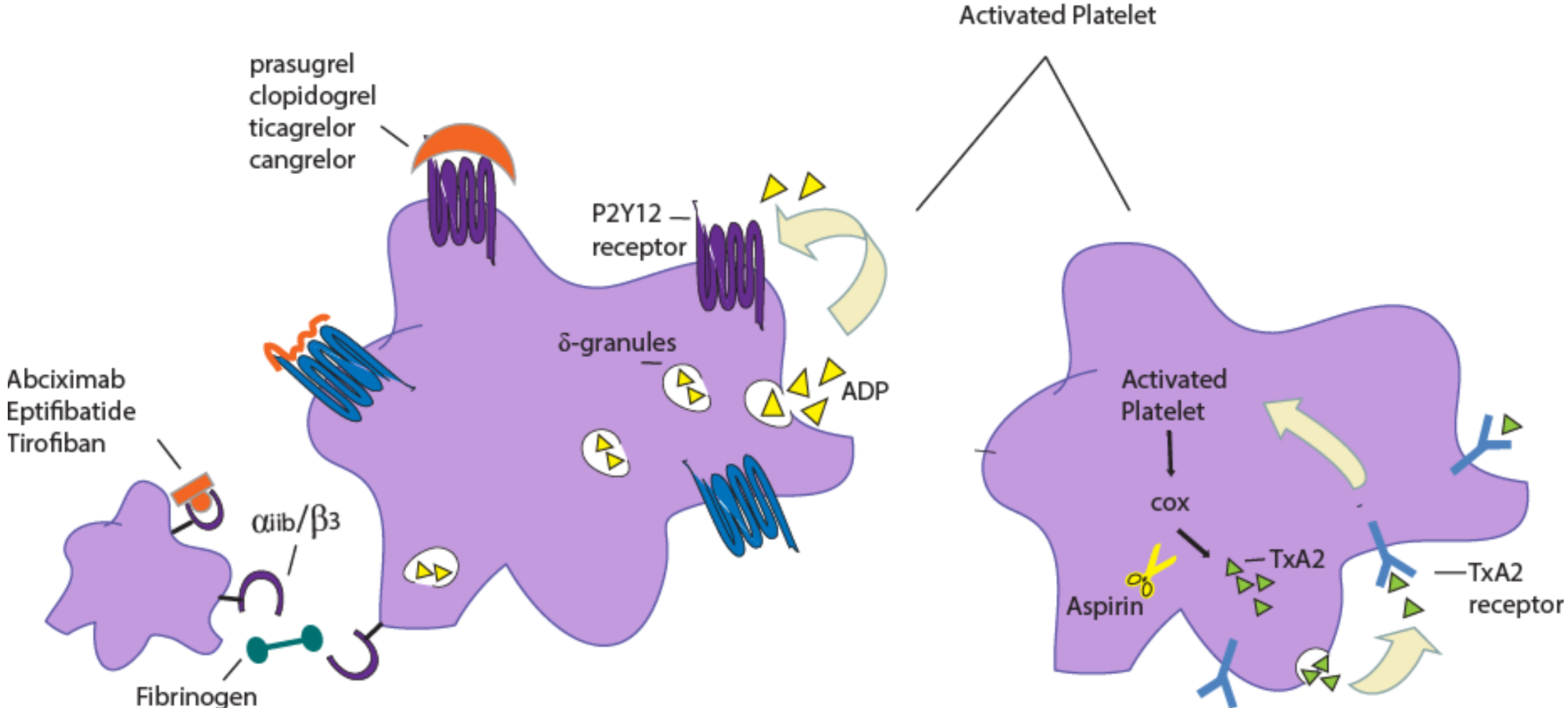
Platelet to lymphocyte ratio as a prognostic predictor of mortality for sepsis



The association between high PLR and mortality was significant in the subgroups without vasopressor use (OR 1.39; 95%CI 1.08 to 1.77) and AKI (OR 1.54; 95%CI 1.20 to 1.99) and with a SOFA score ≤ 10 (OR 1.51; 95%CI 1.17 to 1.94)

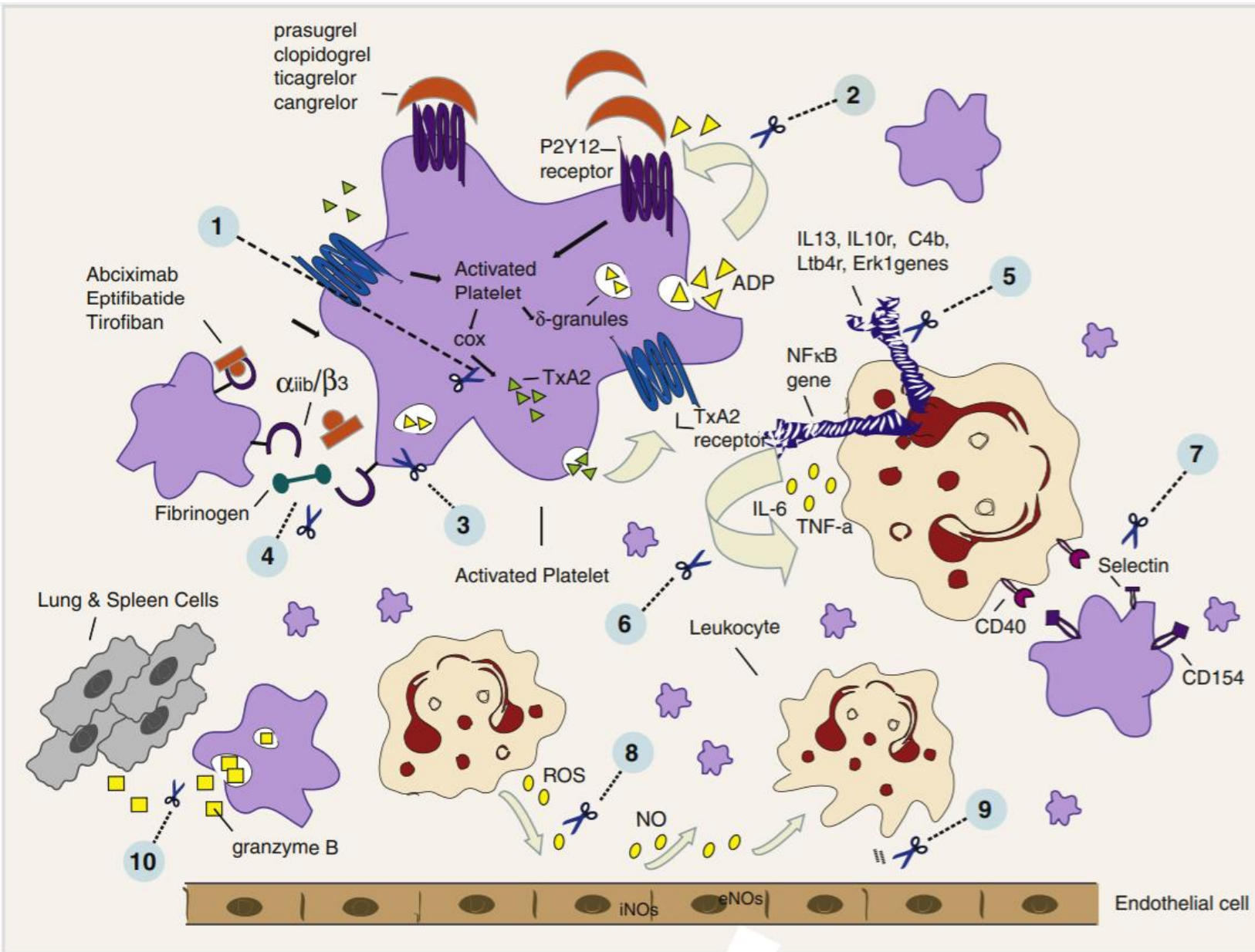
4. ANTI-platelets...

A quick reminder on platelets and platelet receptors



ADP and TxA2 stimulates amplification of platelet activation response

Antiplatelets is sepsis

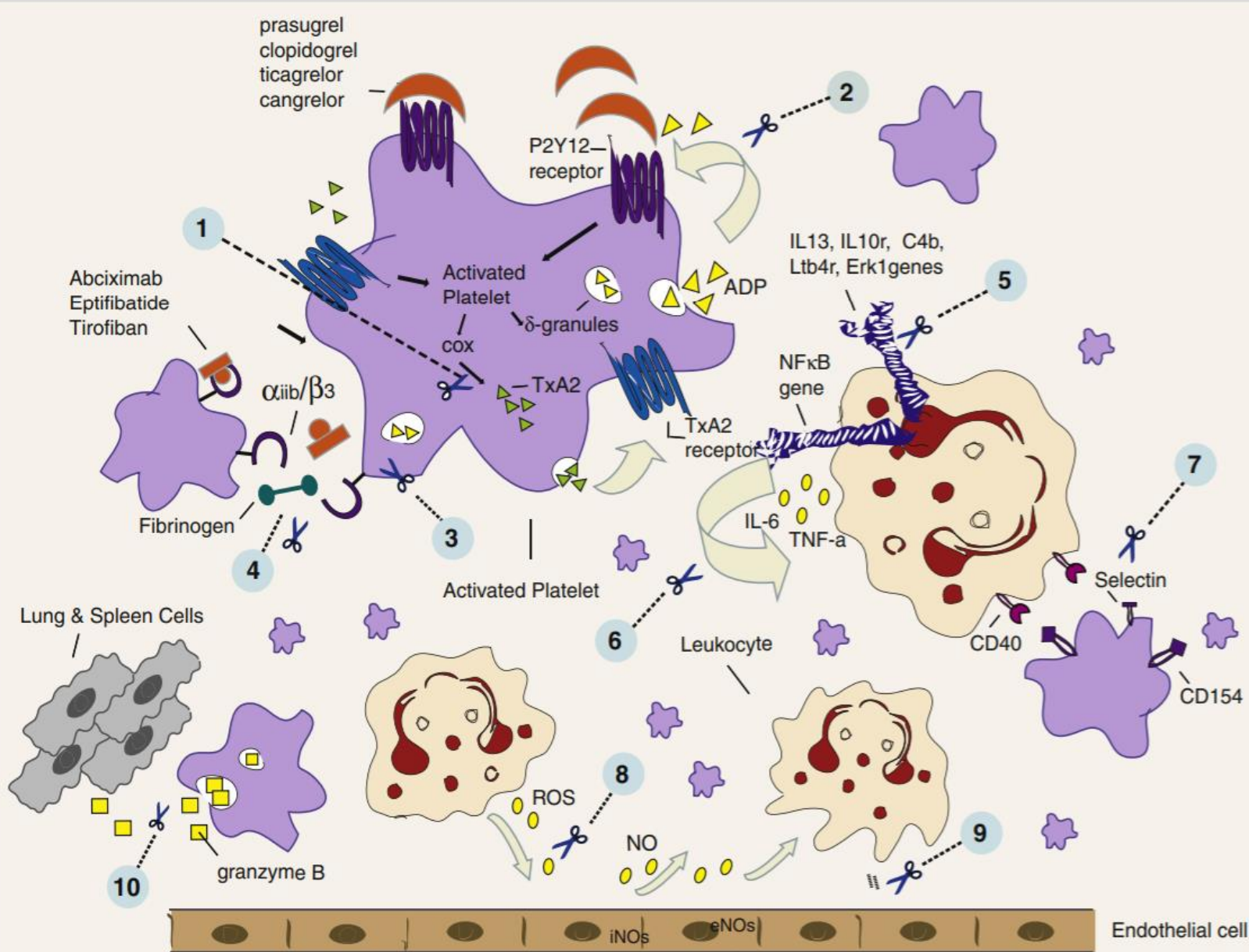


(1,2) Amplification of platelet activation response hampered by ASA and P2Y12 inhibitors, through blockade of TxA2 production and ADP binding to P2Y12 receptors respectively

(3,4) Platelet aggregation attenuated following blockade of ADP mediated GPIIb/IIIa receptor maturation by P2Y12 inhibitors and prevention of aggregate formation by GPIIb/IIIa antagonist administration

(5,6) ASA and clopidogrel down regulate expression of pro inflammatory mediators

Antiplatelets is sepsis



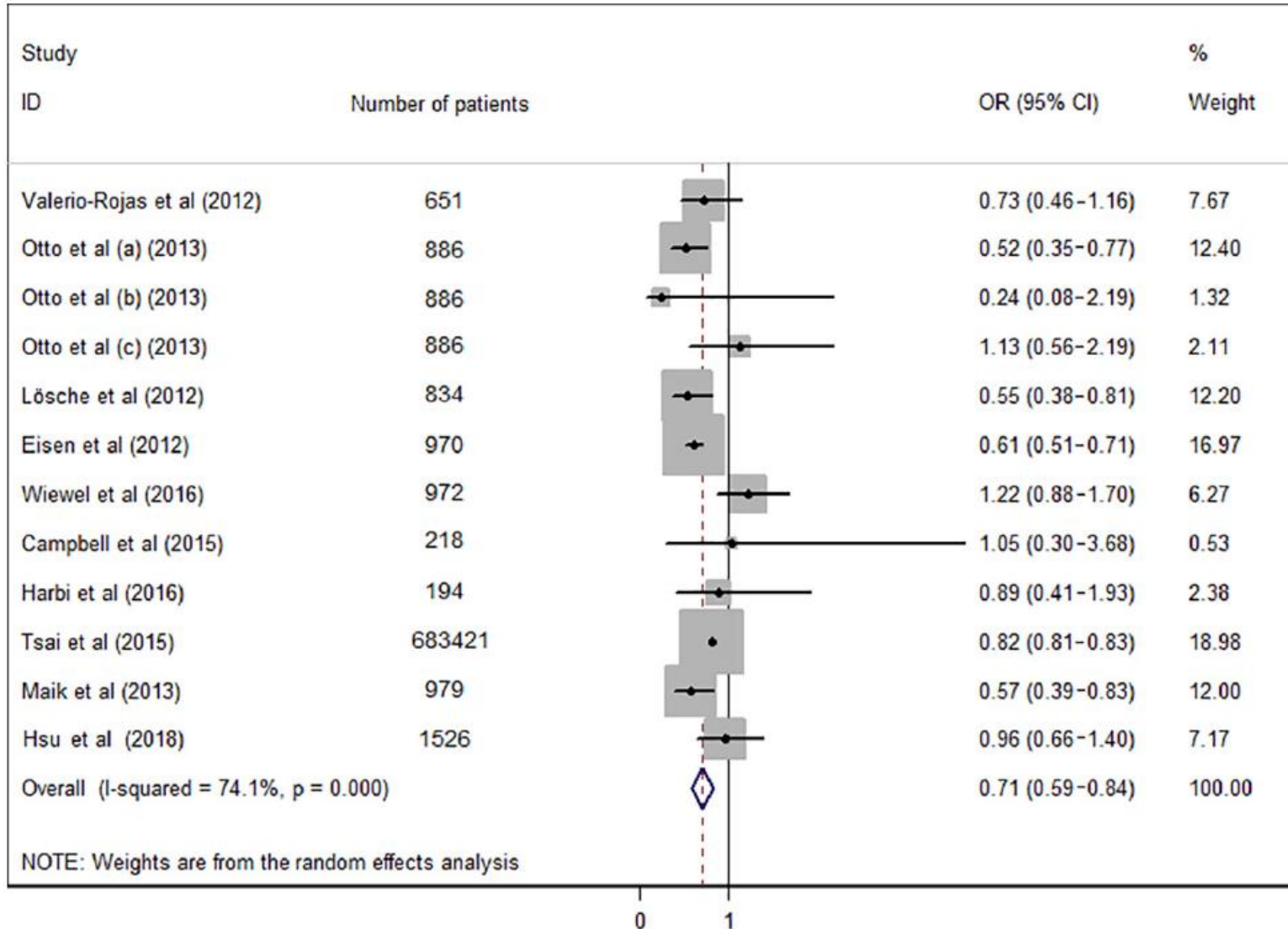
(7) ASA and P2Y12 inhibitors alter platelet-leukocyte interactions through decreased expression of cell ligands

(8) P2Y12 inhibitors attenuate endothelial damage caused by neutrophil ROS production.

(9) ASA promotes anti-adhesive NO production preventing leukocyte tethering, adhesion and emigration.

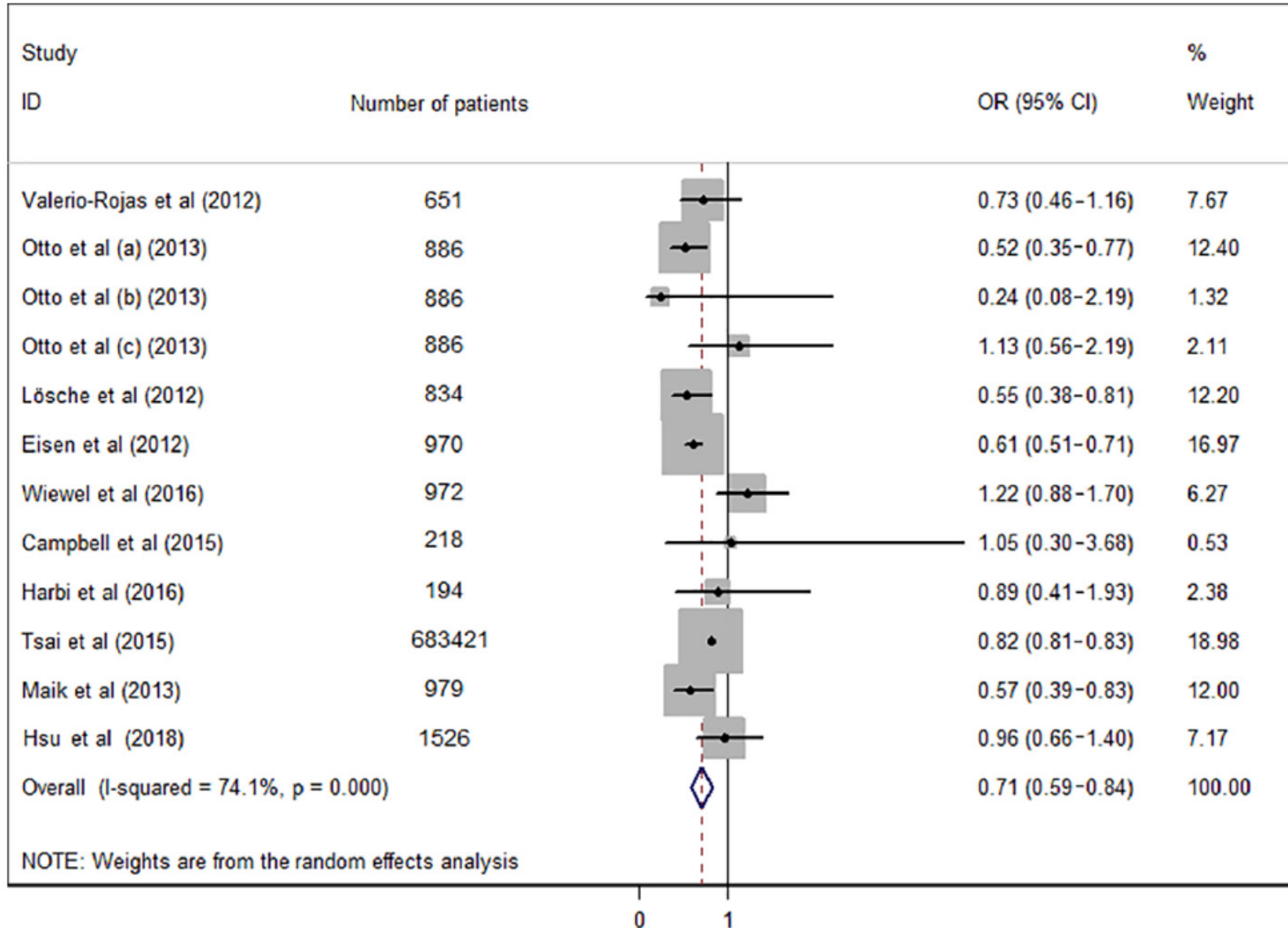
(10) GPIIb/IIIa antagonists to decrease platelet mediated cytotoxicity caused by granzyme-B secretion

The effect of antiplatelet therapy on the mortality rate of patients with sepsis



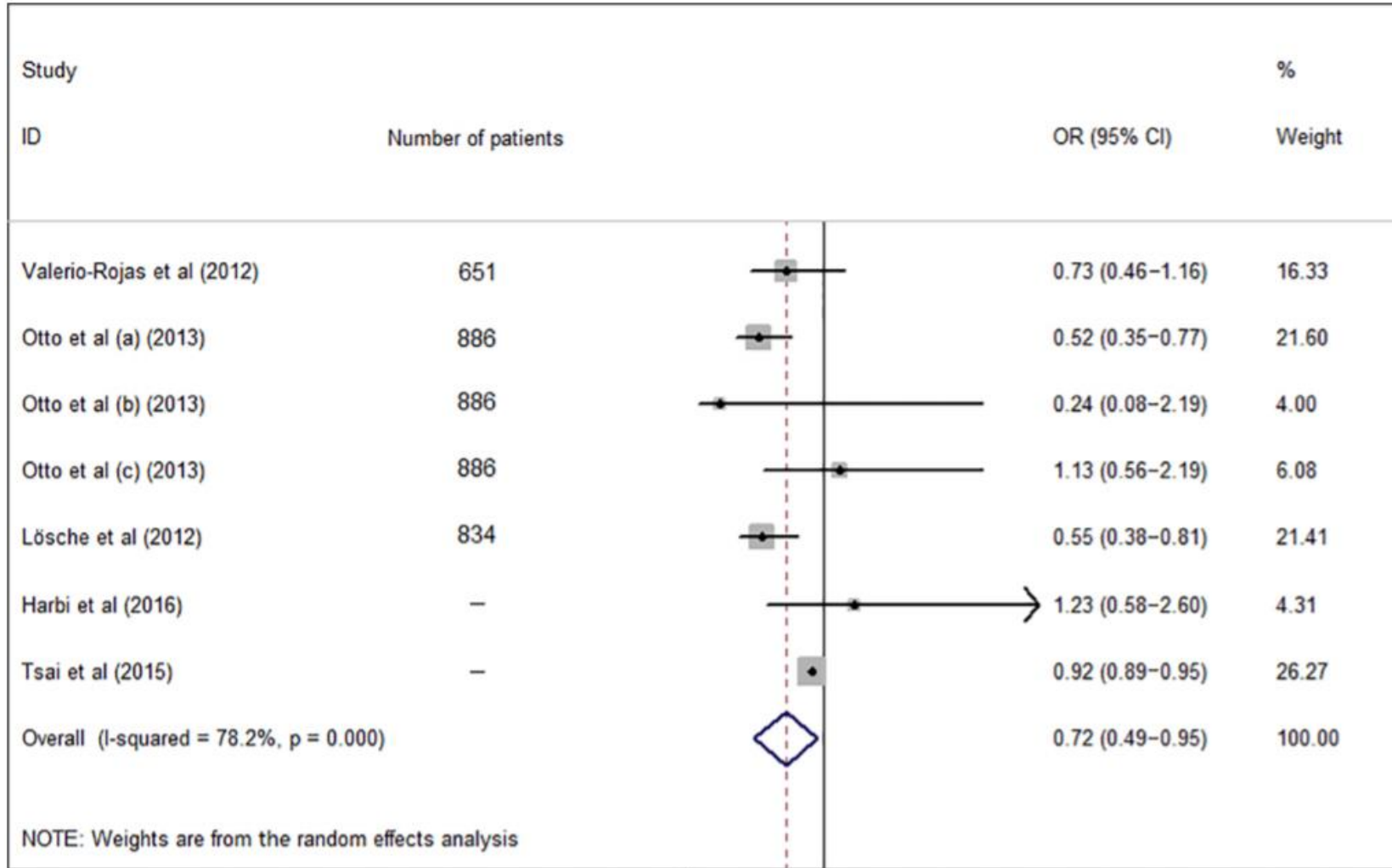
The use of antiplatelet drugs can effectively reduce the mortality of patients with sepsis (OR=0.82, 95% CI: 0.81–0.83, p < 0.05)

The effect of aspirin on the mortality rate of patients with sepsis



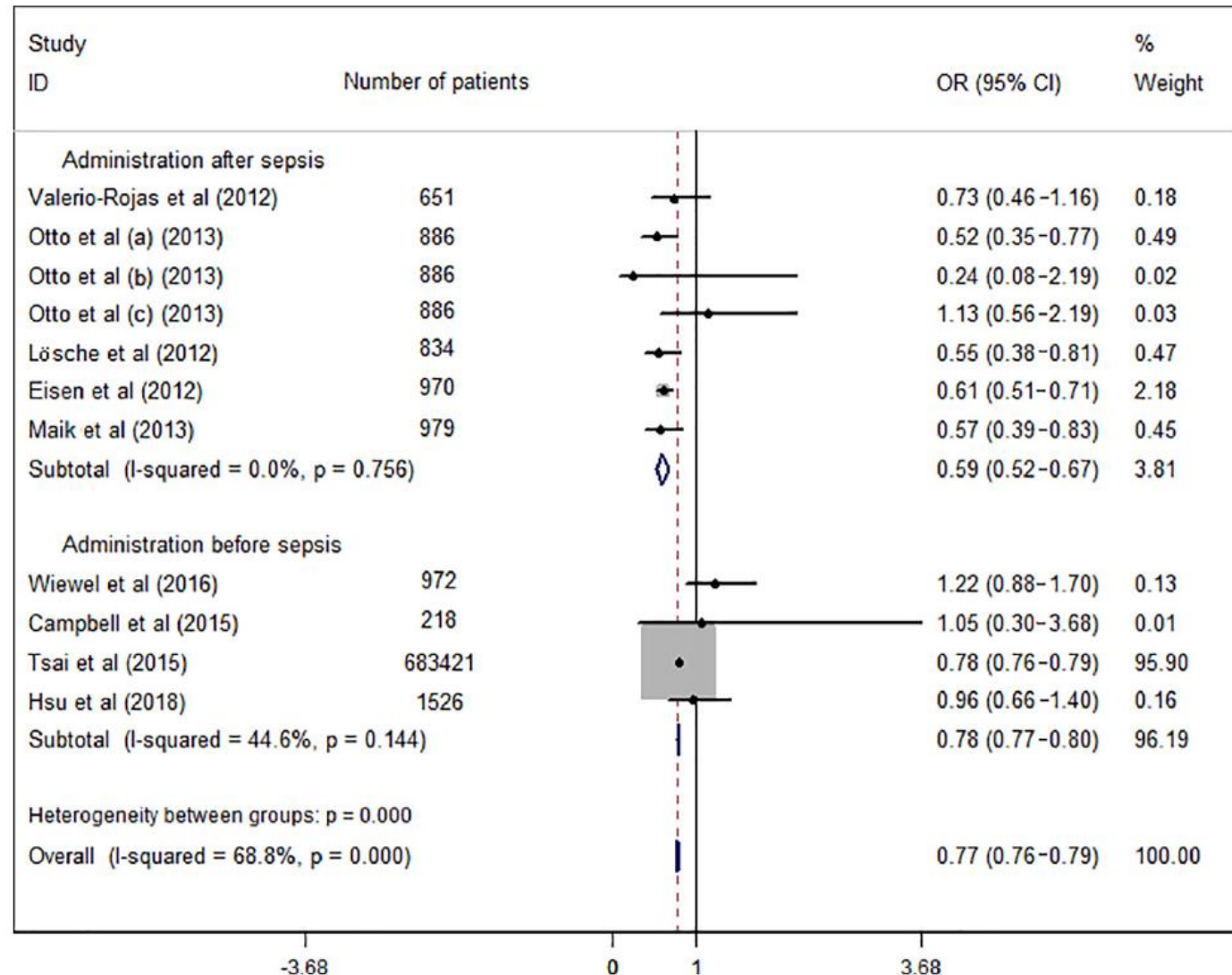
Aspirin effectively reduced mortality in patients with sepsis (OR =0.60, 95% CI: 0.53–0.68, p<0.05)

The effect of aspirin on the mortality rate of patients with sever sepsis & septic shock



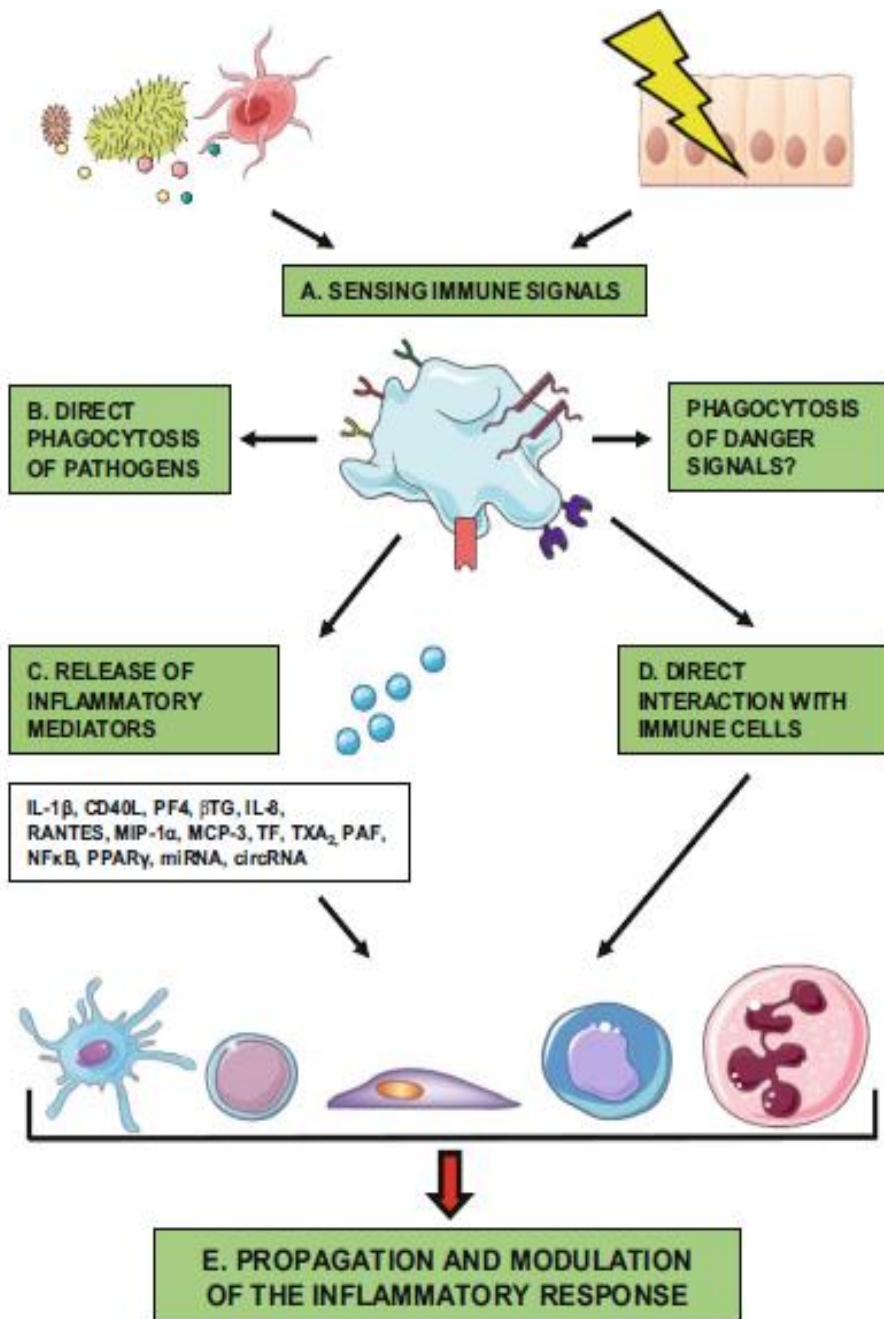
Aspirin effectively reduced mortality in patients with severe sepsis and septic shock (OR =0.72, 95% CI: 0.49–0.95, p<0.05)

Effect of timing of antiplatelet therapy on the mortality rate of patients with sepsis

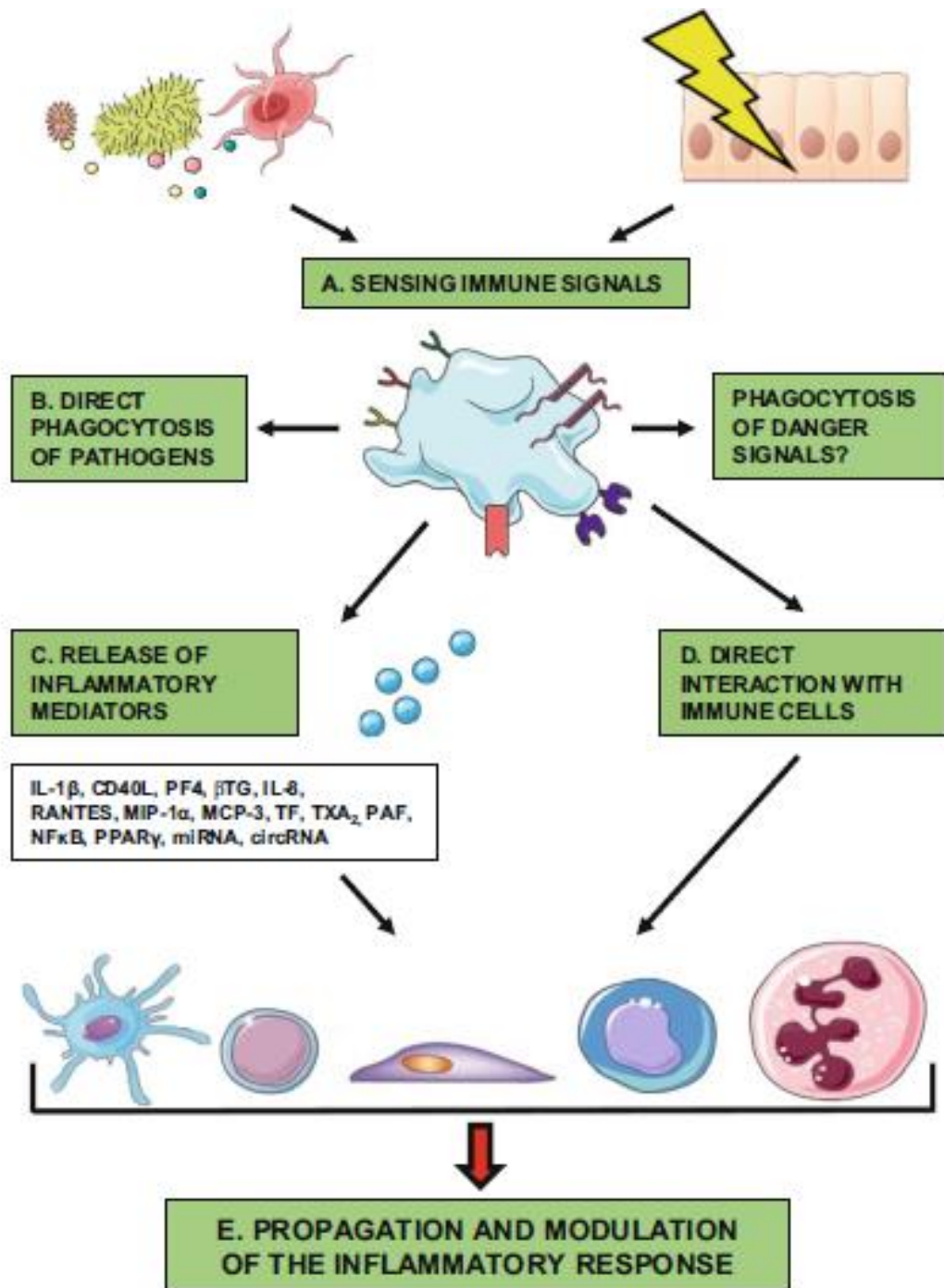


Antiplatelet drugs can reduce mortality when administered either before (OR = 0.78, 95% CI: 0.77–0.80) or after sepsis (OR = 0.59, 95% CI: 0.52–0.67)

5. To cut the long story short...



- PLTS are targeted to sites of infection → **directly interact with microbes** (cluster, encapsulate, & facilitate pathogen clearance)
- Direct contact of PLTs with bacteria, viruses, & parasites **may induce phagocytosis of pathogen** by PLTs (incl. Staph, HIV, influenza, dengue, HCV, P. vivax, Toxoplasma)
- **PLTs produce several bactericidal and fungicidal proteins and peptides**, defensins, thrombocidins (thrombin-induced platelet microbicidal proteins), and kinocidins (chemokines with microbicidal activity) allowing direct pathogen killing.
- PLTs contribute to elimination of pathogens also indirectly by **interaction with other immune cells** promoting their inflammatory response to pathogens.

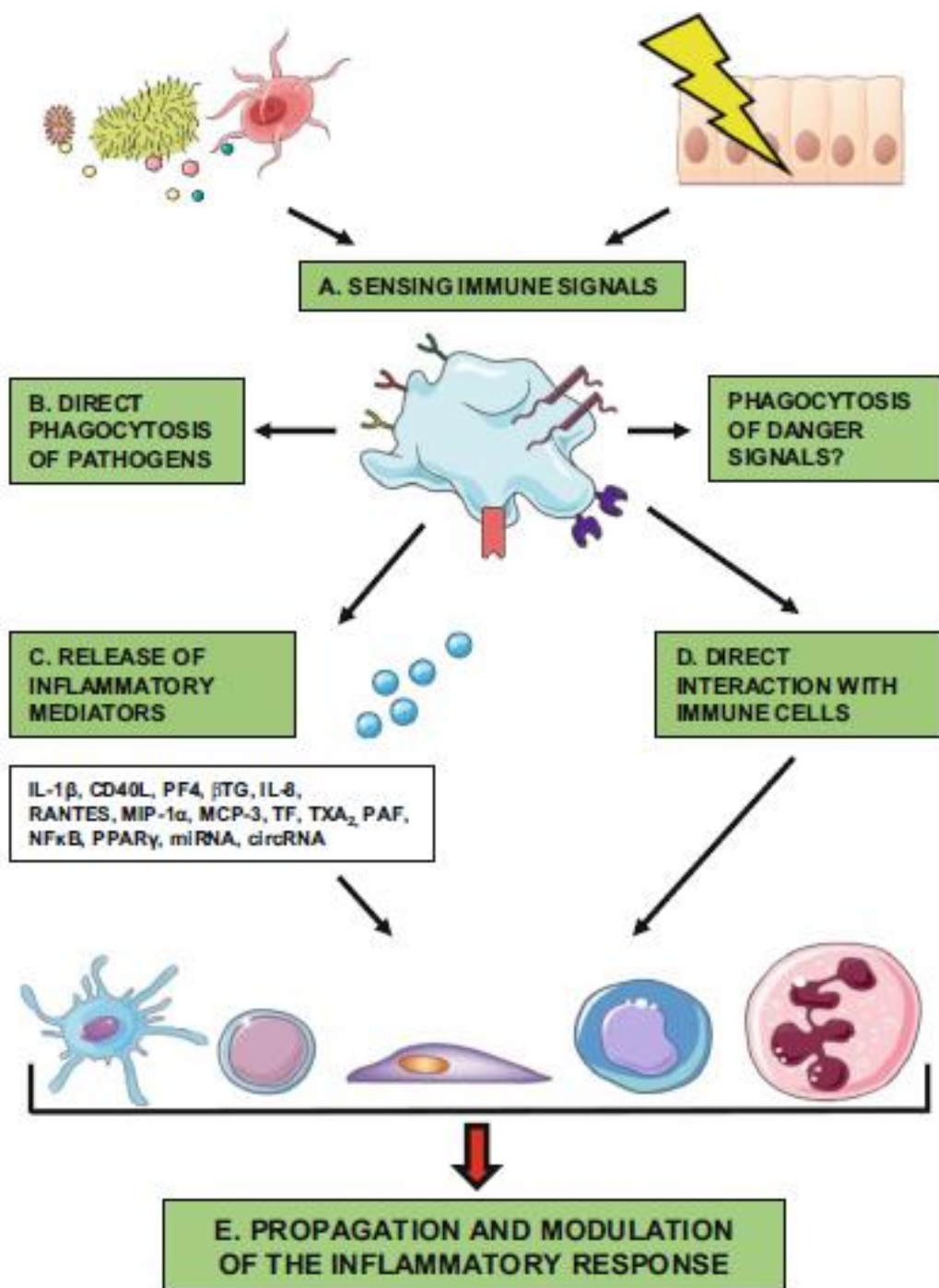


- PLTs-NEU interaction in bacterial and viral infections results in **NET formation and entrapment of the microorganisms.**

Integrins involved are LFA-1 in bacterial and Mac-1 in viral infection.

- PLTs were identified to be an important **surveillance mechanism to resident liver macrophages** (Kupffer cells) during blood-borne infection.

- PLT were found to be indispensable in host defense; however in some infections, like HIV or Streptococcus pyogenes, **platelets were reported to augment dissemination of infection.**



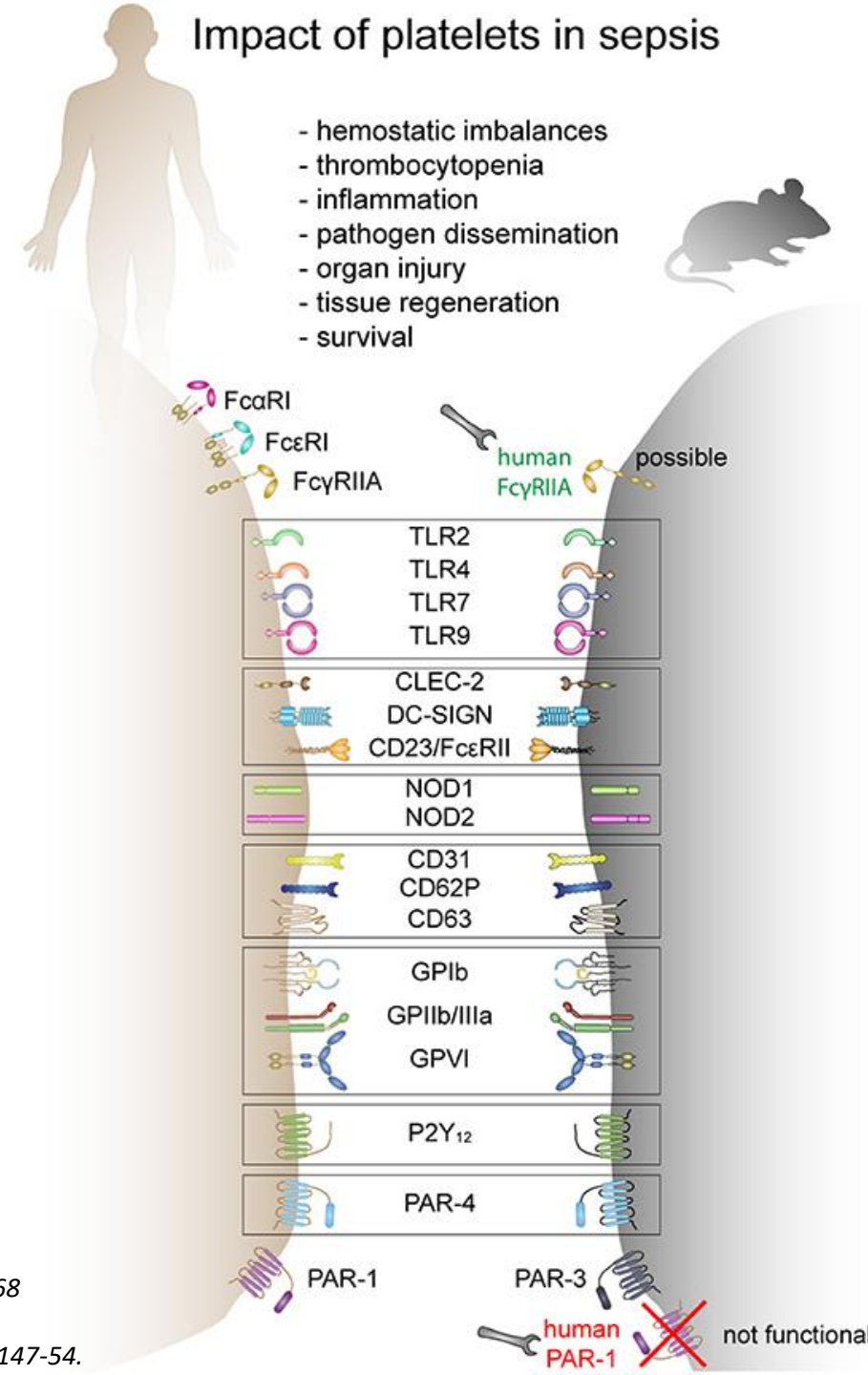
Platelets in Adaptive Immunity

- Platelets have been shown to be involved in T cell responses, to induce B cell isotype switch and dendritic cell maturation.
- Some of the mediators secreted by platelets, such as IL-1 β , CD40L, PF4, RANTES, and TXA₂, create a **direct link between innate and adaptive immunity.**
- Platelets are involved in **bidirectional signaling and direct cell interactions** with adaptive immune cells.
- Platelets were shown to **process and present antigen** in MHC class I and directly activate naive T cells in a platelet MHC class I-dependent manner

Mind The GAP: Results vary depending on species, settings and regimen

- Favorable results in animal models but humans ?
- ASA vs other anti-platelets ? What pathway ? What dose ?
 - Clopidogrel increasing CAP incidence
 - Ticagrelor reducing infection related death in PLATO study following CABG
- Setting
 - Patients with sepsis → ASA ↑ duration of mechanical ventilation and incidence of severe sepsis
 - Patients with severe sepsis / shock → ASA ↓ duration of mechanical ventilation and incidence of ARDS

Impact of platelets in sepsis



Crit Care 2013;17:402

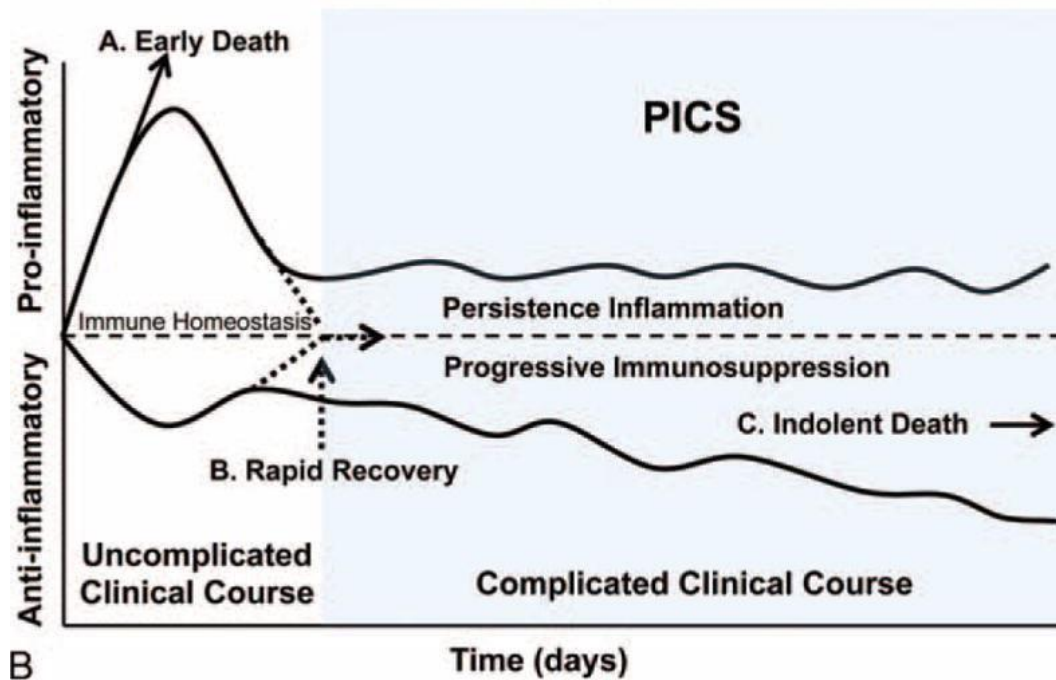
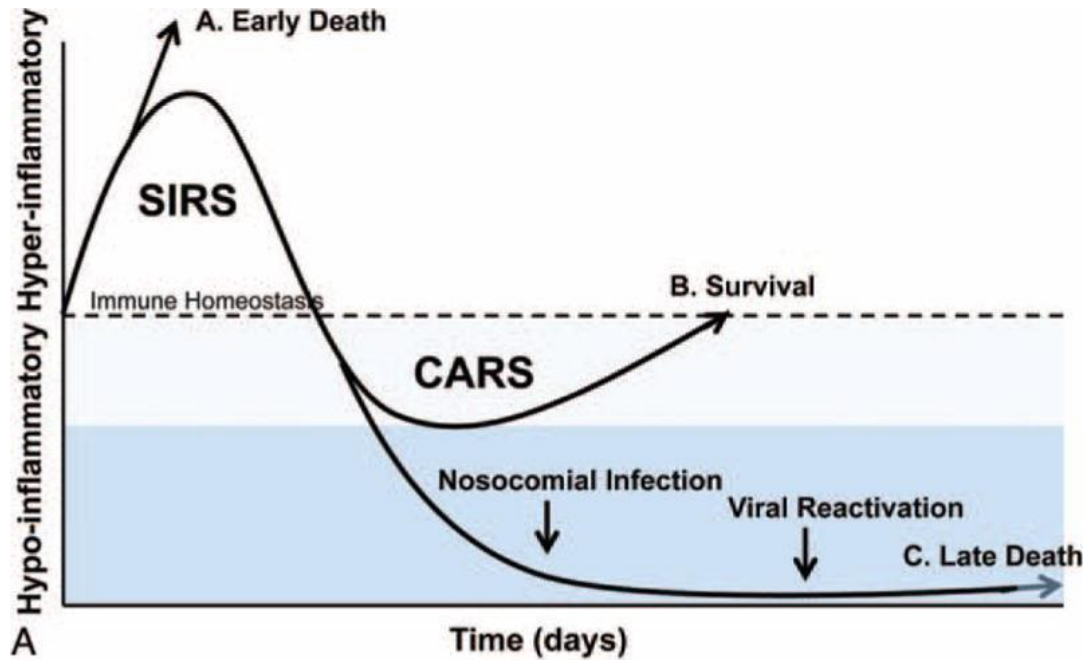
Journal of Critical Care 50 (2019) 162–168

Front Immunol. 2019 Jul 17;10:1687

J Thromb Thrombolysis. 2013 Feb;35(2):147-54.

Intervene : When and How ?

- Variation according to timing of study, location of PLTS in circulation and pathogenesis



- When anti-platelets administered as preventive therapy and stopped their impact on mortality remains unclear
- Anti-platelets did not reduce level of plasma pro-inflammatory cytokines
- Patients with high risk of bleeding excluded a priori in clinical studies



Thank you for your patience & attention