Le plasma de patients COVID : impact sur les cellules endothéliales

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Hémostase et Transfusion

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Disclosures for SUSEN

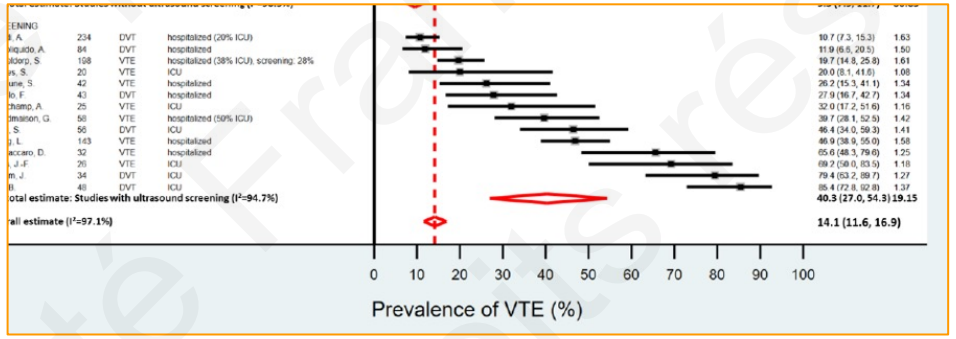
Research Support/PI	**Carmat, CorWave, Roche-Chugai, Stago Investigator for: Biomarin, Bioverativ, CSL Behring, LFB, Pfizer, Roche-Chugaî, Sanofi, Shire/Takeda, Siemens Healthiners and Sobi**
Employee	**Lille University**
Major Stockholder	**Laelaps Therapeutics (Co-owner/founder)**
Honoraria	**No relevant conflicts of interest to declare**
Scientific Advisory Board	**Biomarin, LFB, Roche, Sanofi, Sobi, Takeda**

All fees go to Lille University

COVID-19 and coagulopathy : high prevalence of venous thromboembolism events

Up to 40% (95%CI 27-54)

DVT screening



Despite the use of thromboprophylaxis

Nopp S et al. *Res Pract Thromb Haemost*. 2020

Circulation

RESEARCH LETTER

Pulmonary Embolism in Patients With COVID-19

Awareness of an Increased Prevalence

American Heart Association.

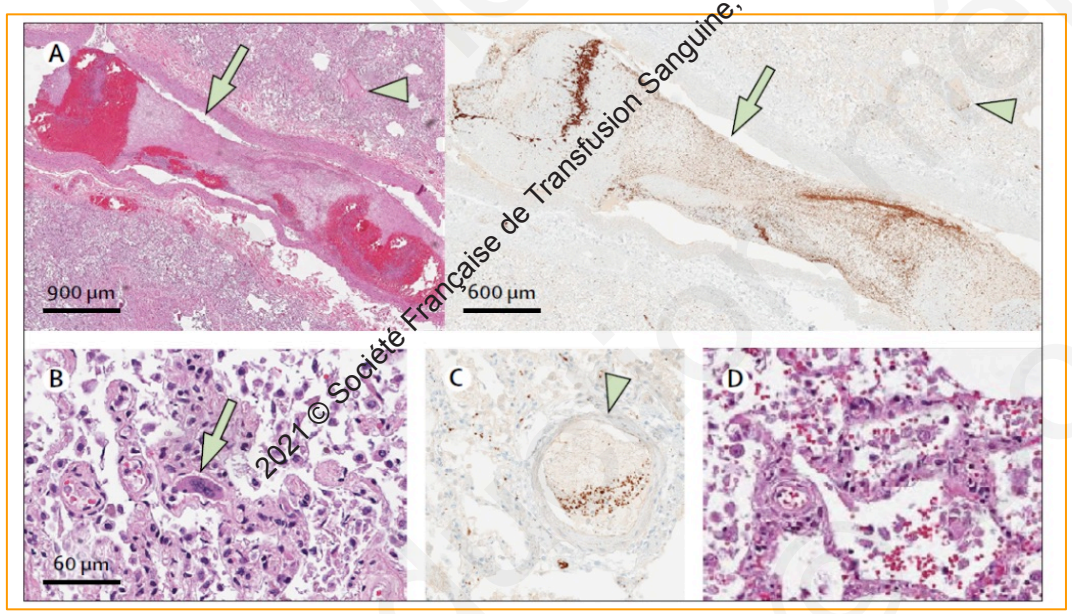
J Poissy, J Goutay, M Caplan, E Parmentier, T Duburcq, F Lassalle, E Jeanpierre, A Rauch, J Labreuche, S Susen, For the Lille Haemostasis COVID-19 Group

Thrombotic risk in intensive care unit COVID-19/ non COVID-19 ≃ x 3

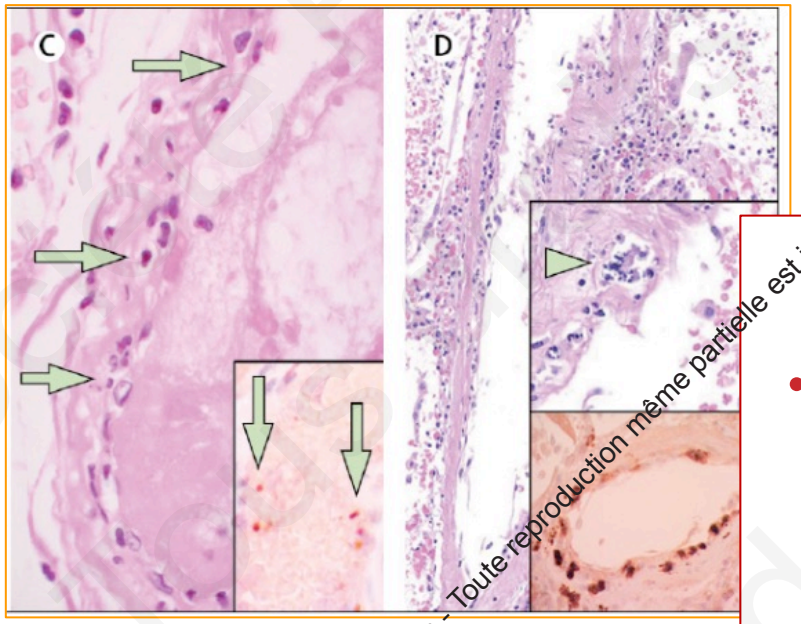
COVID-19 coagulopathy: autopsy studies

Large vessels

Microcirculation



Severe endothelial cell damages



- Apoptosis
- Loss of tight junctions
- Separation from basement membranes

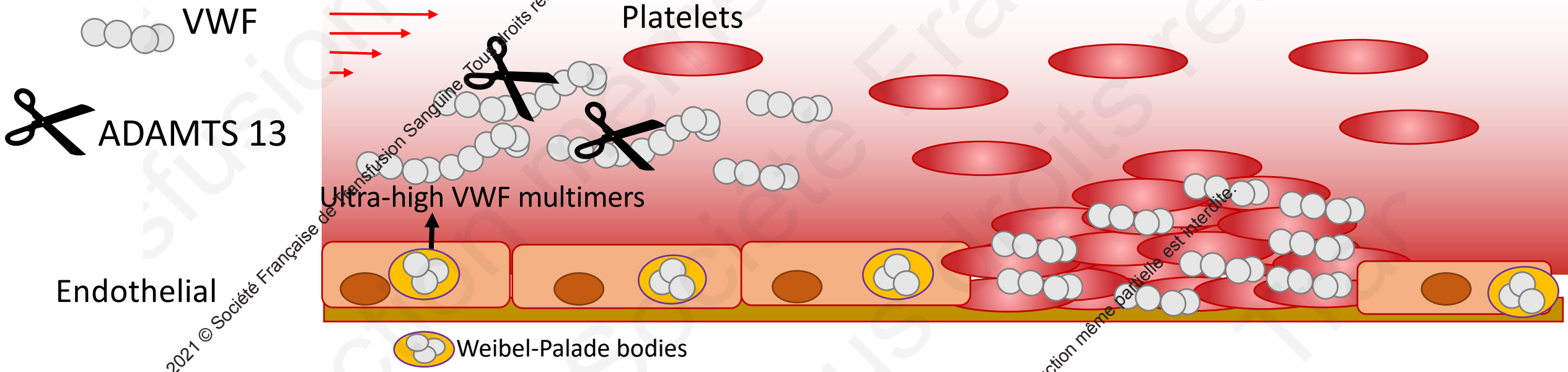
Varga Z et al, *Lancet,* 2020

Fox SE et al, *Lancet respir med*. 2020,
Ackermann M et al, *N Engl J Med*,.2020,
Nicolaï, L et al *J Thromb Haemost*. 2020

Diffuse endotheliopathy

Trigger thrombi formation

Von Willebrand factor (VWF)

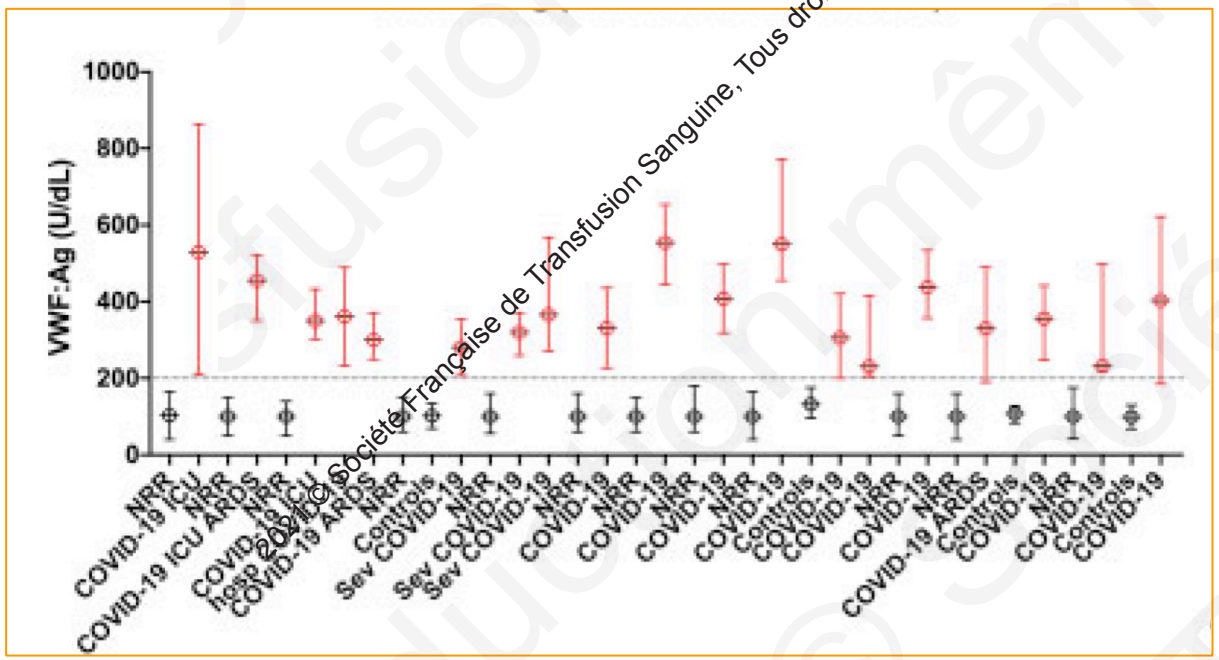


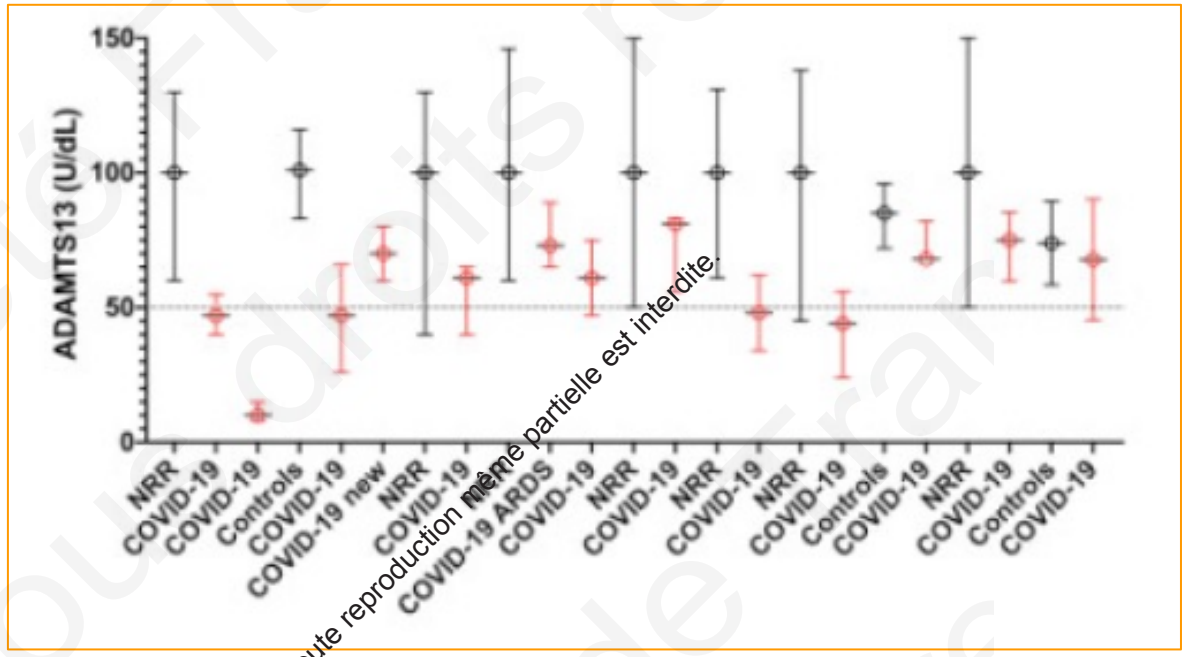
- Important role in haemostasis, particularly within microvasculature

Contribution to COVID-19-associated thrombi formation ?

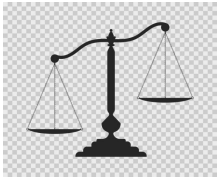
COVID-19 : high VWF/ADAMTS 13 ratio

VWF ■ COVID-19 ■ Controls ***ADAMTS 13***





Favaloro EJ et al, *Semin Thromb Hemost*, 2021



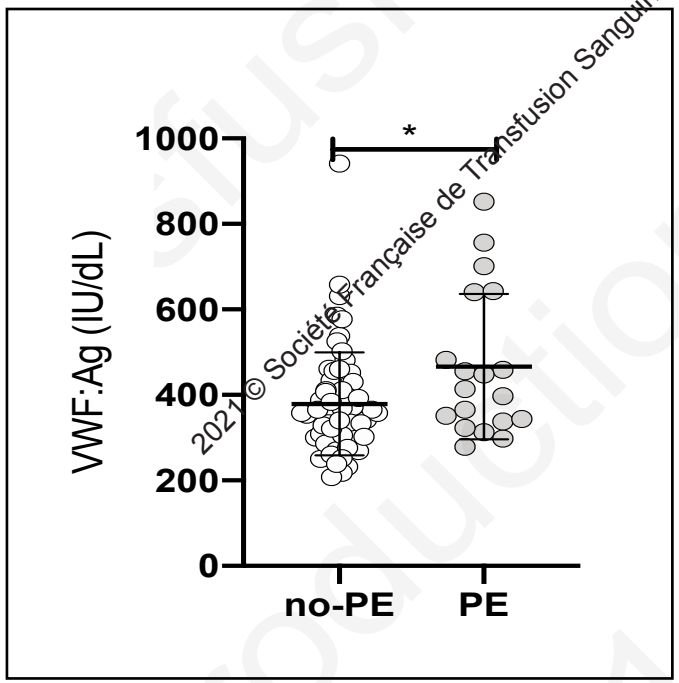
Markedly ↗↗ VWF and mild to moderate ADAMTS 13 deficiency

Hypercoagulable state – high risk of thrombus formation

VWF levels and severity of COVID-19

Pulmonary embolism (PE)

107 intensive care unit COVID-19 patients



Poissy J et al, *Circulation*, 2020

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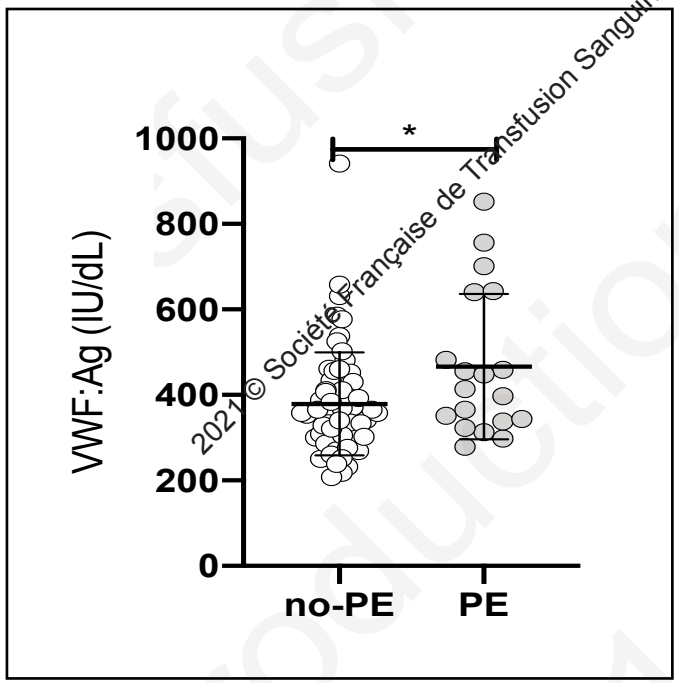
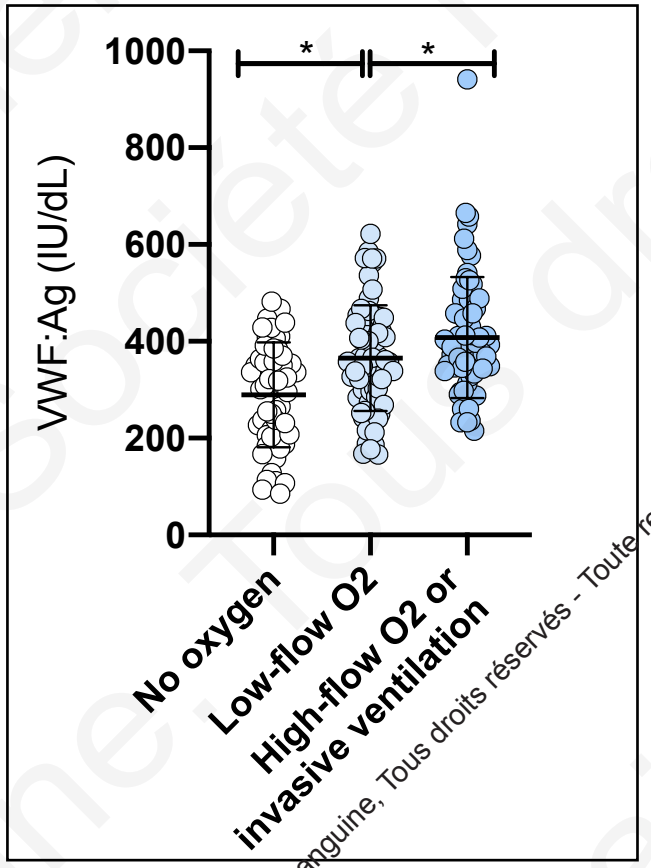


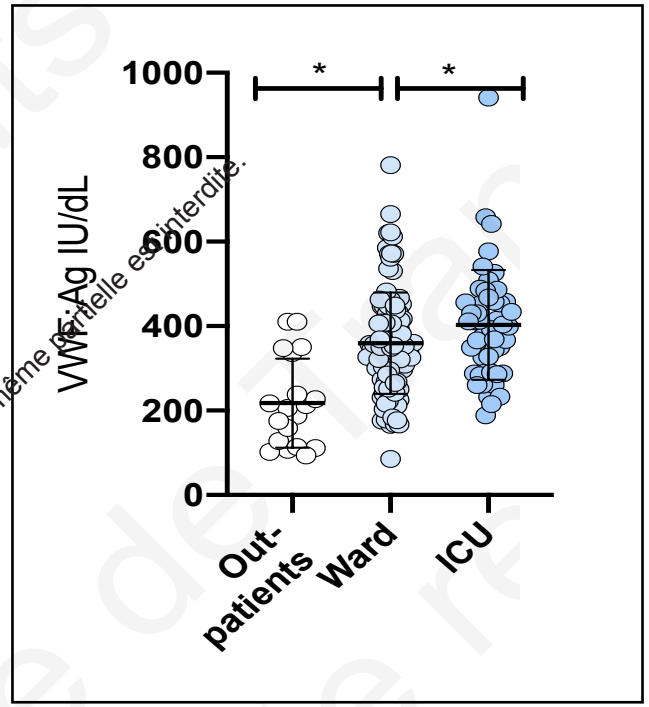
Poissy J et al, *Circulation*, 2020

Oxygen requirements

243 COVID-19 patients



Care intensity



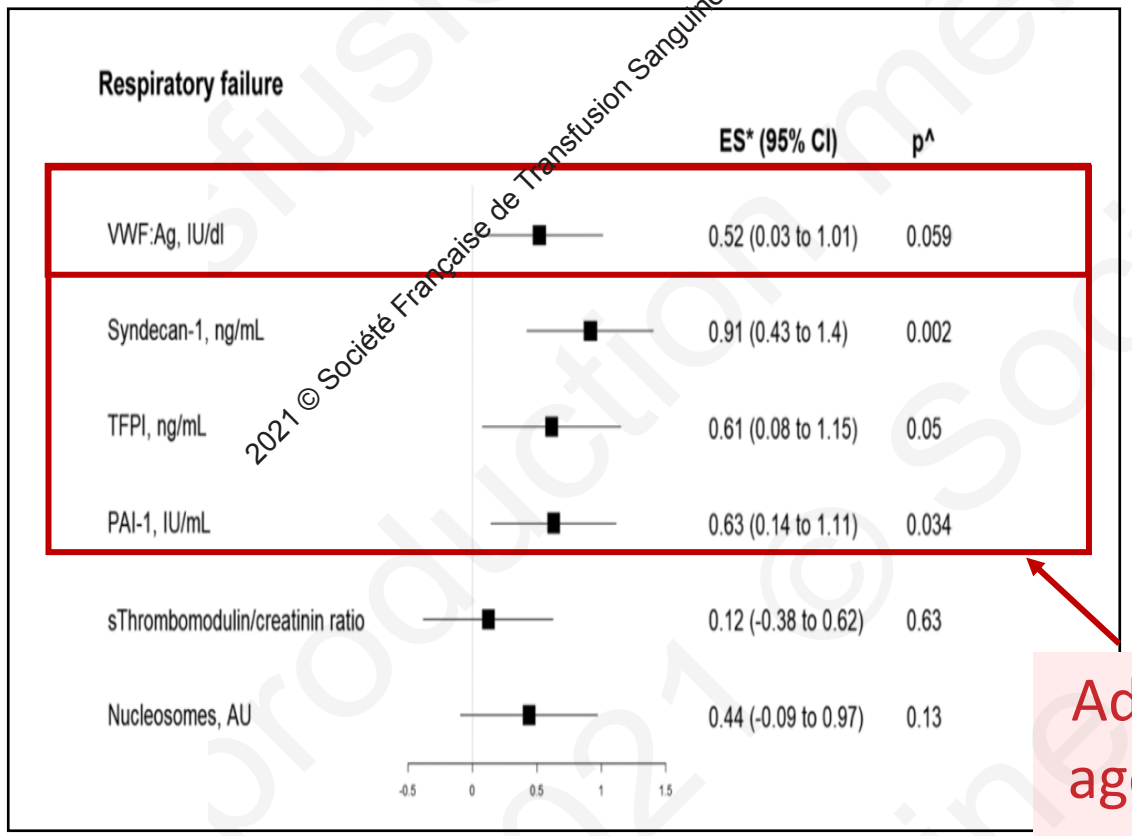
Rauch A et al, *J Thromb Haemost*, 2020

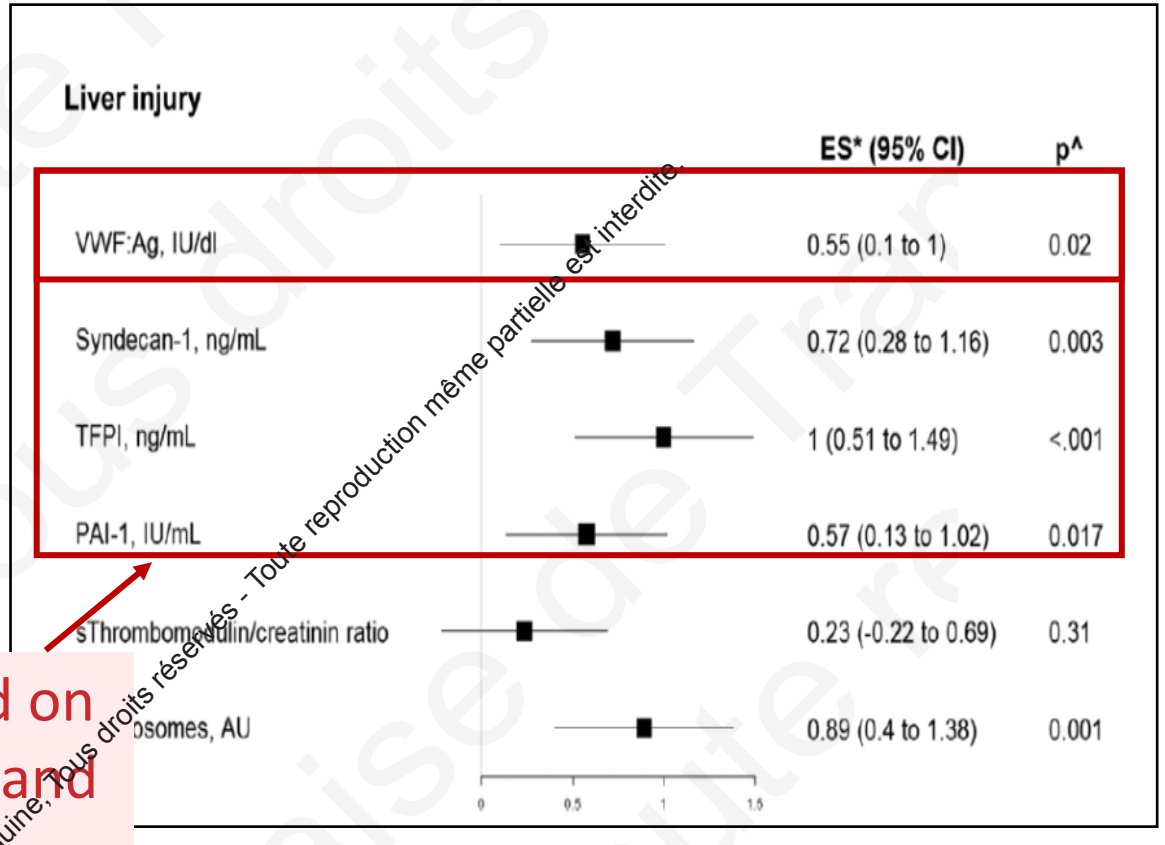
VWF levels and severity of COVID-19

Respiratory failure

Liver injury

82 ICU COVID-19 patients – occurrence within 14 day of ICU admission





Adjusted on age, sex and BMI

Dupont A et al, *Arterioscler Thromb Vasc Biol,* 2021

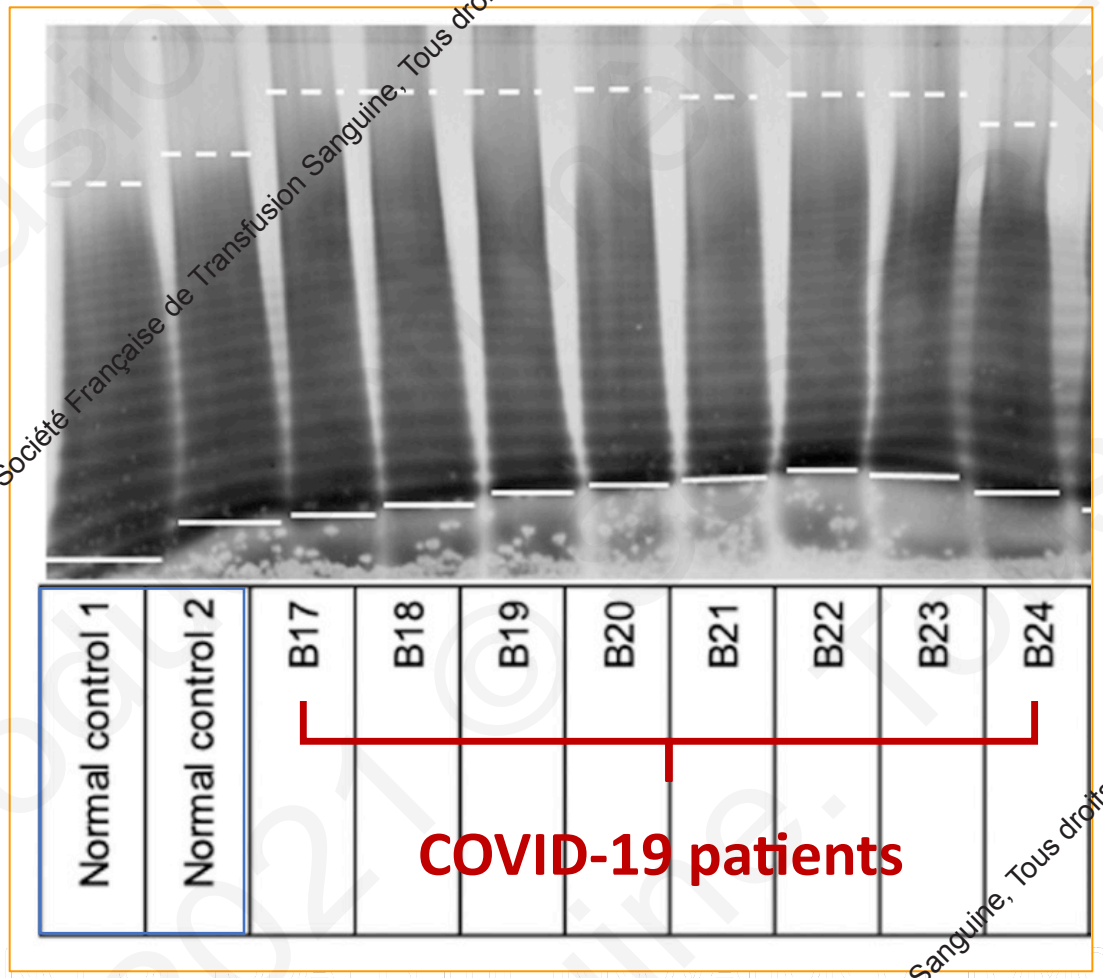
Presence of ultra-high molecular weight VWF multimers in COVID-19 patients plasma

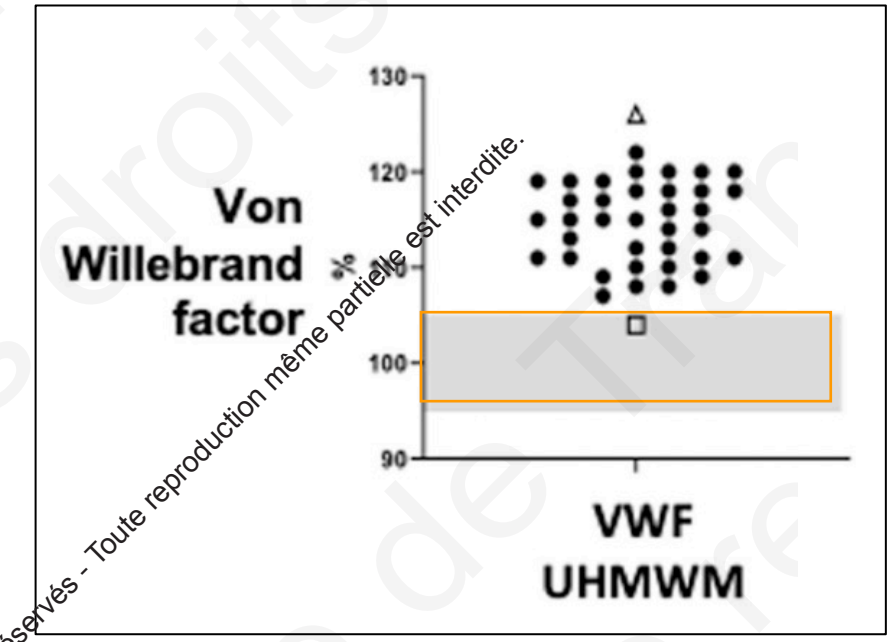
Agarose gel electrophoresis + anti VWF antibody

36 severe COVID-19 patients

Migration

multimers



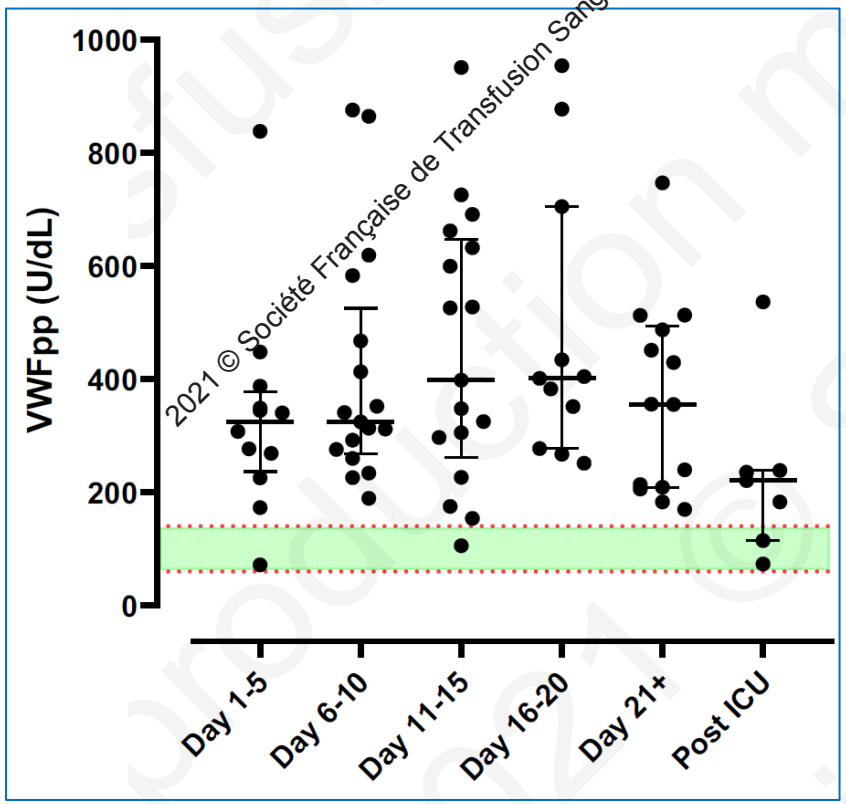


Turecek PL et al, *Thromb Res* 2021

ISTH 2021

VWF propeptide (VWFpp) and severity of COVID-19

Propeptide (pp) VWF

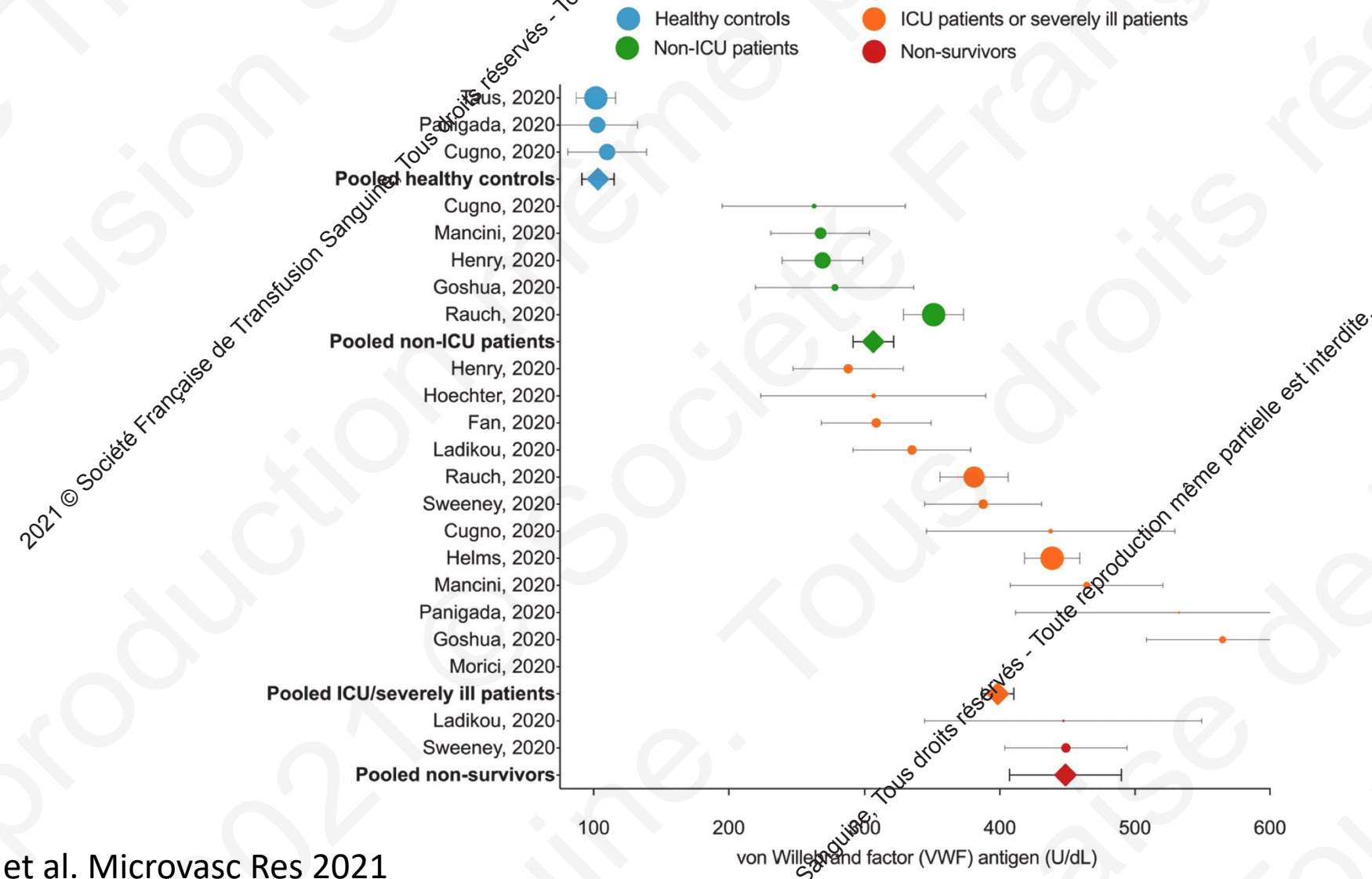


Markedly ↗ plasma VWFpp

- Venous thromboembolism events
- Severity of disease
- Oxygen requirements
- High care intensity

Ward SE et al, *Br J Haematol*, 2021, Mancini I et al, *J Thromb Haemost*, 2021

Meta-analysis of VWF and outcome in COVID-19



Adrianto et al. Microvasc Res 2021

VWF propeptide (VWFpp) and severity of COVID-19

Presence of UHMV VWF multimers in plasma

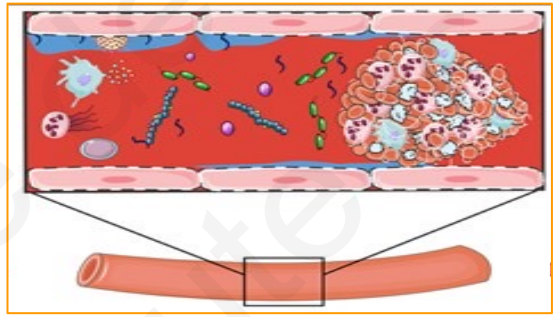
Markedly ↗ plasma VWFpp

Substantial endothelial cell damages

High plasma VWF levels

Hypercoagulable state

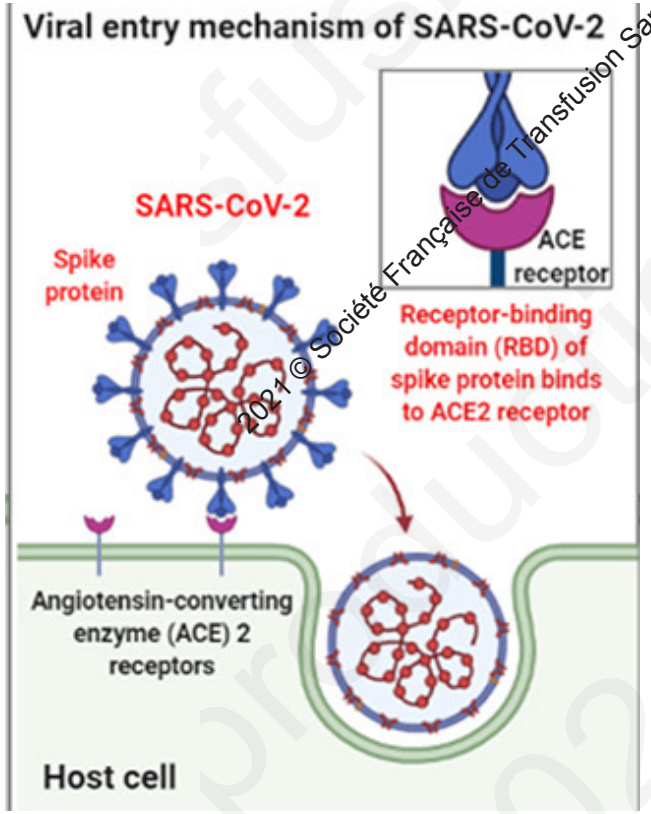
Thrombosis



Dupont A et al, *Arterioscler Thromb Vasc Biol*, 2021

Endothelial cell damages and COVID-19

Direct viral infection ?



Substantial endothelial cell damages

↓

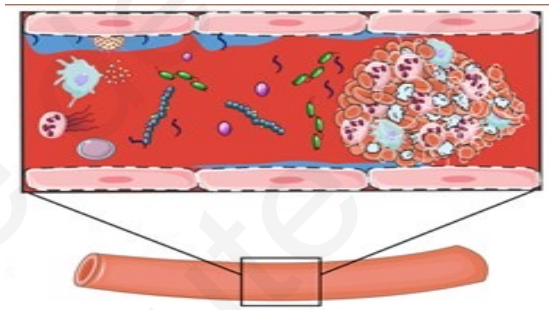
High plasma VWF levels

↓

Hypercoagulable state

↓

Thrombosis



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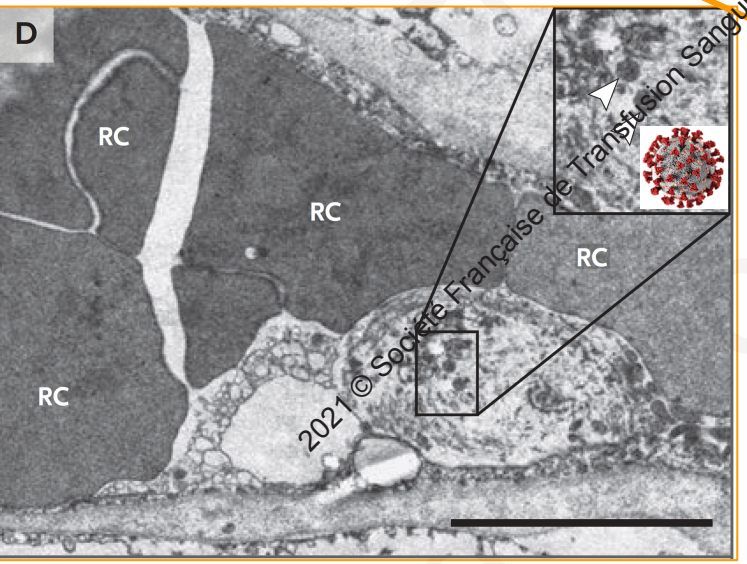

Post-mortem studies

Varga Z et al, *Lancet,* 2020
Ackermann M et al, *N Engl J Med*,.2020,
Fox SE et al, *Lancet respir med*, 2020

Substantial endothelial cell damages

↓

High plasma VWF levels

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Hypercoagulable state

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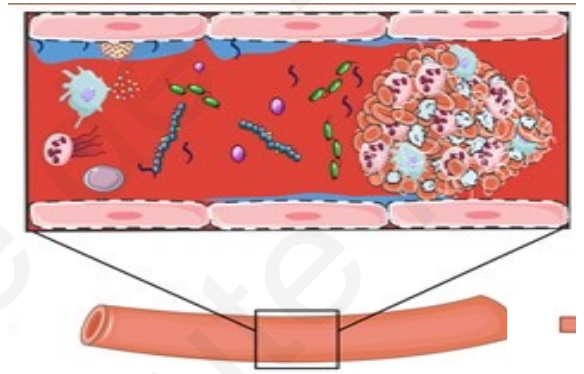
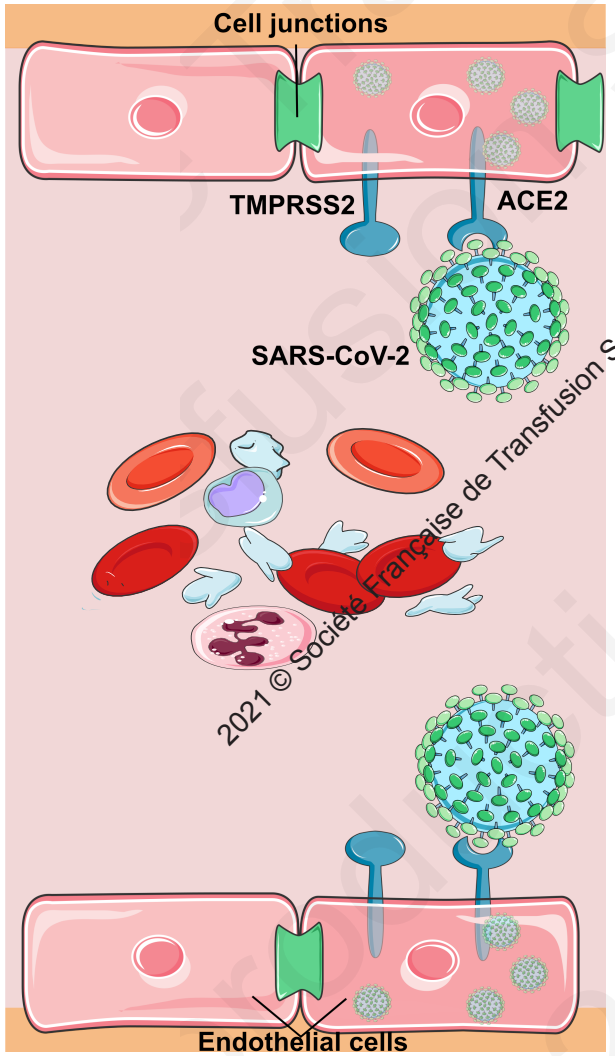
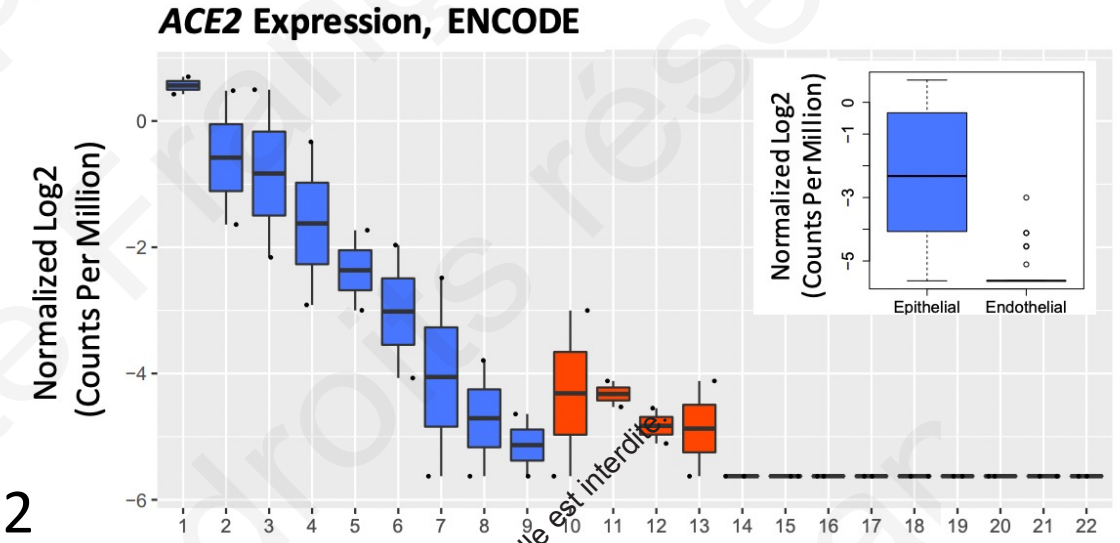
Thrombosis

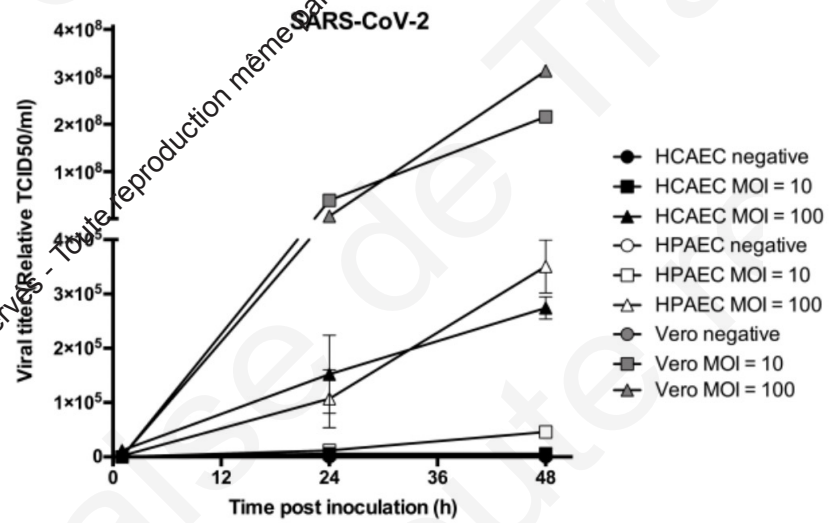


COVID-19 endotheliopathy : 1/No direct viral infection



Lack of Evidence of ACE2 Expression and Replicative Infection by SARSCoV-2 in Human Endothelial Cells

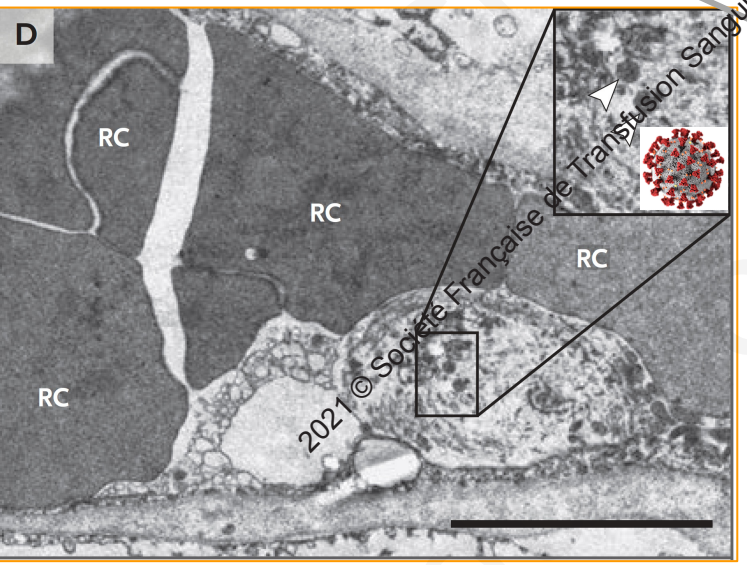




McCracken et al. Circulation 2021

Endothelial cell damages and COVID-19

Direct viral infection ?



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Varga Z et al, *Lancet,* 2020
Ackermann M et al, *N Engl J Med*,.2020,
Fox SE et al, *Lancet respir med*, 2020

Circulation

RESEARCH LETTER

Lack of Evidence of Angiotensin-Converting Enzyme 2 Expression and Replicative Infection by SARS-CoV-2 in Human Endothelial Cells

McCracken IR, *Circulation,* 2021

Substantial endothelial cell damages

↓

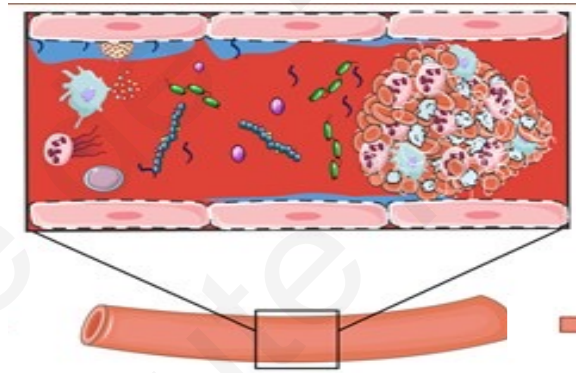
High plasma VWF levels

↓

Hypercoagulable state

↓

Thrombosis



Endothelial cell damages and COVID-19

Direct viral infection ?

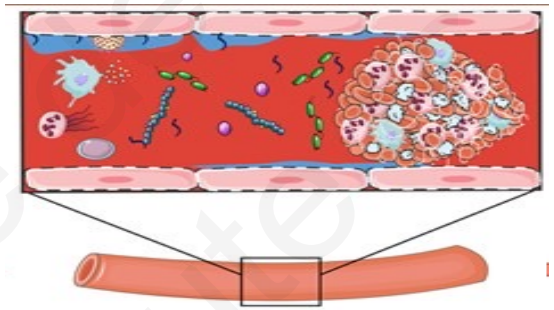
Indirect via circulating mediators ?

Substantial endothelial cell damages

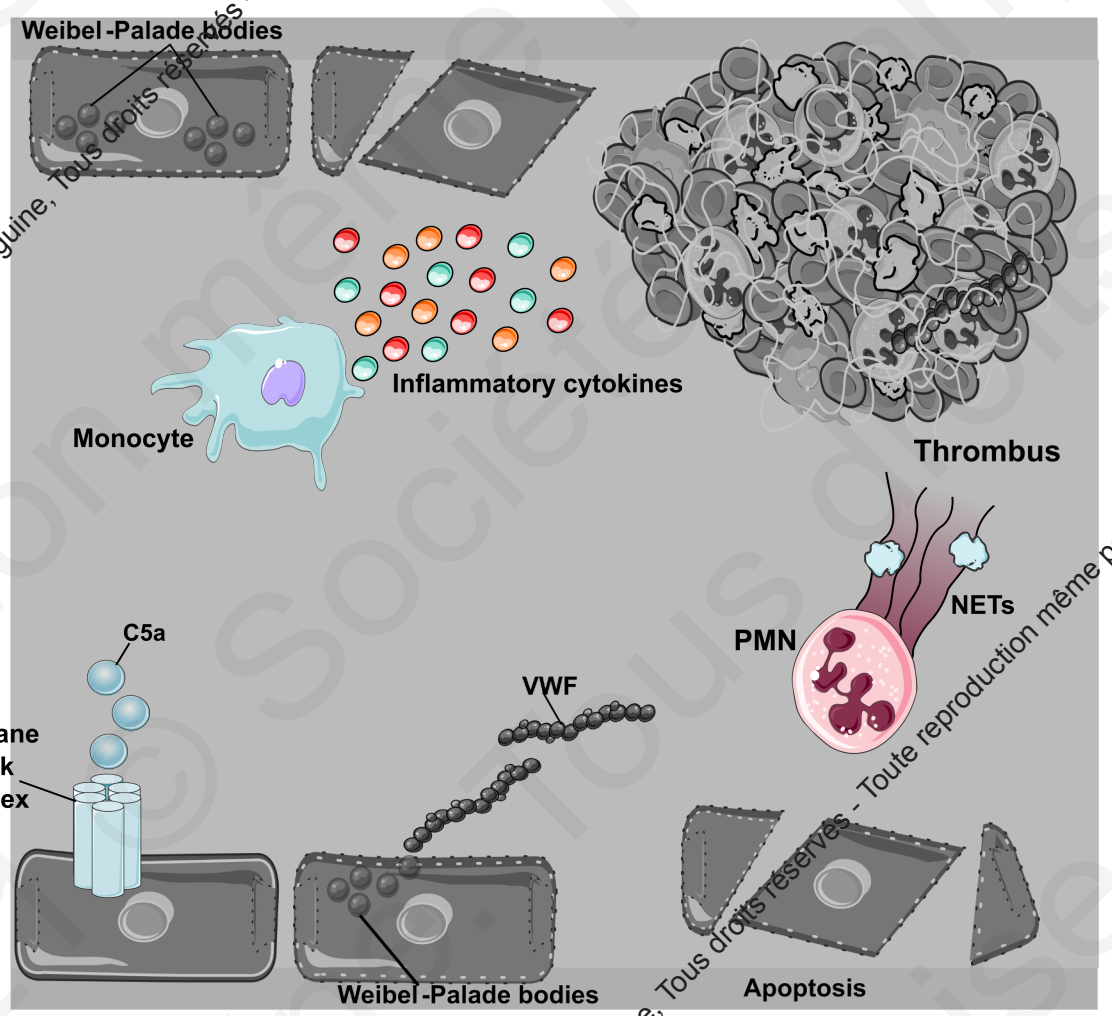
High plasma VWF levels

Hypercoagulable state

Thrombosis



Endothelial cell damages and COVID-19: Direct inflammatory cytotoxycity?



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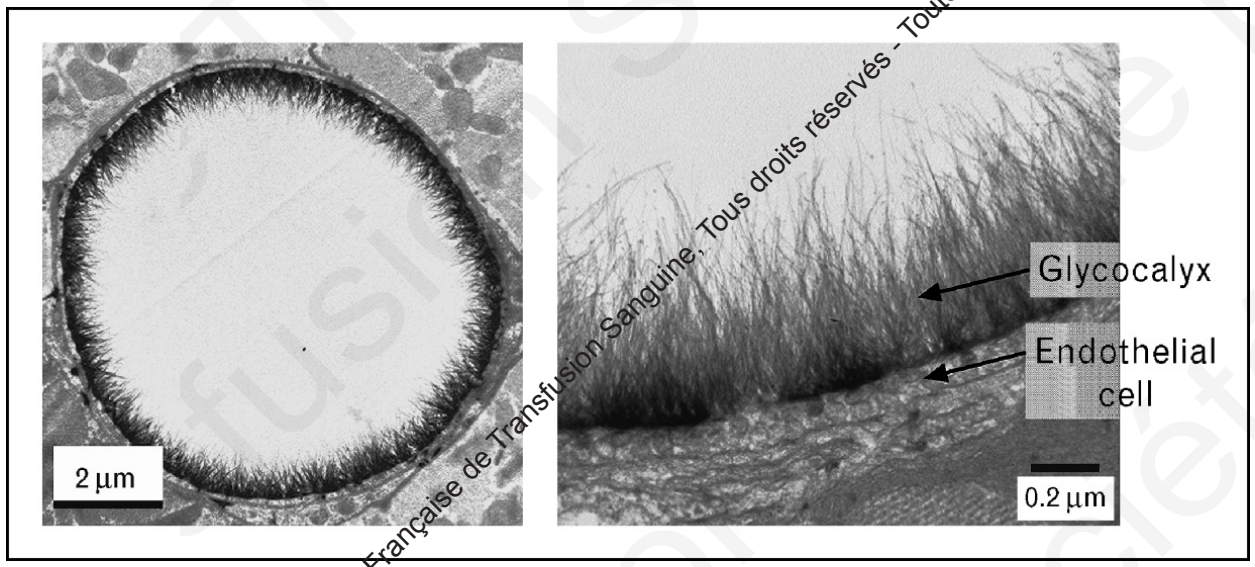
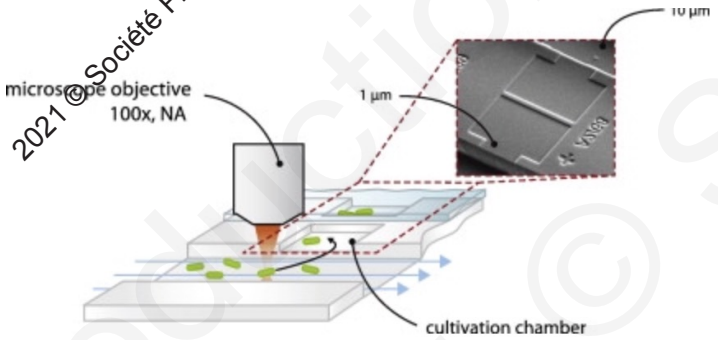
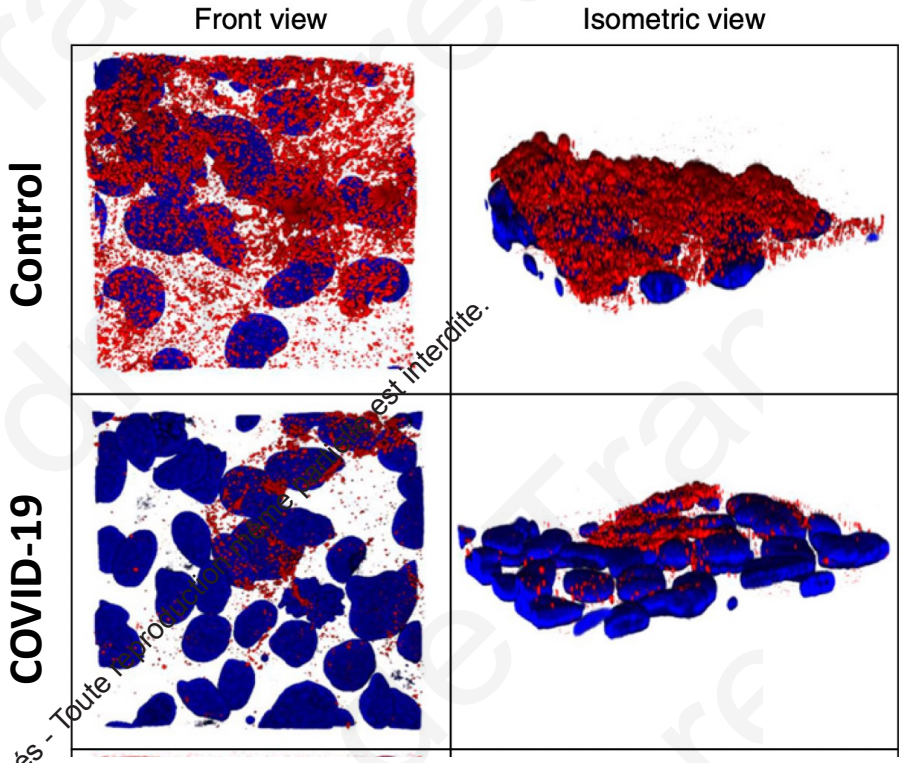




Microfluidic chamber with cultured endothelial cells perfusion with serum

Injury to glycocalyx in severe COVID-19



Stahl et al. Am J Respir Crit Care Med 2020

Endothelial cell damages and COVID-19: role of plasma

- Plasma sampled on admission from 28 consecutive patients (non-ICU, n=16; ICU, n=12)
- In convalescent patients (n=6 from the 12 patients in the ICU) sampled after ICU discharge (mean±SD, 21±7 days)
- Control healthy donors (n=8)
- Positive control = shigatoxin

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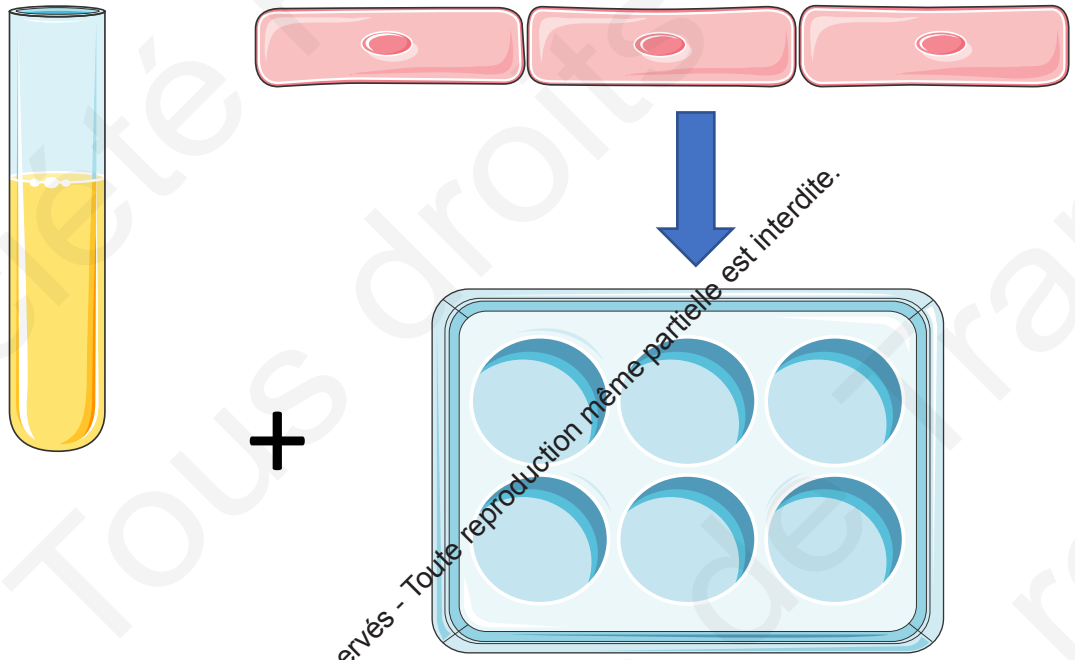
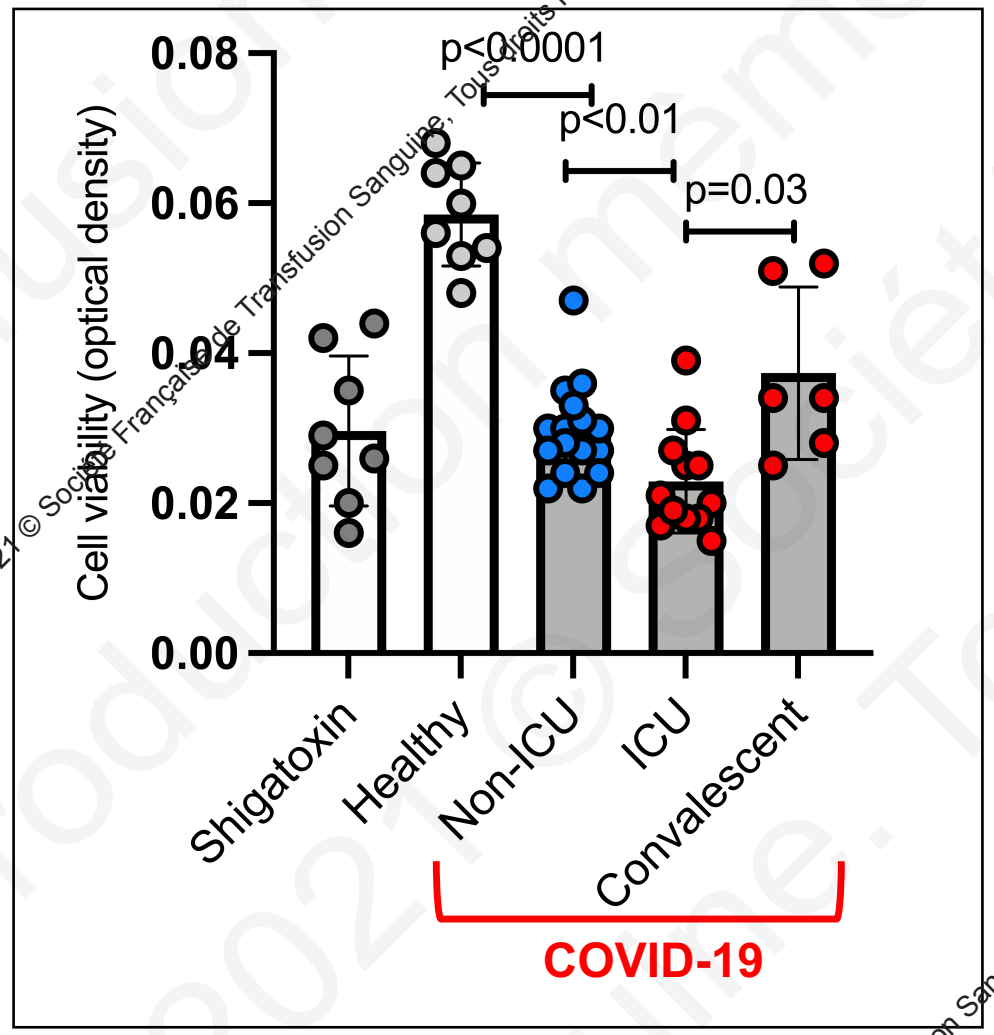
Human pulmonary microvascular endothelial cells



mitochondrial activity (WST-1 test)
1 hour after incubation of cells with plasma

Endothelial cell damages and COVID-19: role of plasma

Human pulmonary microvascular endothelial cells viability after exposure to plasma (1 hour)

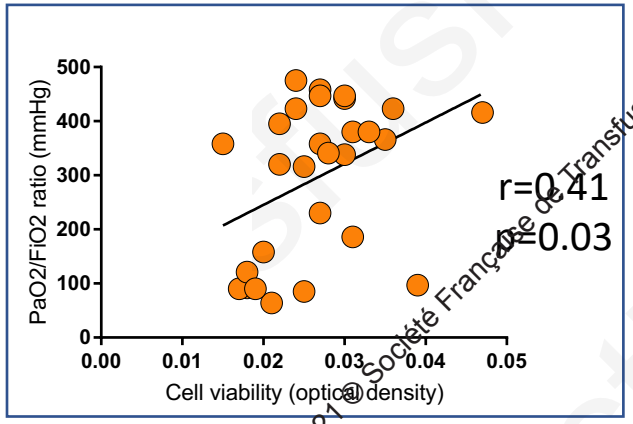


Rapidity of the effect
excludes a direct cytopathic effect
by SARS-COV-2

Rauch A, Dupont A et al, *Circulation*, 2020

HPMVEC viability and COVID-19

Partial pressure of oxygen /Fraction of inspired oxygen

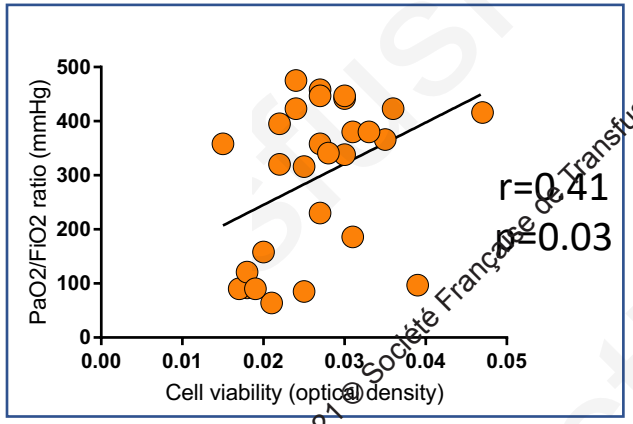


r =Spearman correlation coefficient

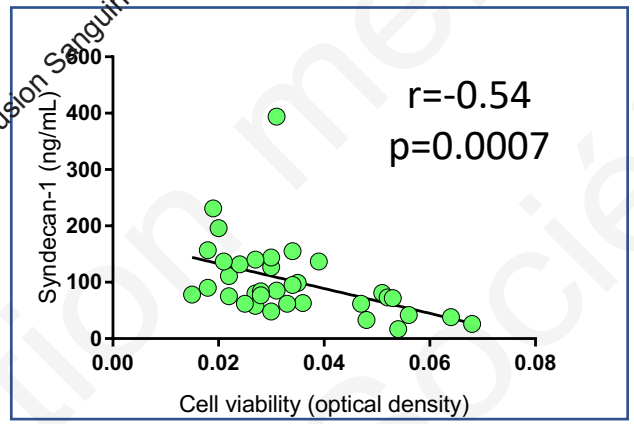
Rauch A, Dupont A et al, *Circulation*, 2020

HPMVEC viability and COVID-19

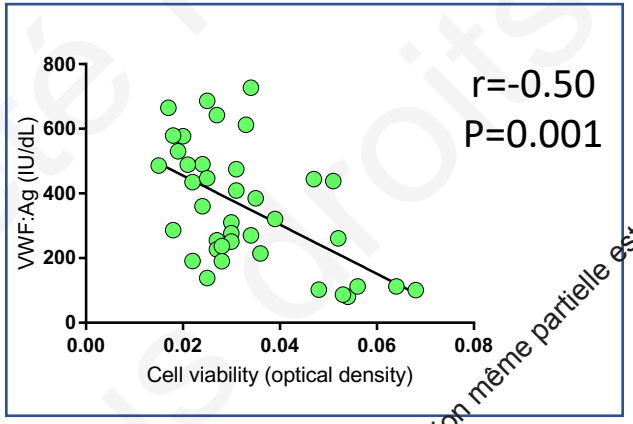
PaO2/FIO2



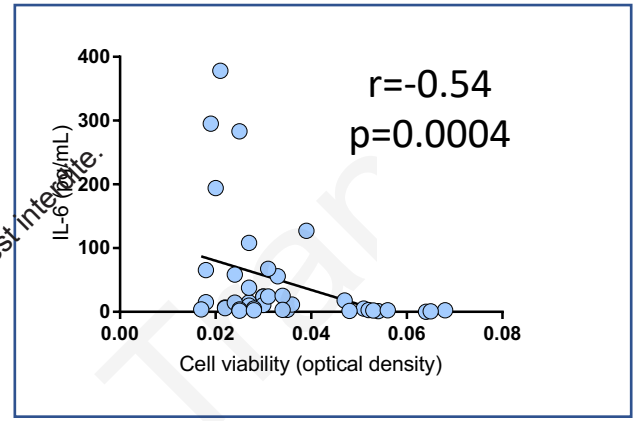
Syndecan



VWF



IL-6



r =Spearman correlation coefficient

Cell viability (Human Pulmonary MicroVascular Endothelial Cells)

Rauch A, Dupont A et al, *Circulation*, 2020

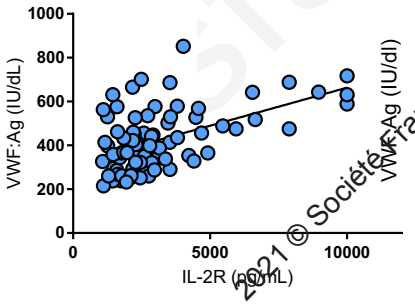
Direct effect of dysregulation of immune response on endothelial damage

VWF, dysregulated immune response and COVID-19

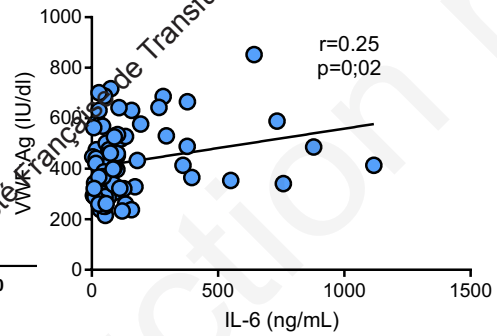
82 ICU COVID-19 patients

Inflammatory cytokines

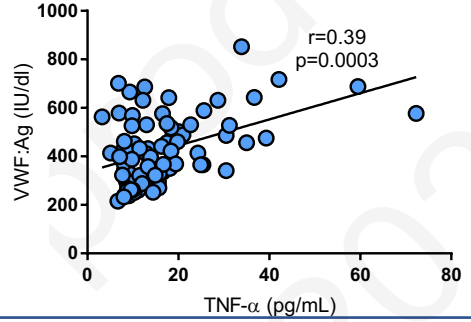
IL-2R



IL-6

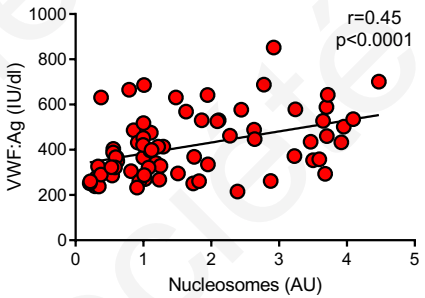


TNF-α

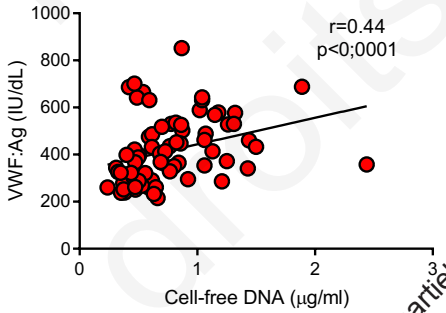


Neutrophil extracellular traps (NETs)

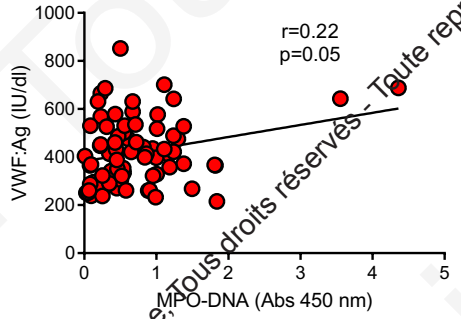
Nucleosomes



Cell-free DNA

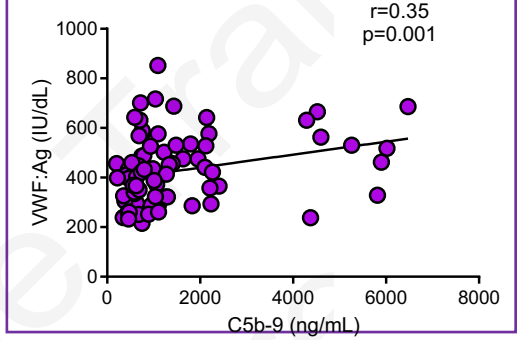


Myeloperoxydase-DNA



Complement

C5b-9



Dupont A et al, *Arterioscler Thromb Vasc Biol*, 2021

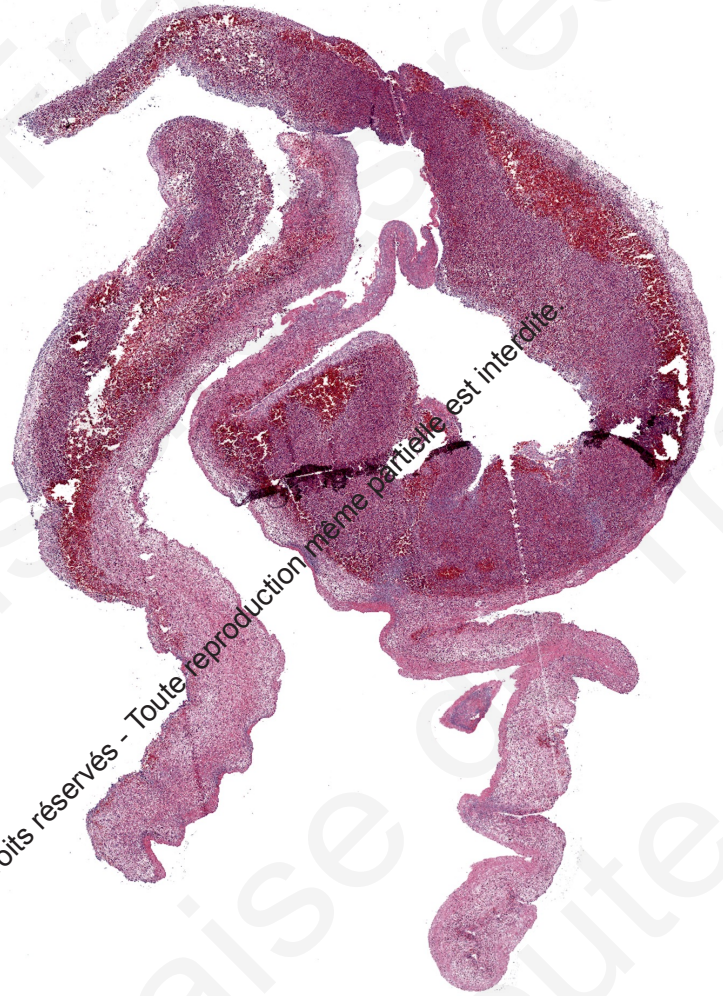
Analysis of Thrombi retrieved from venovenous ECMO circuits

Non COVID

COVID

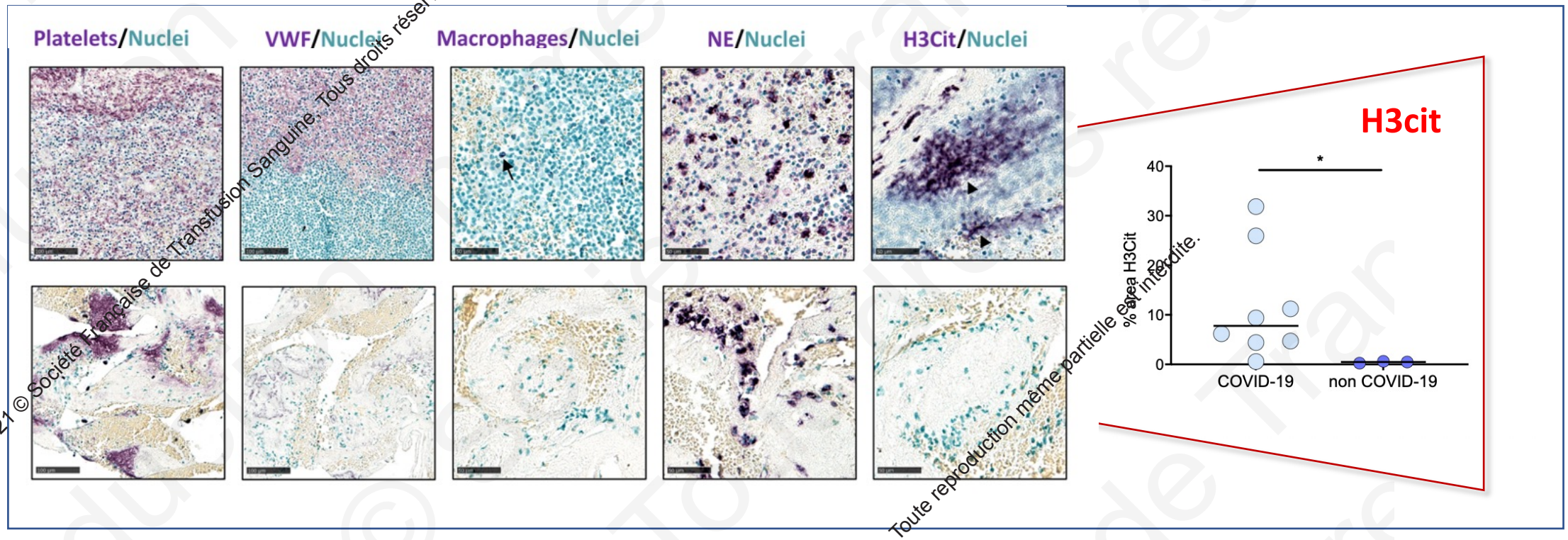
Red blood cells

Fibrin



Stainings Senna Staessens, Unpublished data

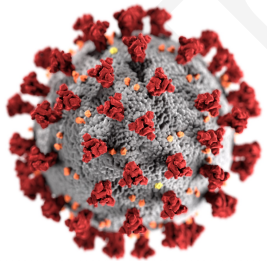
Analysis of thrombi collected in critically ill living patients on extracellular membrane oxygenation



Accumulation of VWF, macrophages, neutrophils and NETs when compared to non-COVID-19 thrombi

Immune response and endotheliopathy promote immunothrombosis

Summary



SARS-CoV-2 infection

Inflammatory cytokines **NETs** **Complement cascade**

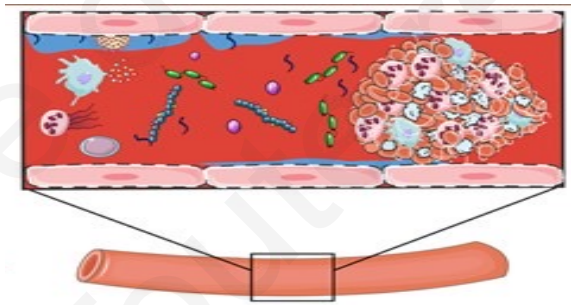
Dysregulation of immune response

Endothelial cell damages

↗ ↗ ↗ VWF plasma levels

Hypercoagulable state

High risk of immunothrombosis



Arteriosclerosis, Thrombosis, and Vascular Biology

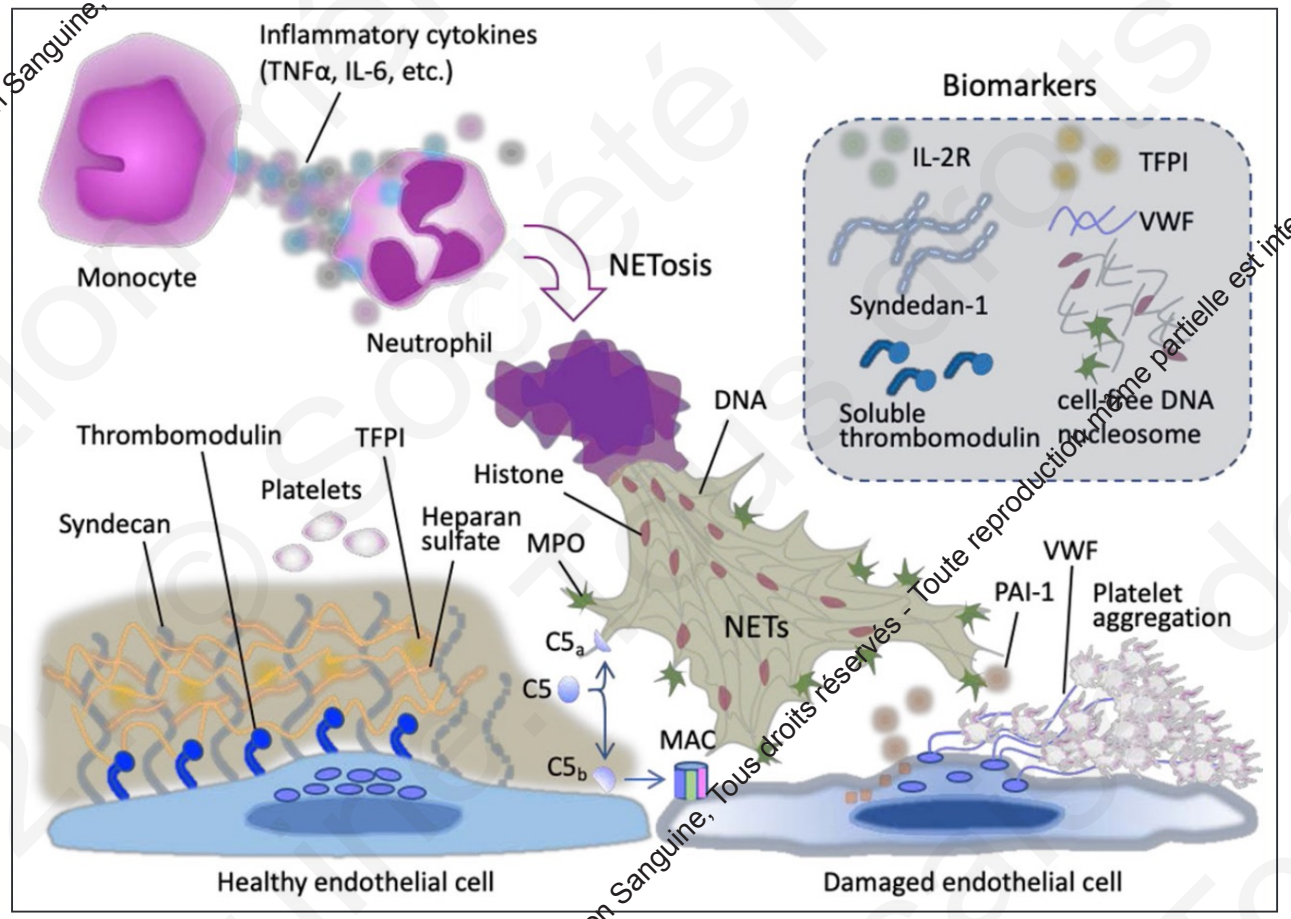
EDITORIAL

Endothelial Injury in COVID-19 and Acute Infections

Putting the Pieces of the Puzzle Together

See accompanying article on page 1750

Jerrold H. Levy, Toshiaki Iba, and Elizabeth E. Gardiner



Future investigations

- Evaluate in prospective studies the possibility of routinely measuring circulating levels of VWF as prognostic predictive markers of severe COVID-19
- Assess whether these mechanisms could be translated to pneumonia related to other viruses (Sars, Mers or Influenza),
- Determine whether therapeutic interventions targeting VWF may have an impact on patient's prognosis recombinant ADAMTS-13, caplacizumab
- Impact of viral mutations on endothelial damage

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