



NETs and Thromboinflammation

Teresa Padro

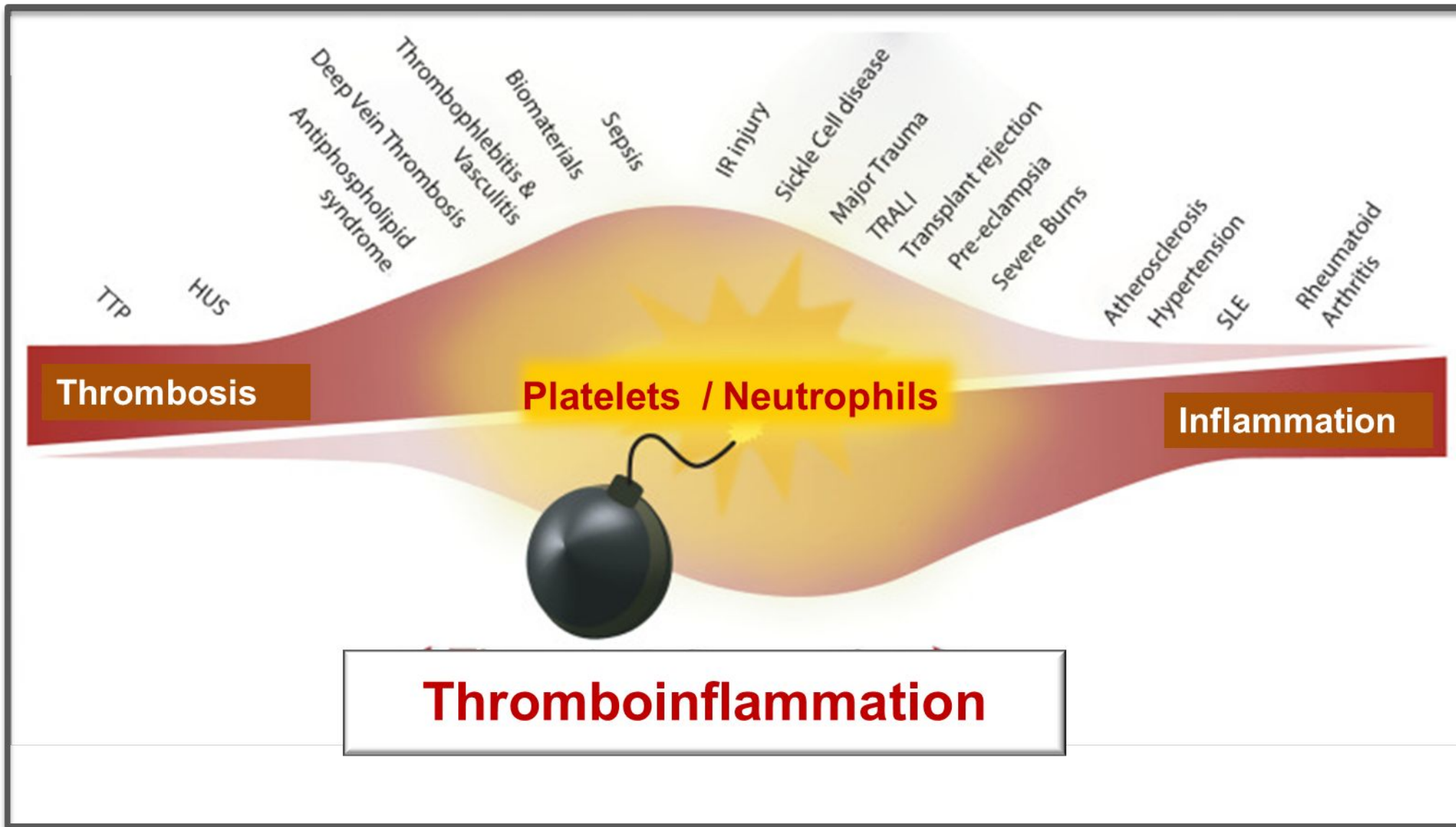
Programa ICCV-Cardiovascular, Institut de Recerca Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, CIBERCV, Barcelona, Spain.

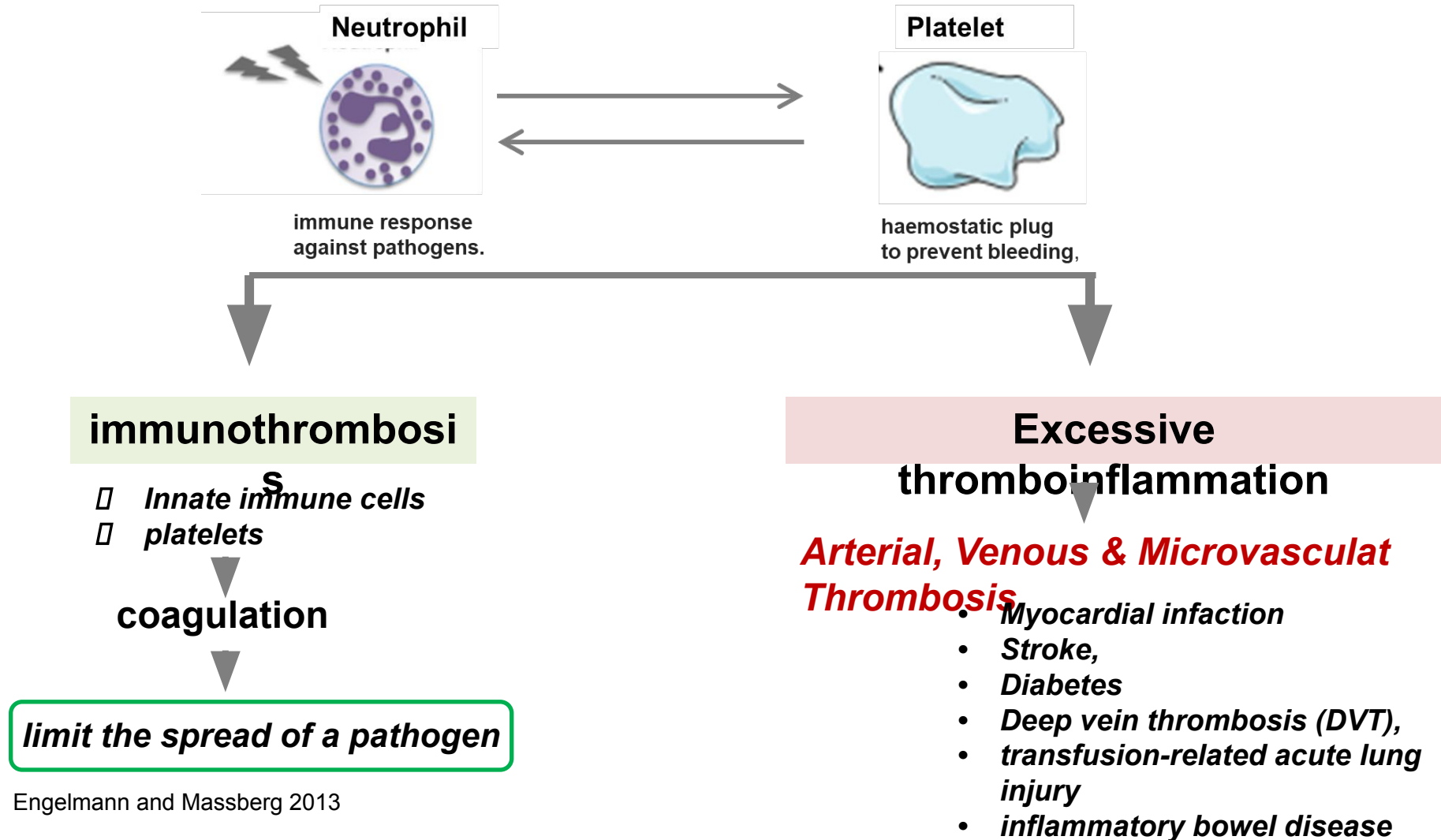




Declaration of Conflict Of Interest

I have no potential conflict of interest to report

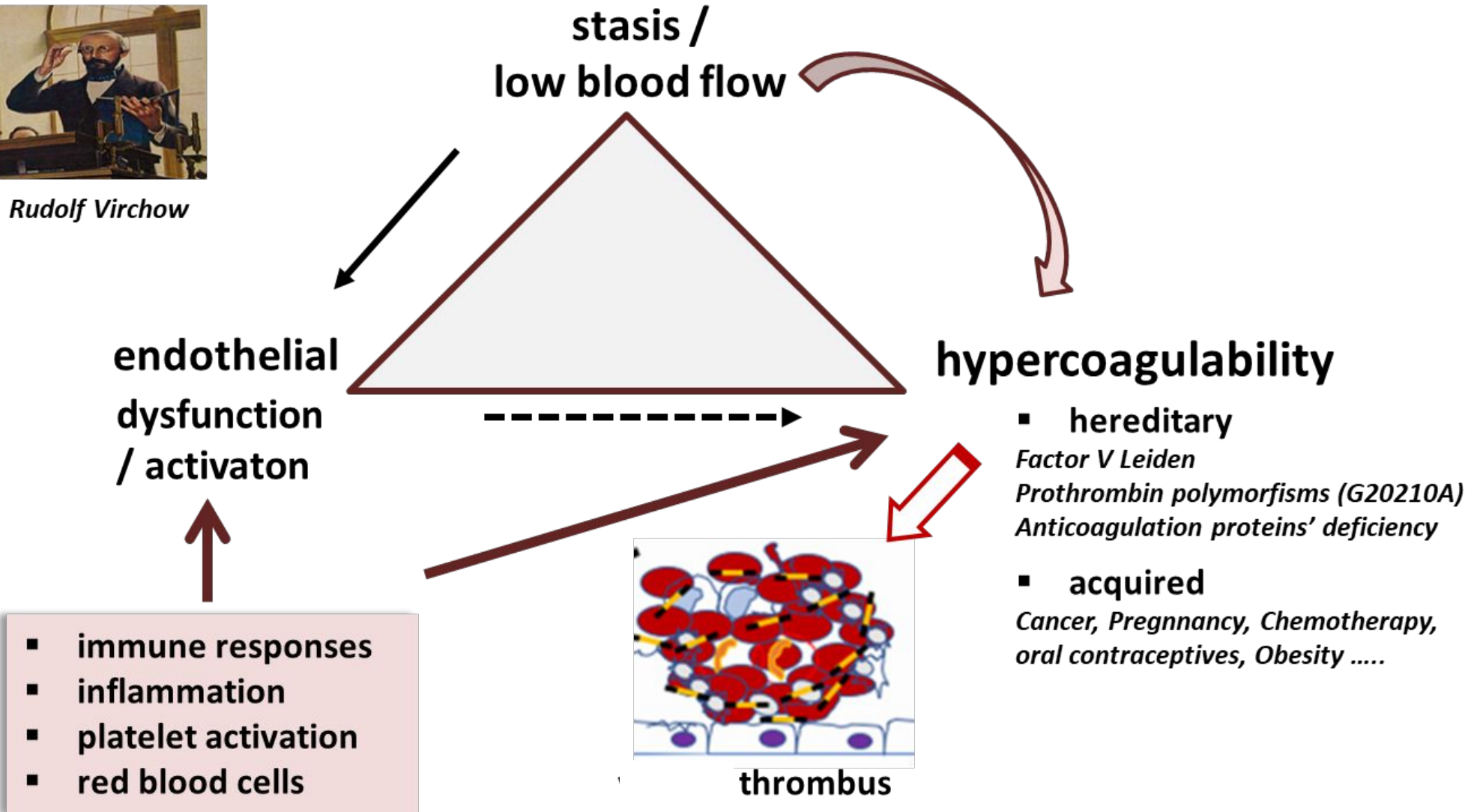




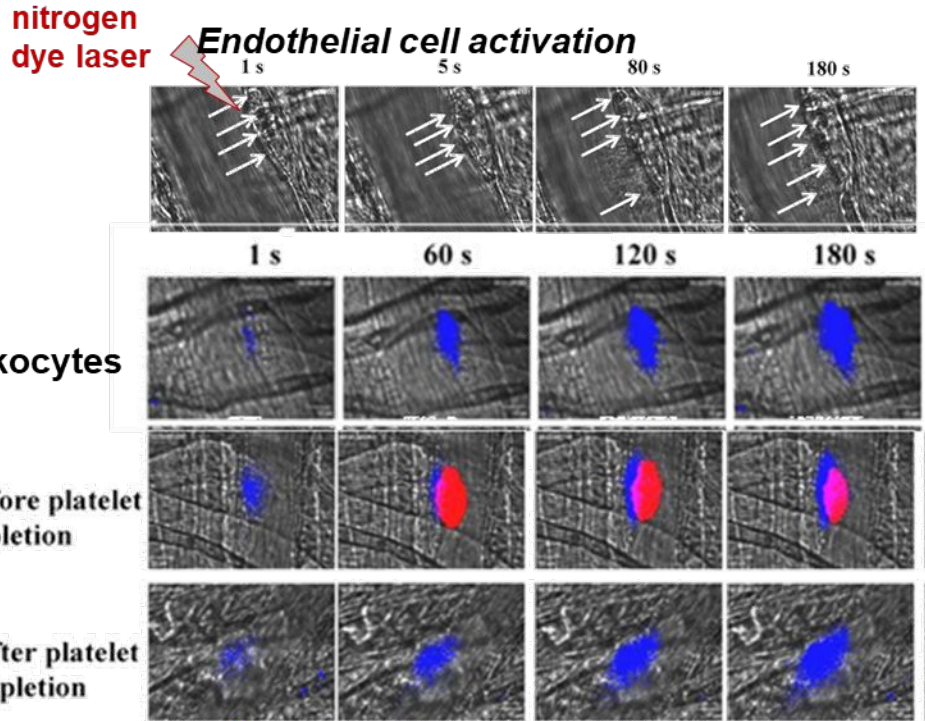
Immune dysregulation in Thrombosis



Rudolf Virchow



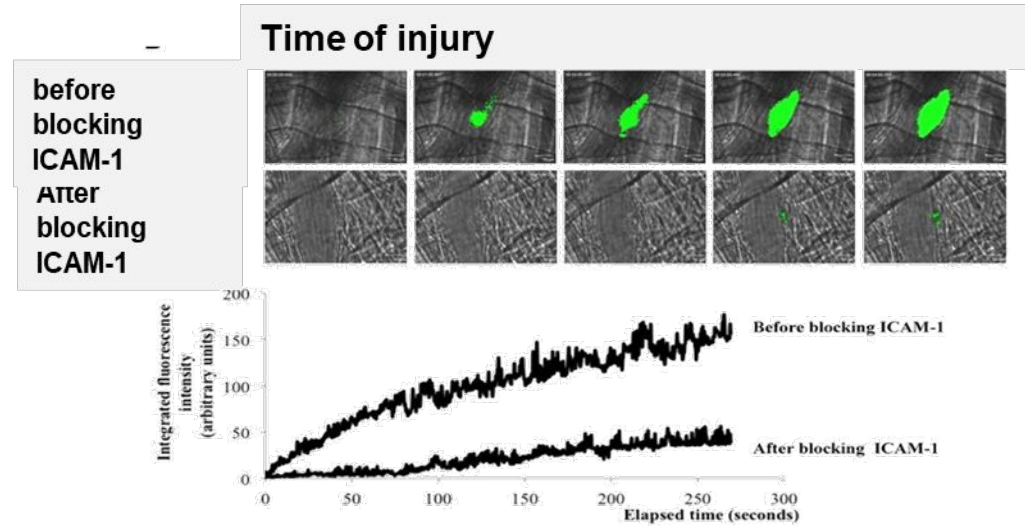
Real-time intravital microscopy Mice cremaster muscle microcirculation



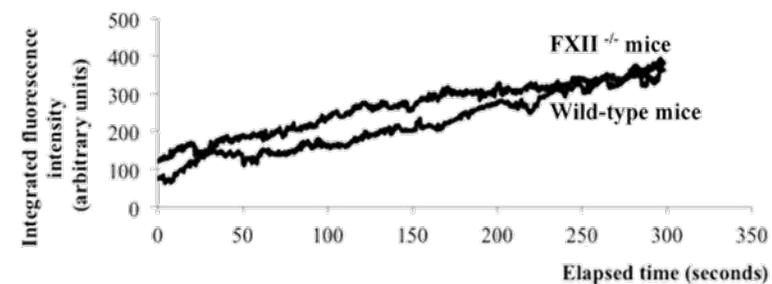
CD11b (Blue) = Leukocyte / Monocyte
CD41 (red) = platelets

NEUTROPHILS
Binding through ICAM-1

Fibrin generation in reduced in the absence of neutrophil adhesion at the site of injury



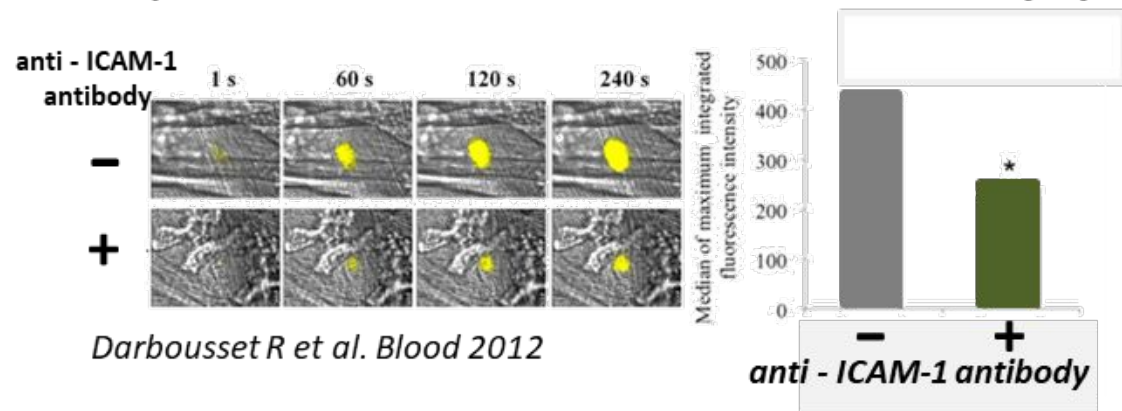
fibrin generation were similar in FXII^{-/-} mice



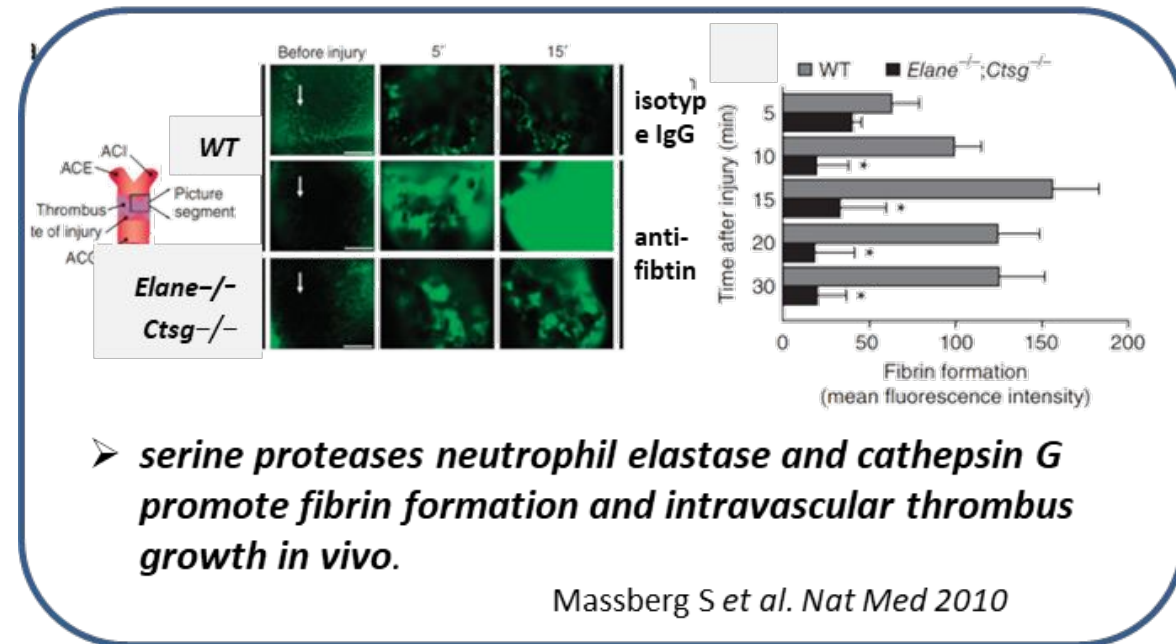
interaction of neutrophils with endothelial cells is a critical step preceding platelet accumulation for initiating thrombosis in injured microvessels.

Mice cremaster muscle microcirculation

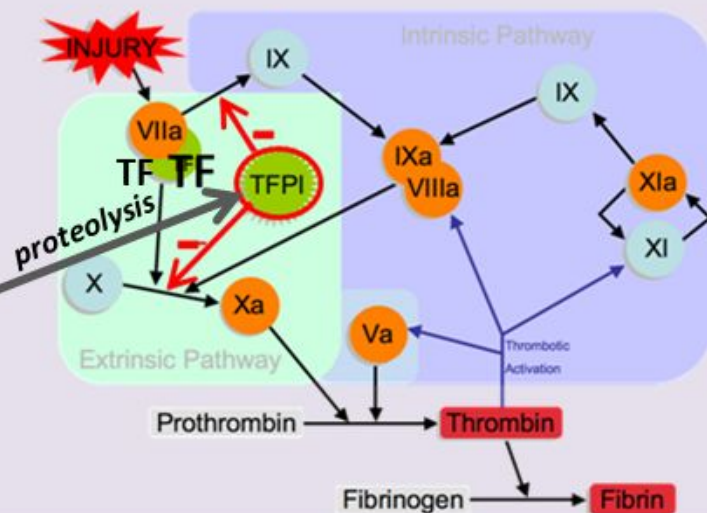
Neutrophils and TF accumulation at site of vascular injury



injured carotid arteries in mice deficient in neutrophil elastase and cathepsin G (Elane^{-/-};Ctsg^{-/-} mice)



Neutrophil



➤ *serine proteases neutrophil elastase and cathepsin G promote fibrin formation and intravascular thrombus growth in vivo.*

Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation.

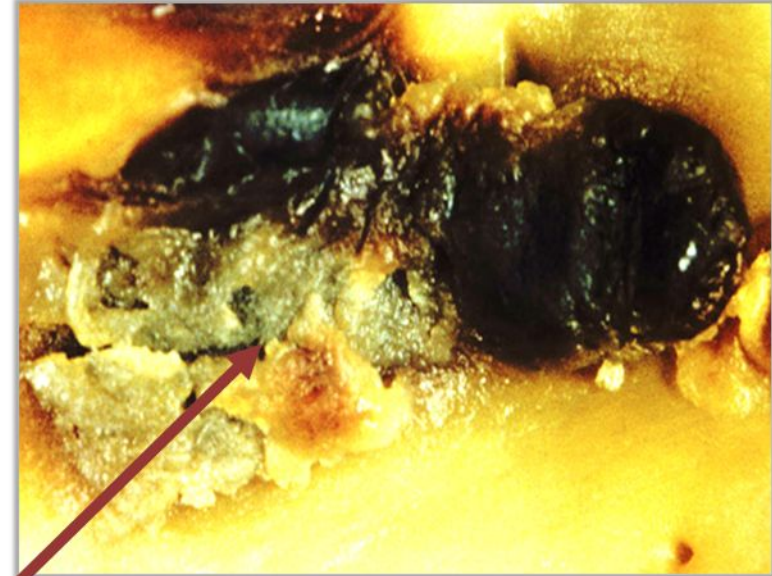
Blood innate immune cells (neutrophils and monocytes) crawling along and adhering to the venous endothelium provide the initiating stimulus venous thrombosis.

Von Brühl ML et al. J ExpMed 2012

Thrombus on atherosclerotic coronary artery plaques

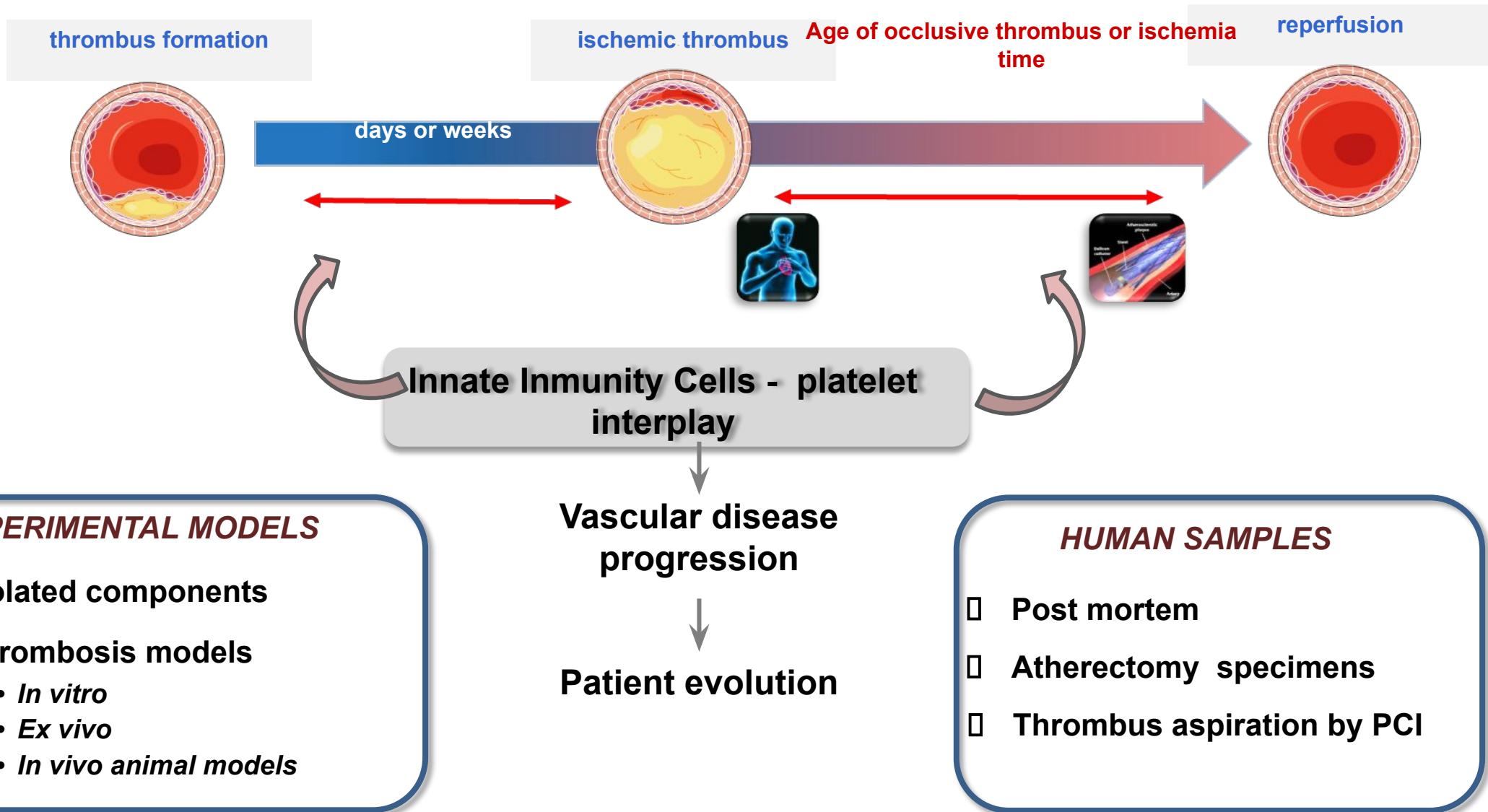
Contenders:

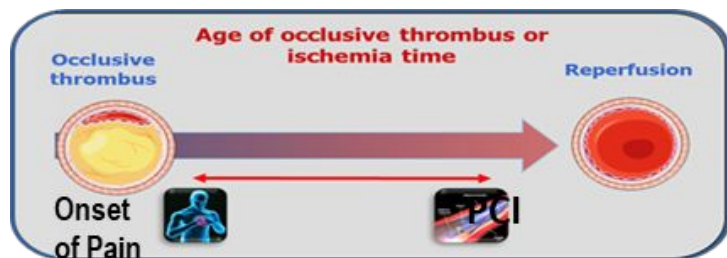
- *Fibrin*
- *Erythrocytes*
- *Platelets*
- *White blood cells*



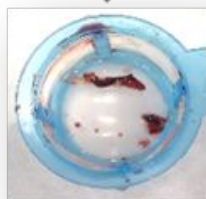
Celular and molecular composition of the thrombi influences their morphology, evolution, and tentative resolution.

Thrombus composition – Dynamic evolution

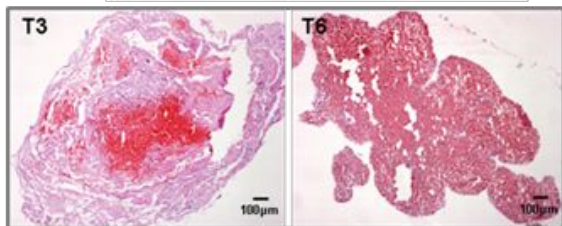




< 3h > 6h



Hematoxilin / eosin



< 3h < 6h

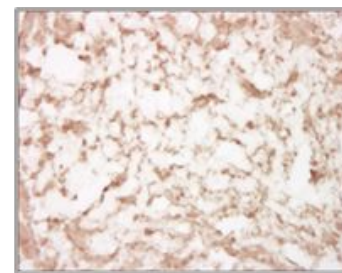
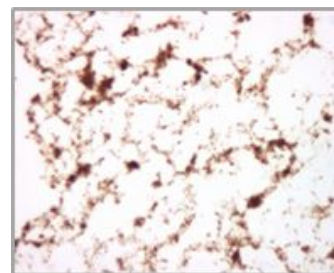
onset-of-pain-to-PCI time

Immunohistology

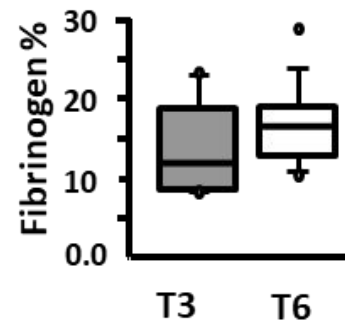
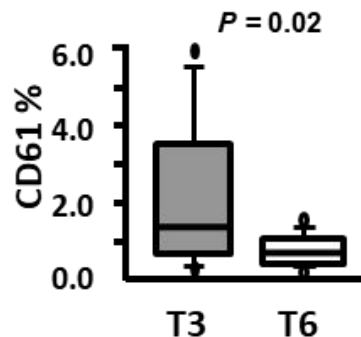
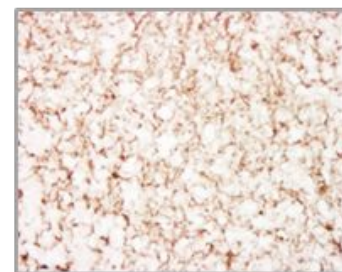
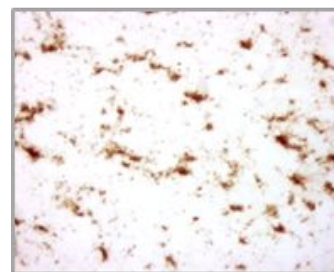
Platelets

Fibrin(ogen)

< 3h

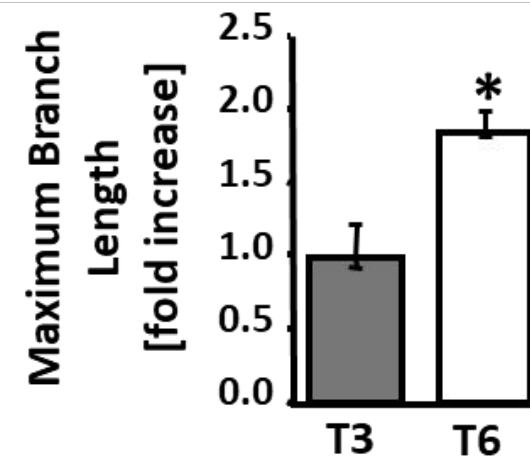
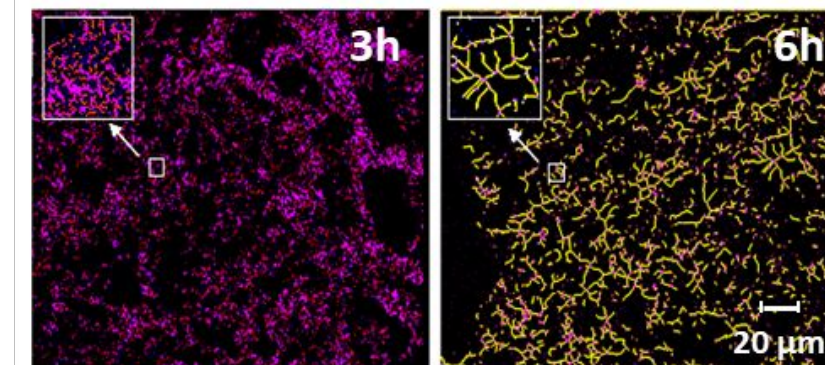


> 6h



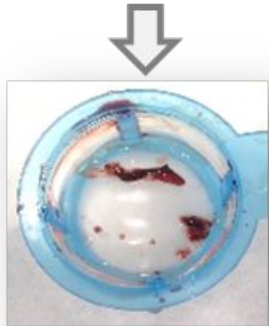
Confocal Microcopy

Fibrin : Branch Legth

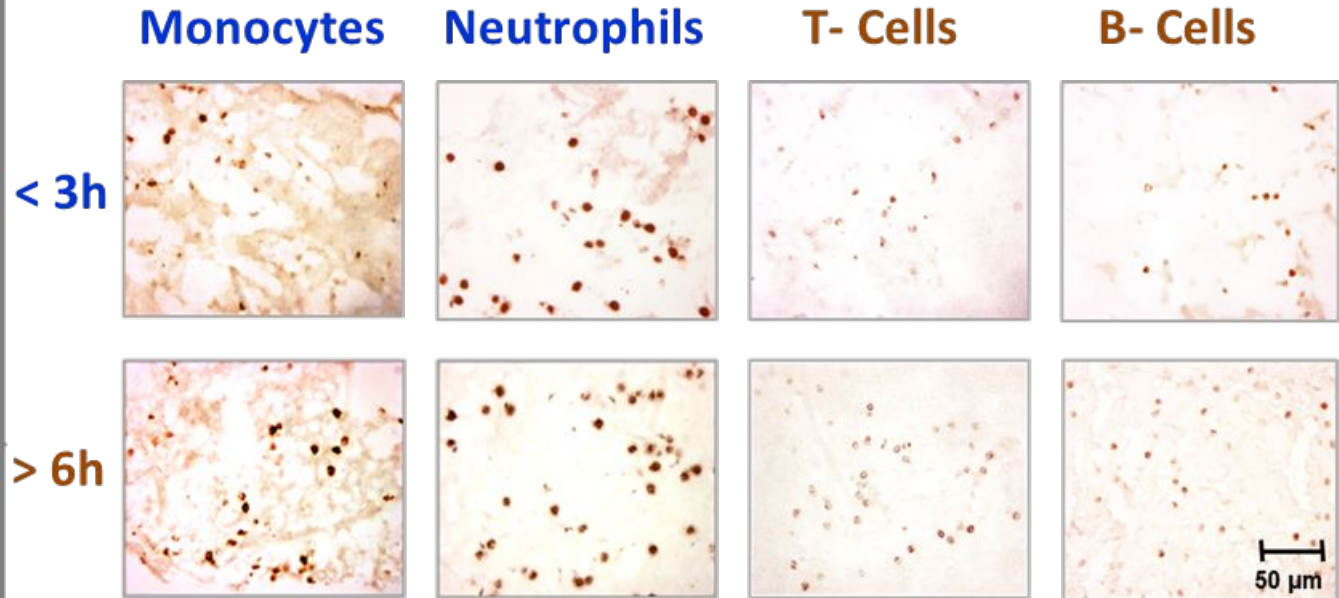


Immune cells in coronary thrombi of STEMI patients

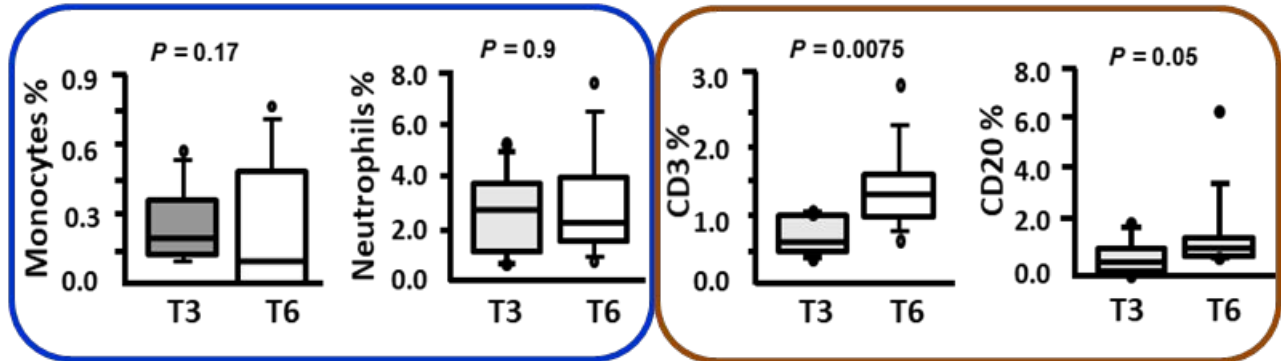
Intracoronary thrombi
STEMI Patients



Immunohistochemical analysis

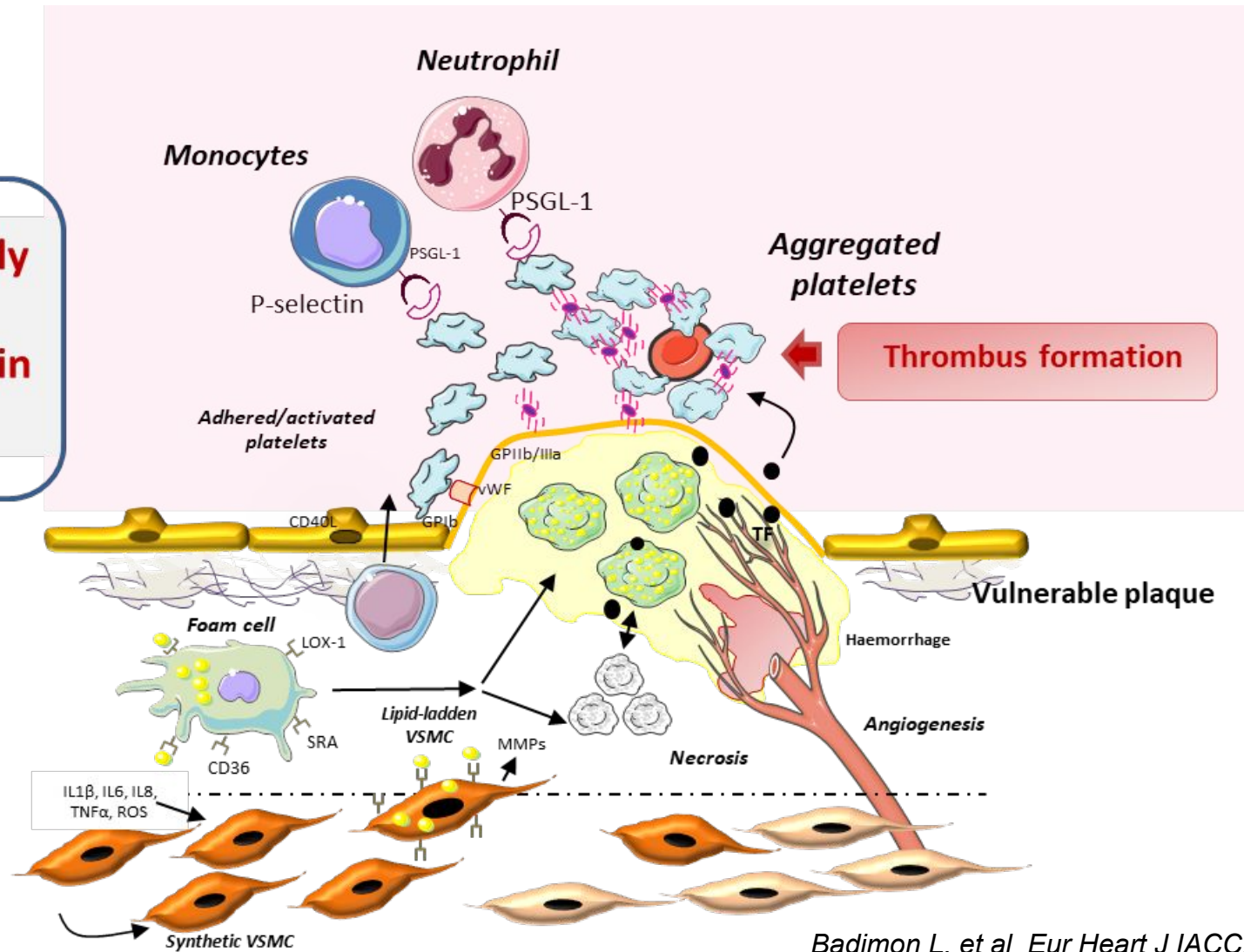


Dynamic change in the infiltration of leucocytes subtypes with time of ischemia



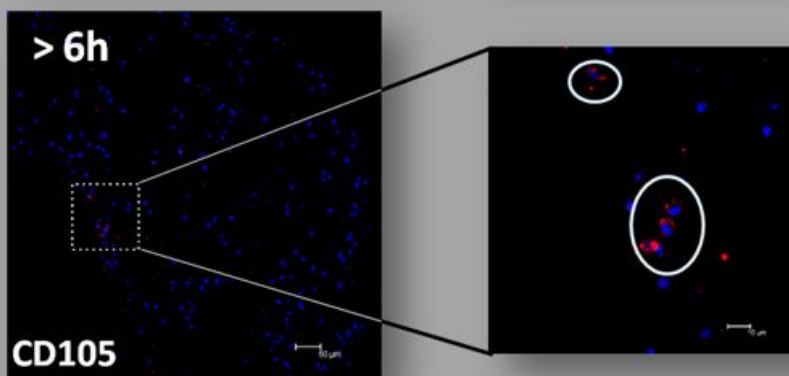
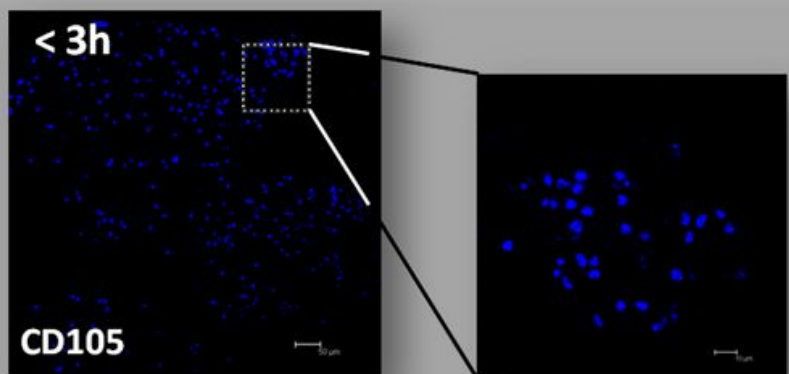
Platelet - Innate immune cell interaction in arterial thrombosis

“Inflammatory cells are not solely passively trapped in developing thrombi, but play an active role in their formation and evolution”



Badimon L. et al *Eur Heart J IACC.* 2014

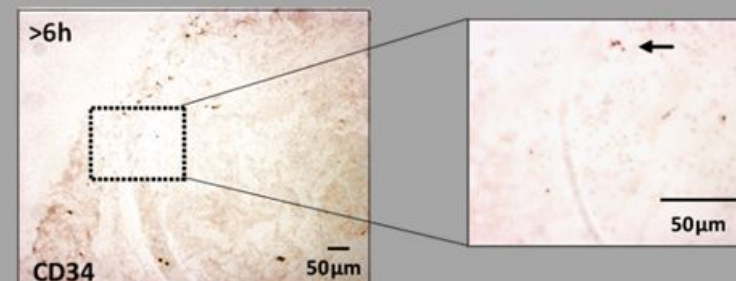
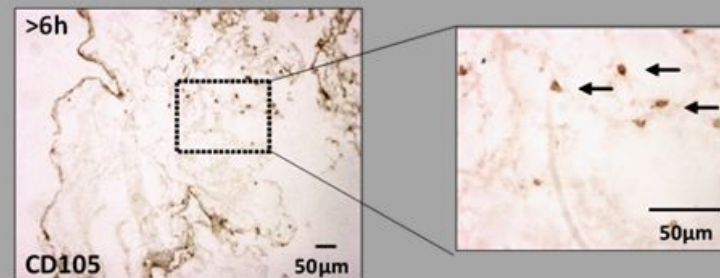
Confocal microscopy



CD105 antibody
Hoechst (Nucleus)

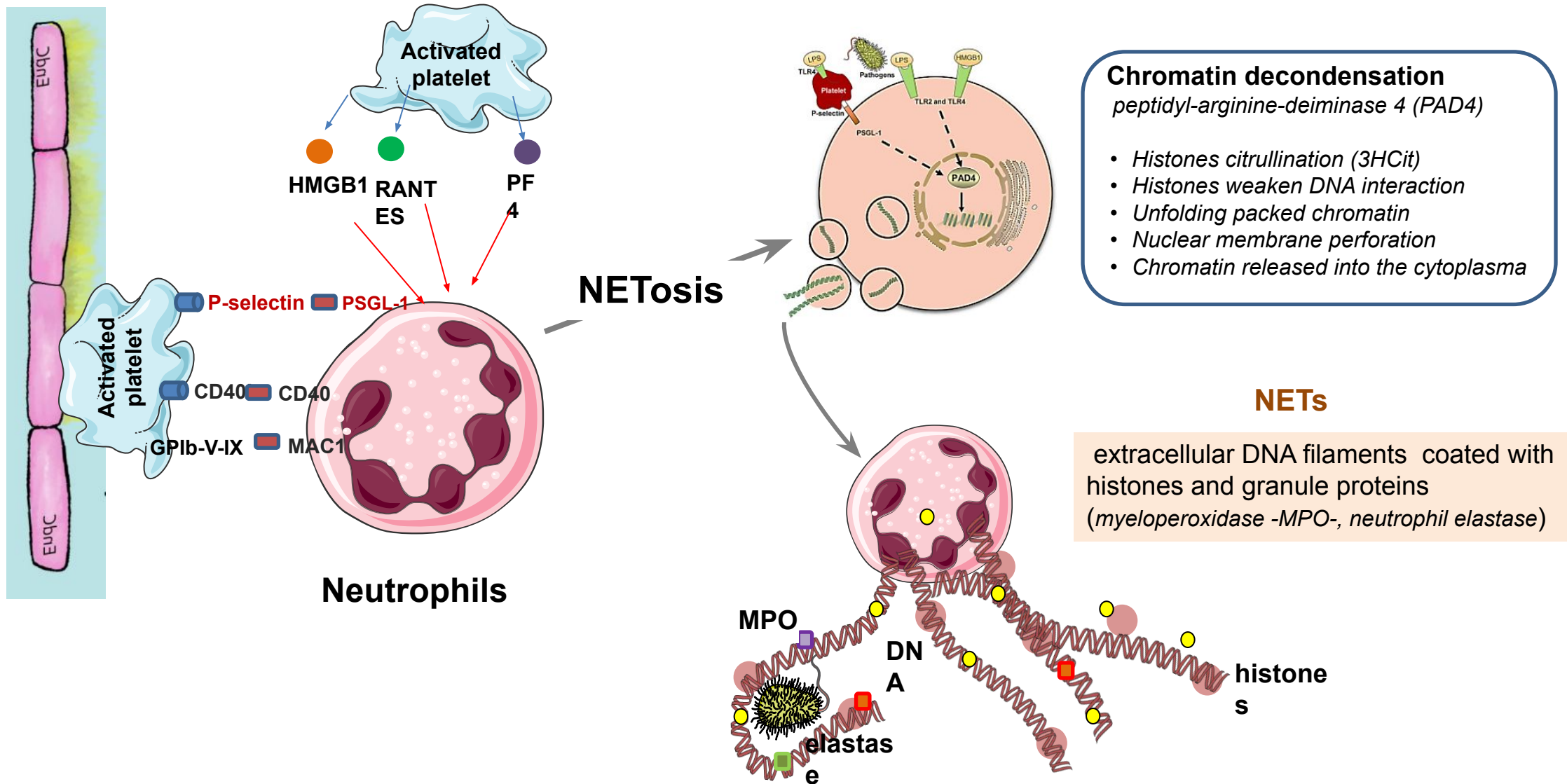
Scale bar = 50 μm.

Immunohistochemistry

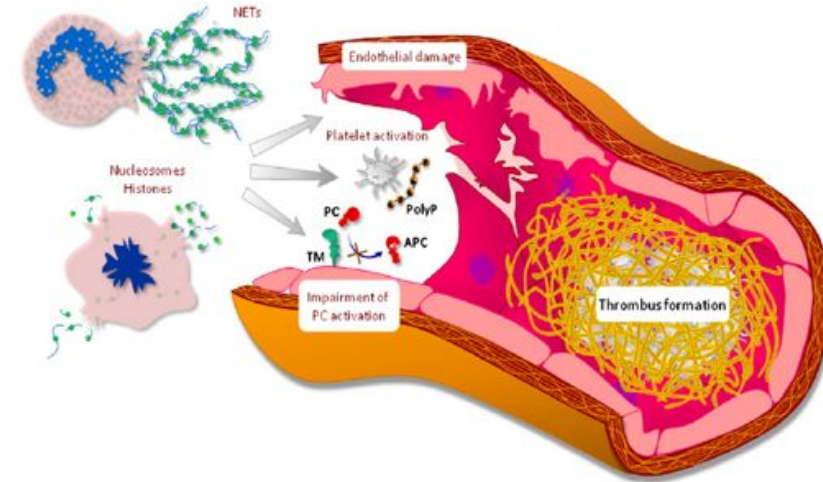
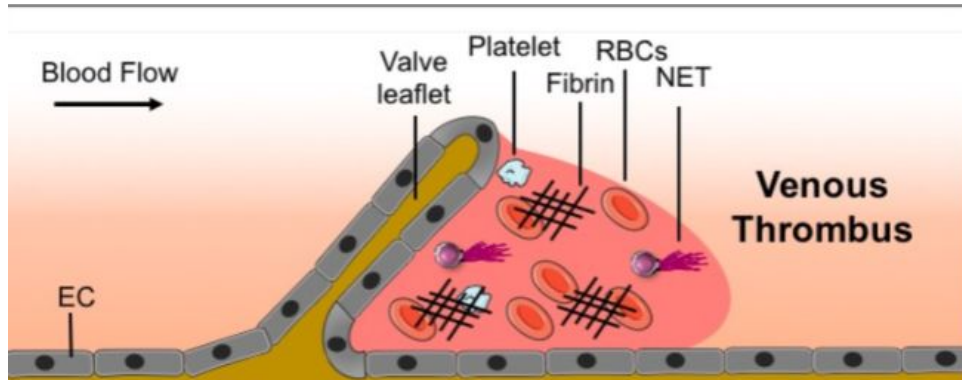


Aged occlusive thrombi (>6h from onset-of-pain) showed infiltration of undifferentiated CD105+ and CD34+ cells that were not present in <3hours occlusive thrombi.

Neutrophil Extracellular Traps (NETs)



Venous thrombi form in valve pockets and contain NETs.



Extracellular traps (NETs), released upon neutrophil activation, promote deep vein thrombosis induced by flow restriction, suggesting possible new targets for drug development.

Brill A et al Atheroscler Thromb Vac Biol 2012

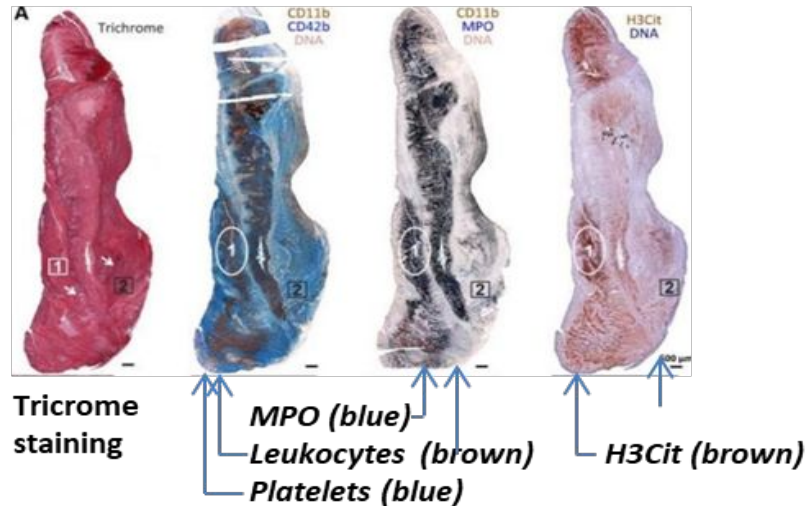
Amplification of intravascular coagulation

- Stimulates TF-dependent extrinsic pathway
- Enhances factor XII activity
- Decreases plasmin generation

NETS are present in organizing thrombi in patients with VTE

Human venous thrombi

Pulmonary embolism thrombus



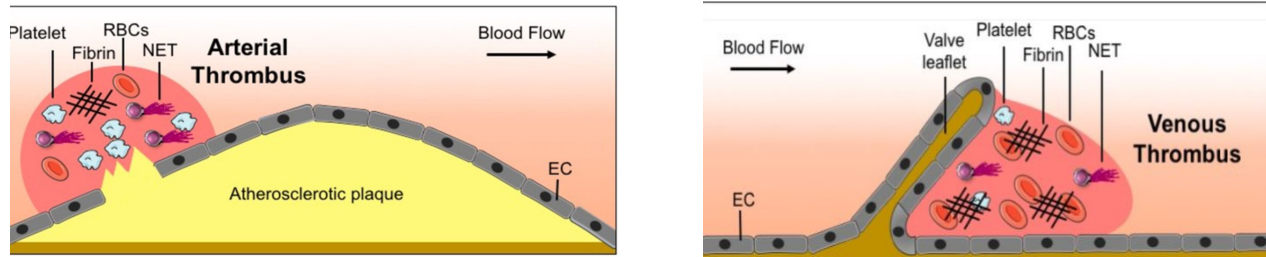
Immunohistochemical staining

MPO: Neutrophil marker
H3Cit: citrullinated histone H3

Exploration of the cellular composition and the presence of NETs within human venous thrombi.

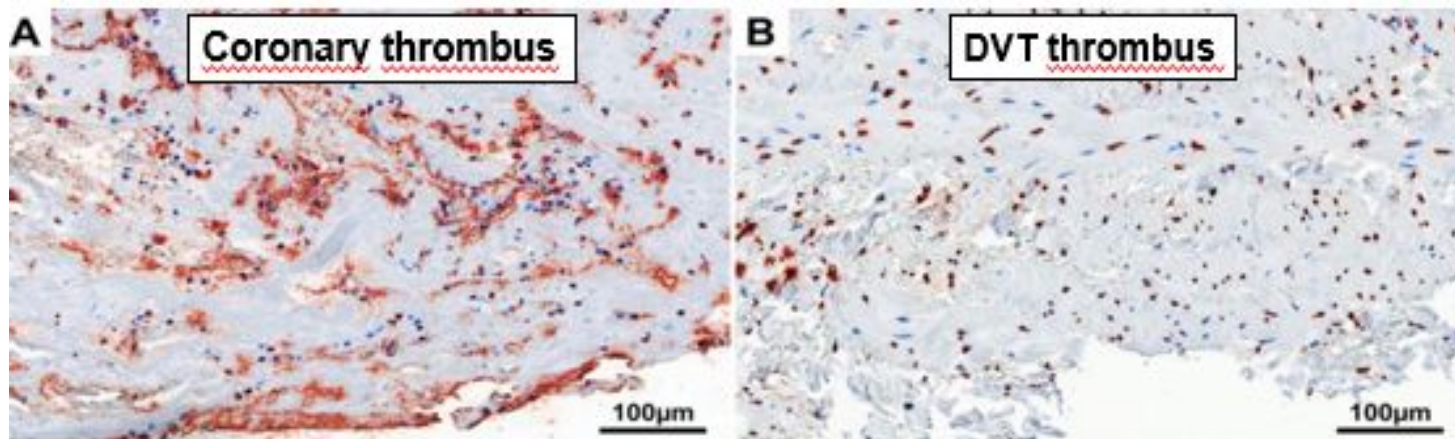
- accumulation of NETs during the organizing stage of pathological venous thrombus development
- differences in platelet and neutrophil deposition within the unorganized, organizing and organized stages of thrombus development.

Arterial thrombi contain NETs.

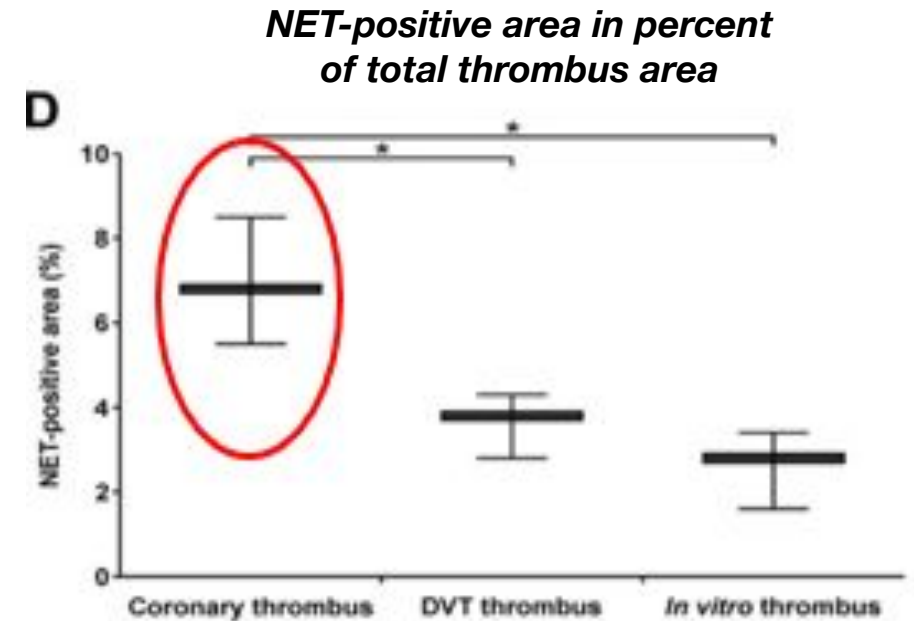


Immunohistochemistry analysis

- coronary thrombi from patients with ST-elevation acute coronary syndrome undergoing primary percutaneous coronary intervention
- deep vein thrombus (DVT)

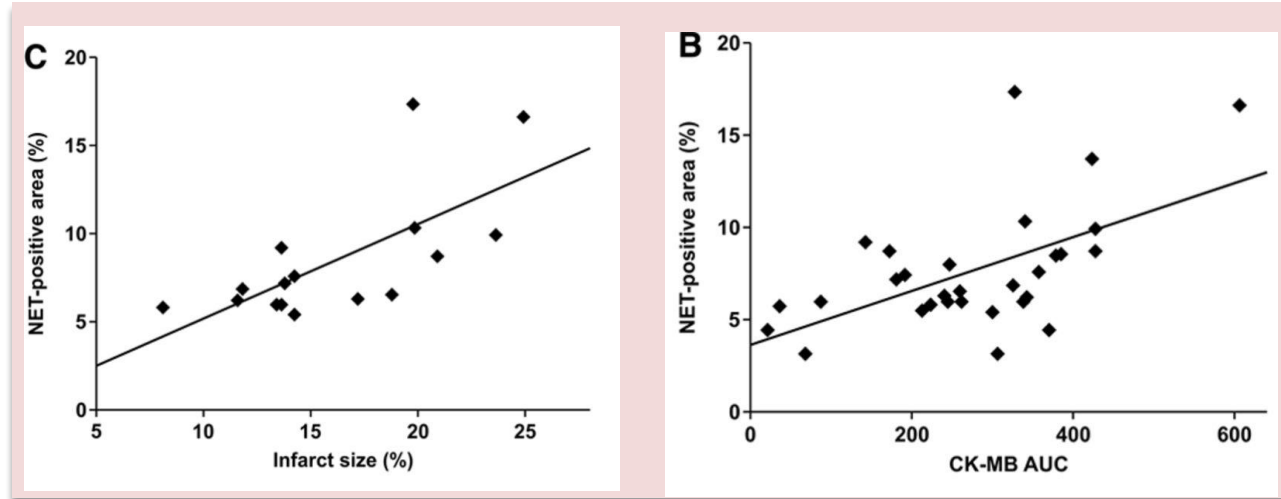


anti-DNA-histone antibody

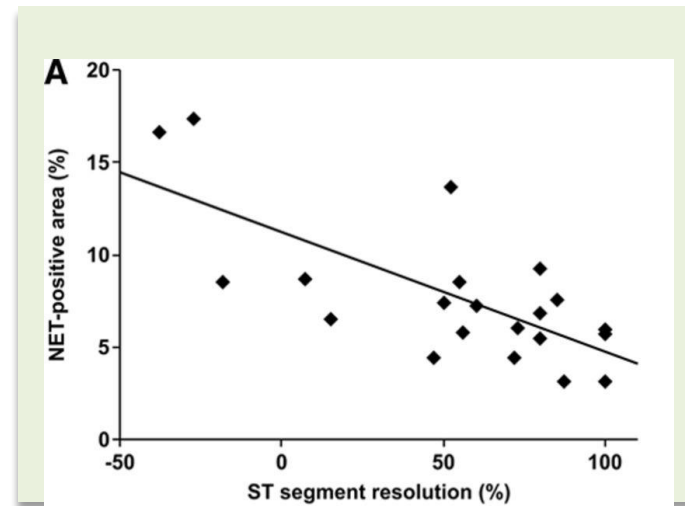


Correlation of NET burden with and infarction size ST-segment resolution

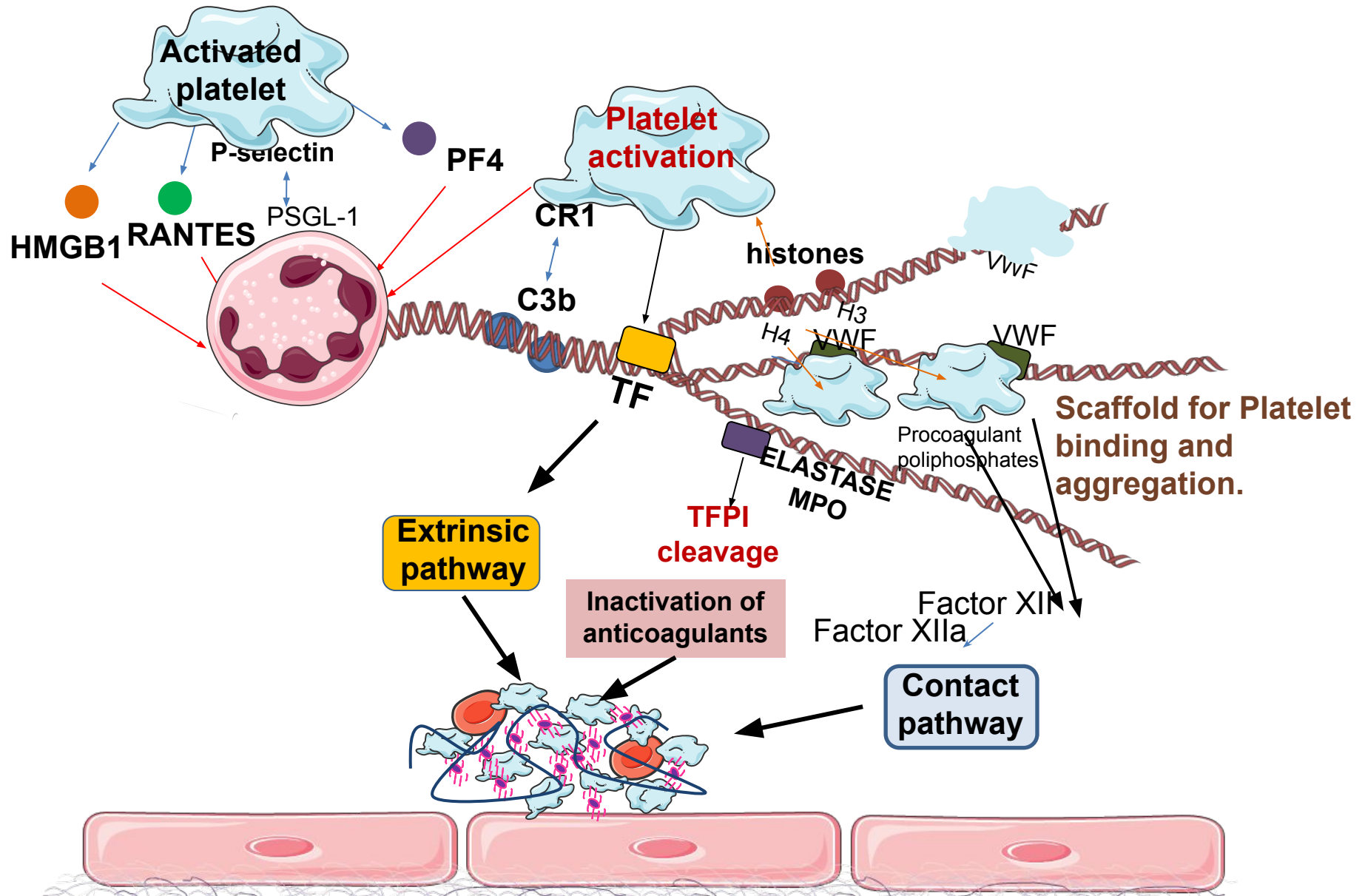
Infarct size



ST segment resolution



How do NETs induce thrombosis in the culprit plaque?



Key take home messages

Concomitant activation of platelets and neutrophils occurs in a variety of inflammatory and atherosclerotic cardiovascular diseases.

The interplay platelet and neutrophils drives immunothrombosis limiting the spread of pathogens,

In addition,

- ✓ **neutrophil-platelet interaction pathways have crucial effects on thromboembolism and chronic vascular disease.**
- ✓ **the platelet-neutrophil crosstalk elicits NETosis**
- ✓ **NETs exacerbates venous thrombosis and provides a scaffold for platelet activation and arterial thrombus formation**

□ **The concept that venous and arterial thrombosis are two different disease entities is currently under revision**

VTE and atherothrombosis shear several key etiologic pathways for disease progression

Lina Badimon

Gemma Vilahur

Ilaria Ramaiola

Oriol Juan-Babot

Esther Peña

Montse Gomez-Pardo

Hospital Clinic Barcelona

Manel Sabate

Victoria Martin

Salvatore Brugaletta

