COVID coagulopathy
Resia Pretorius

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Photo by Anton Jordaan

Collaborators

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- Prof Douglas B Kell (University of Liverpool)
- Dr Amy Proal (Polybio Research Foundation)
- Dr Martin Kraeter (Max Planck institute)
- Dr Asad Khan (NHS Respiratory physician) and Dr Jaco Laubscher (Mediclinic: Stellenbosch)
- Dr Mare Vlok (Proteomics lab: Stellenbosch University)
- Prof Bruce Watson and Prof Maritha Kotze (Stellenbosch University)
- Dr Arneaux Kruger (Clinician and MSc student: Stellenbosch University)
- Dr Chantelle Venter (Blood lab manager: Stellenbosch University)
- Massimo Nunes (PhD student: Stellenbosch University)
- Simone Turner (MSc student: Stellenbosch University and Biocode Technologies)
- Tom Usher (MSc student: Stellenbosch University)

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Research Focus

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- Inflammatory molecules that may cause pathological clotting, including both viral and bacterial inflammagens

- Effects of circulating inflammatory molecules on platelets, RBCs and fibrin(ogen) the main clotting protein

- Development of novel methods to study clotting pathology

Platelets, circulating inflammatory molecules and abnormal blood clotting

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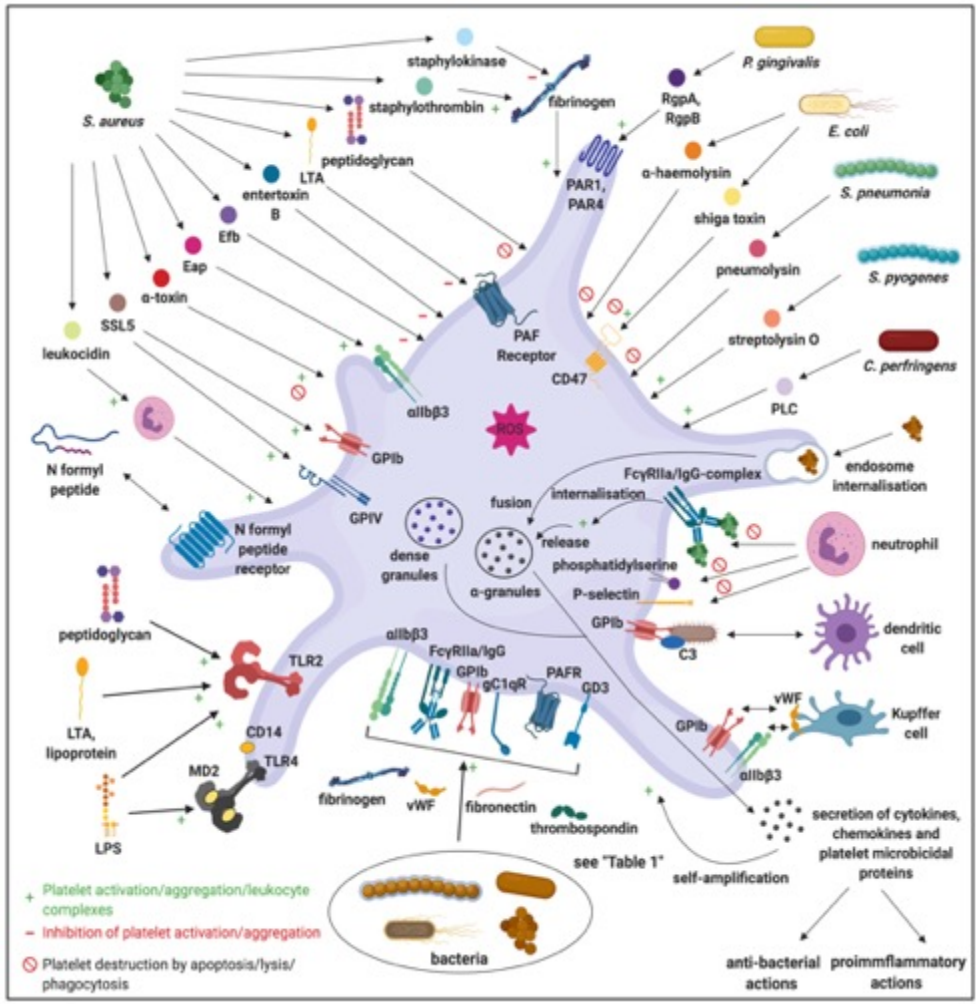
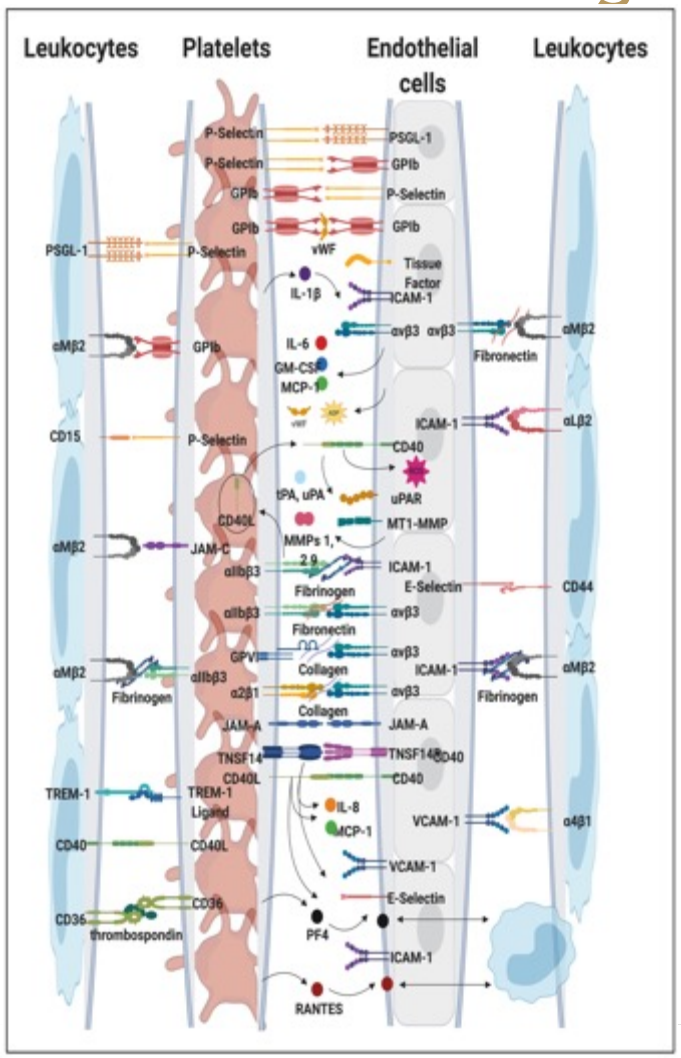
Platelet receptors

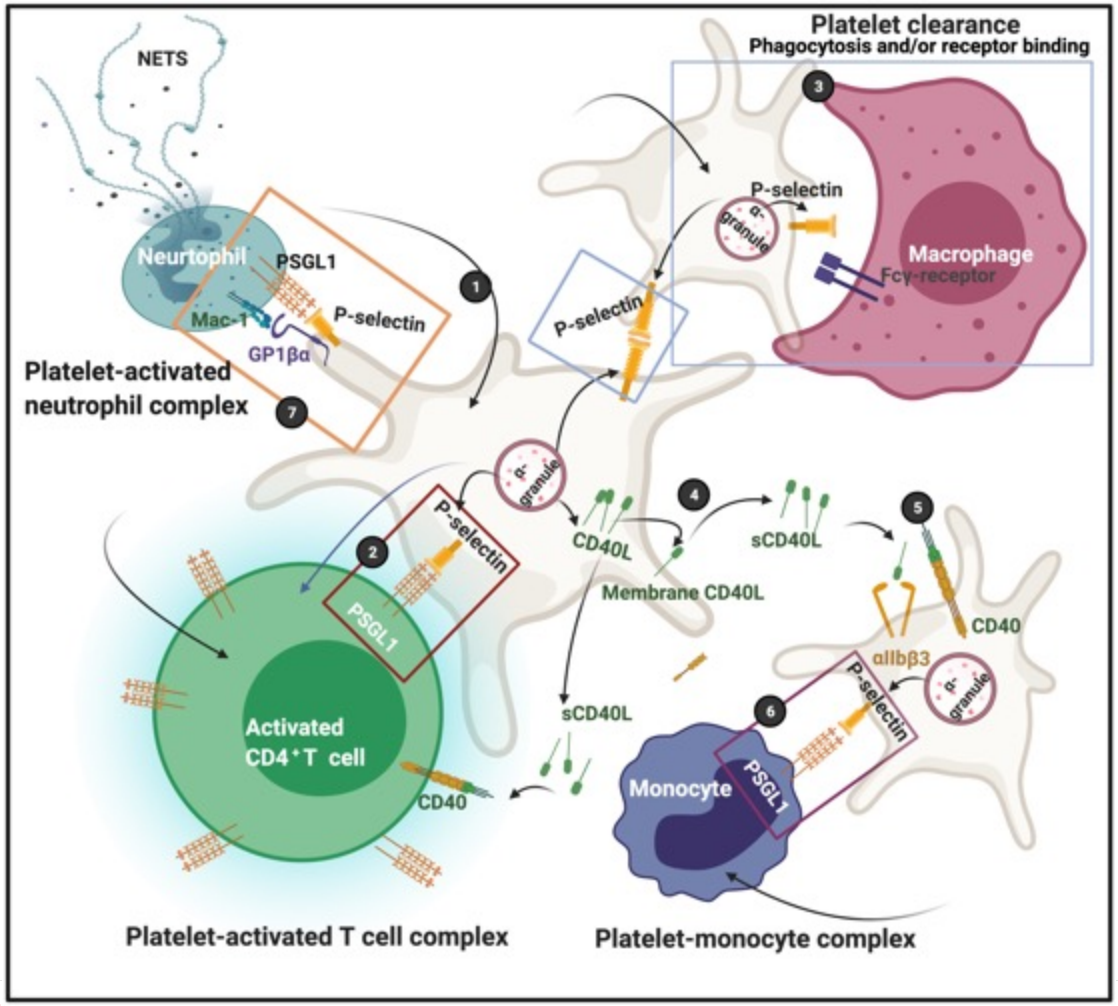


Platelet/endothelial cell signalling

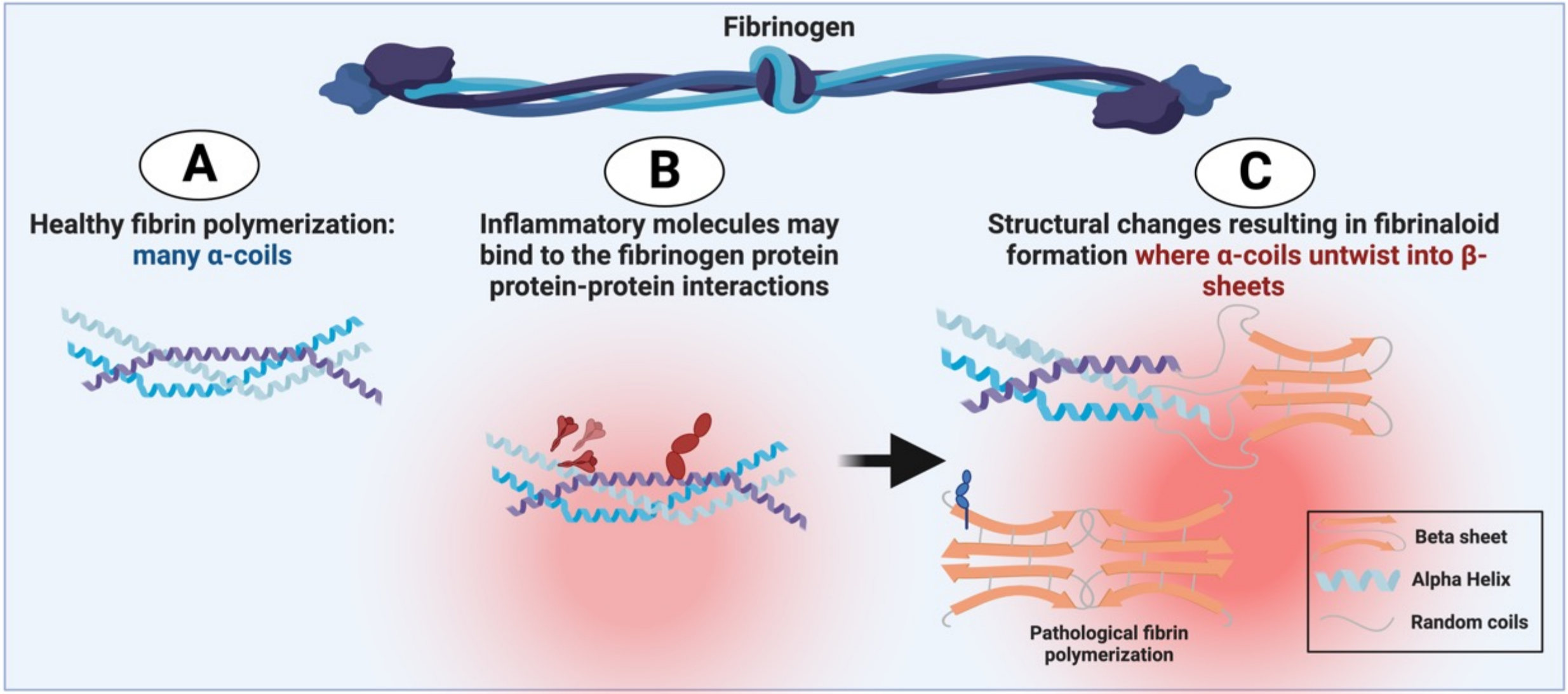


Platelets Interactions

Platelet-Immune Cell Complexes

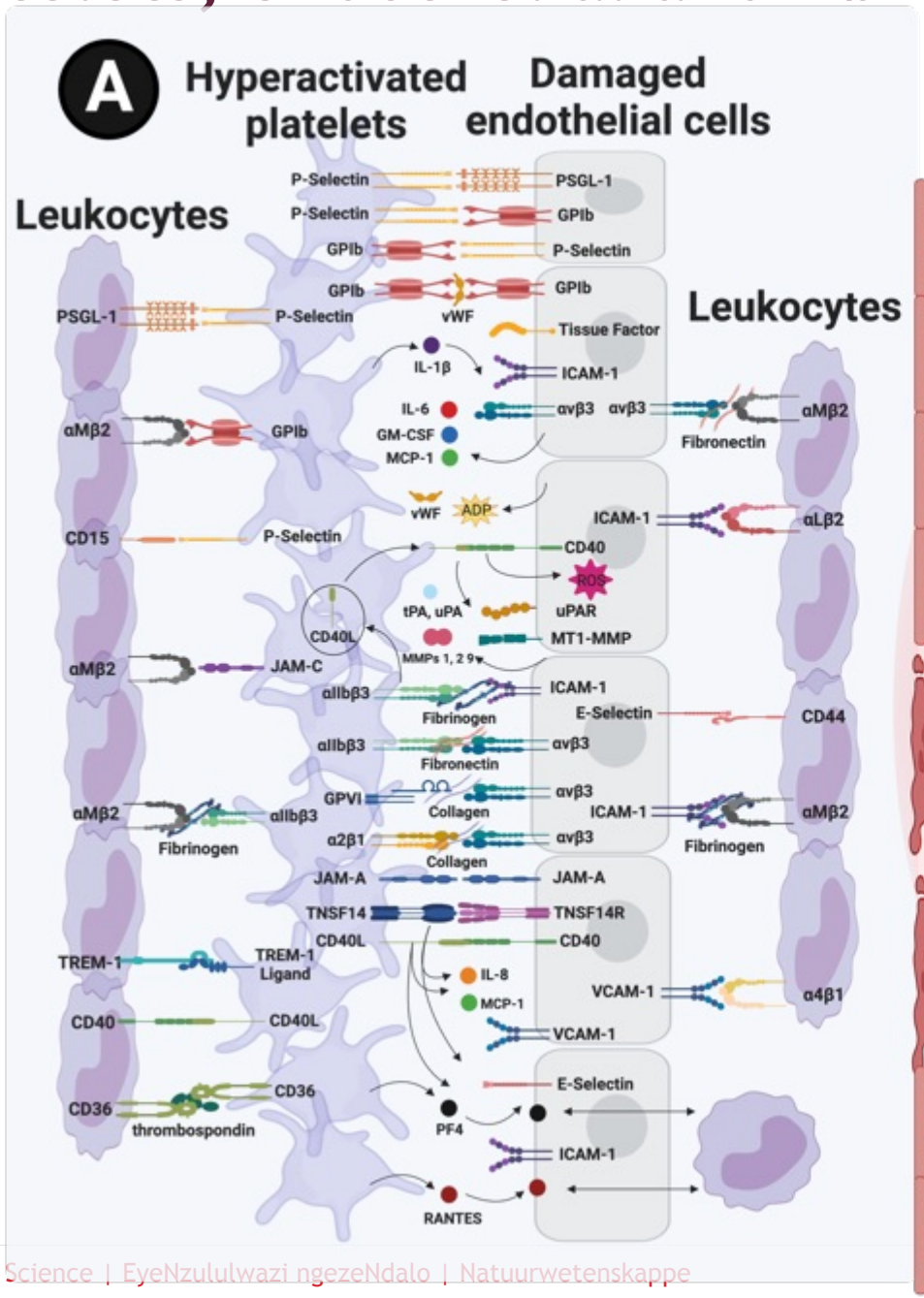


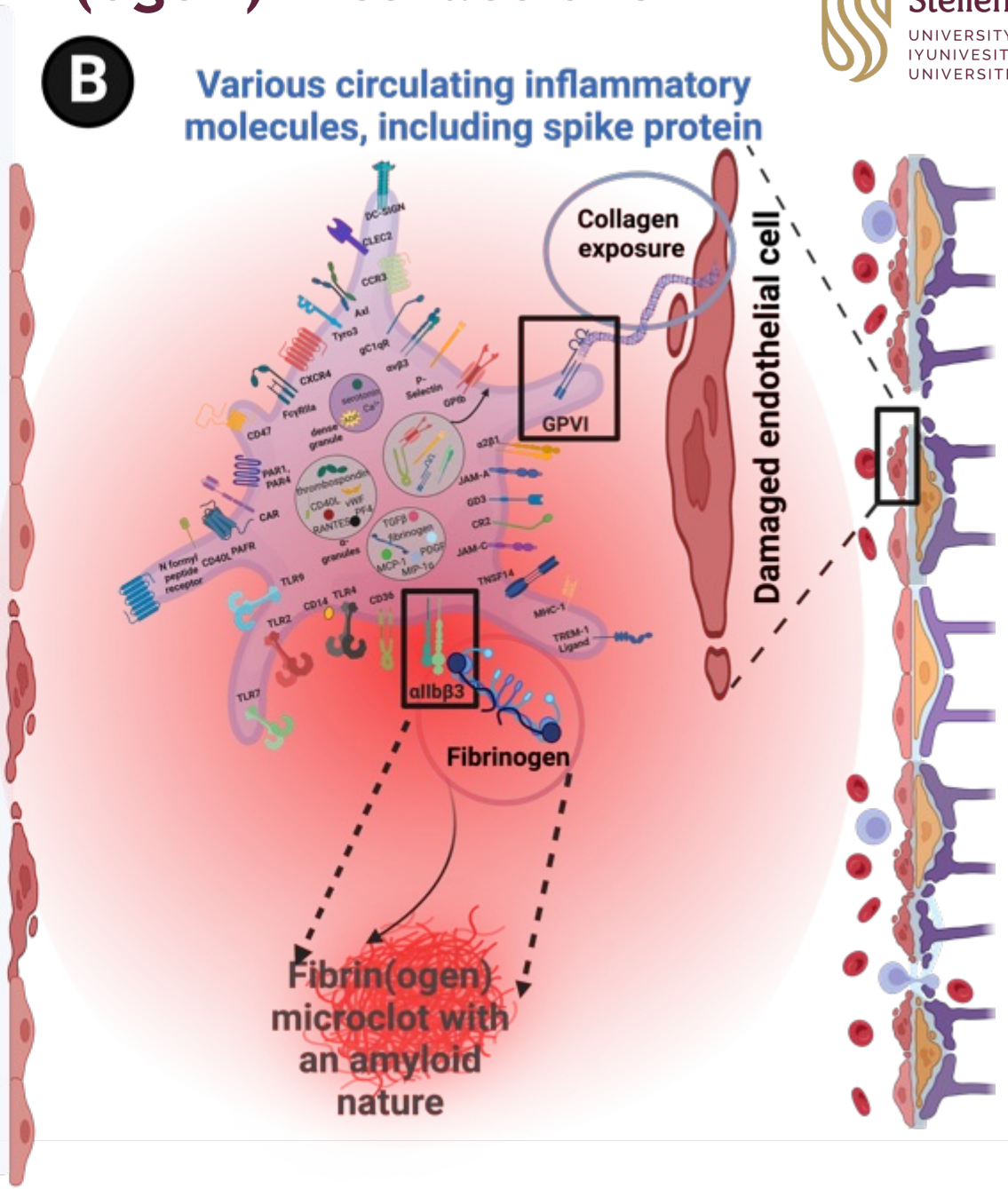
Pathological Clotting



Kell DB, Pretorius E. Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. *Progress in Biophysics and Molecular Biology* 2017; 123: 16-41.

Platelets, endothelial and fibrin(ogen) interactions





Long COVID estimates: why should we worry?

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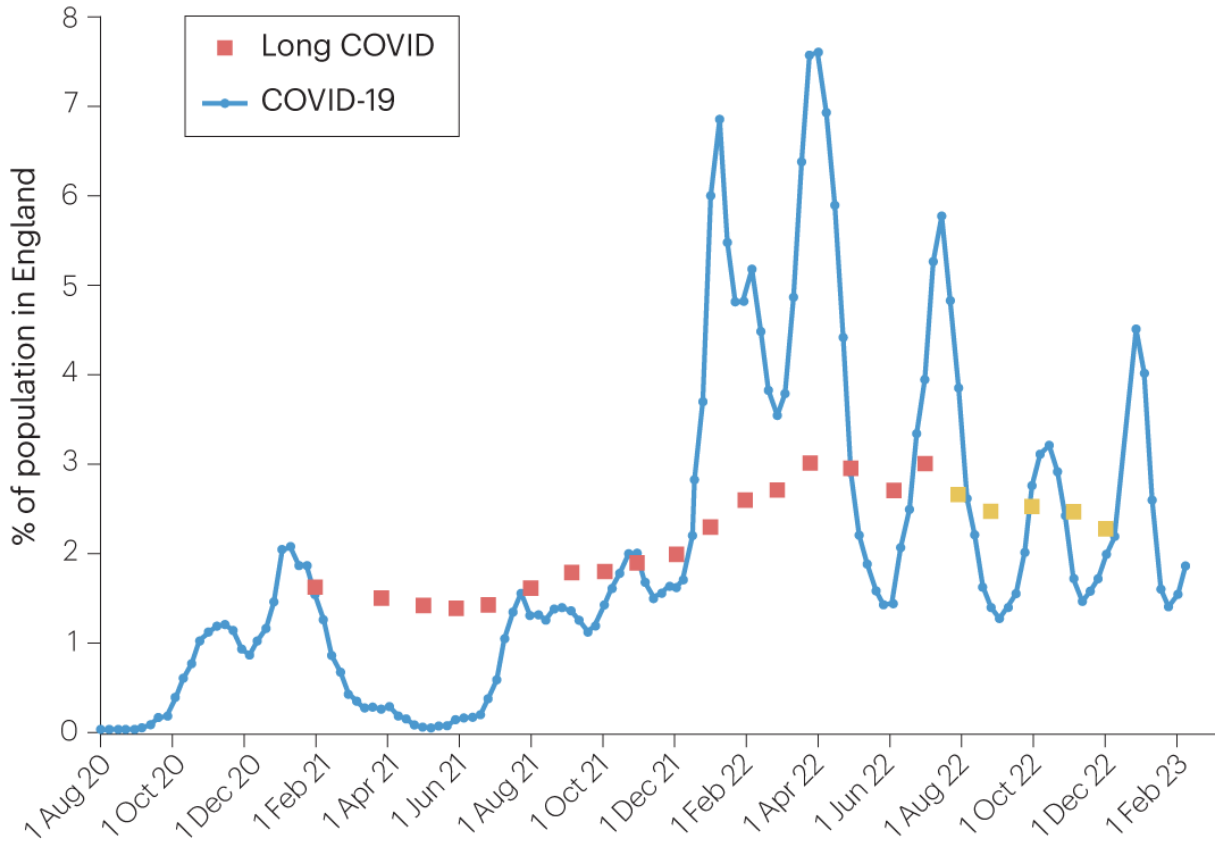
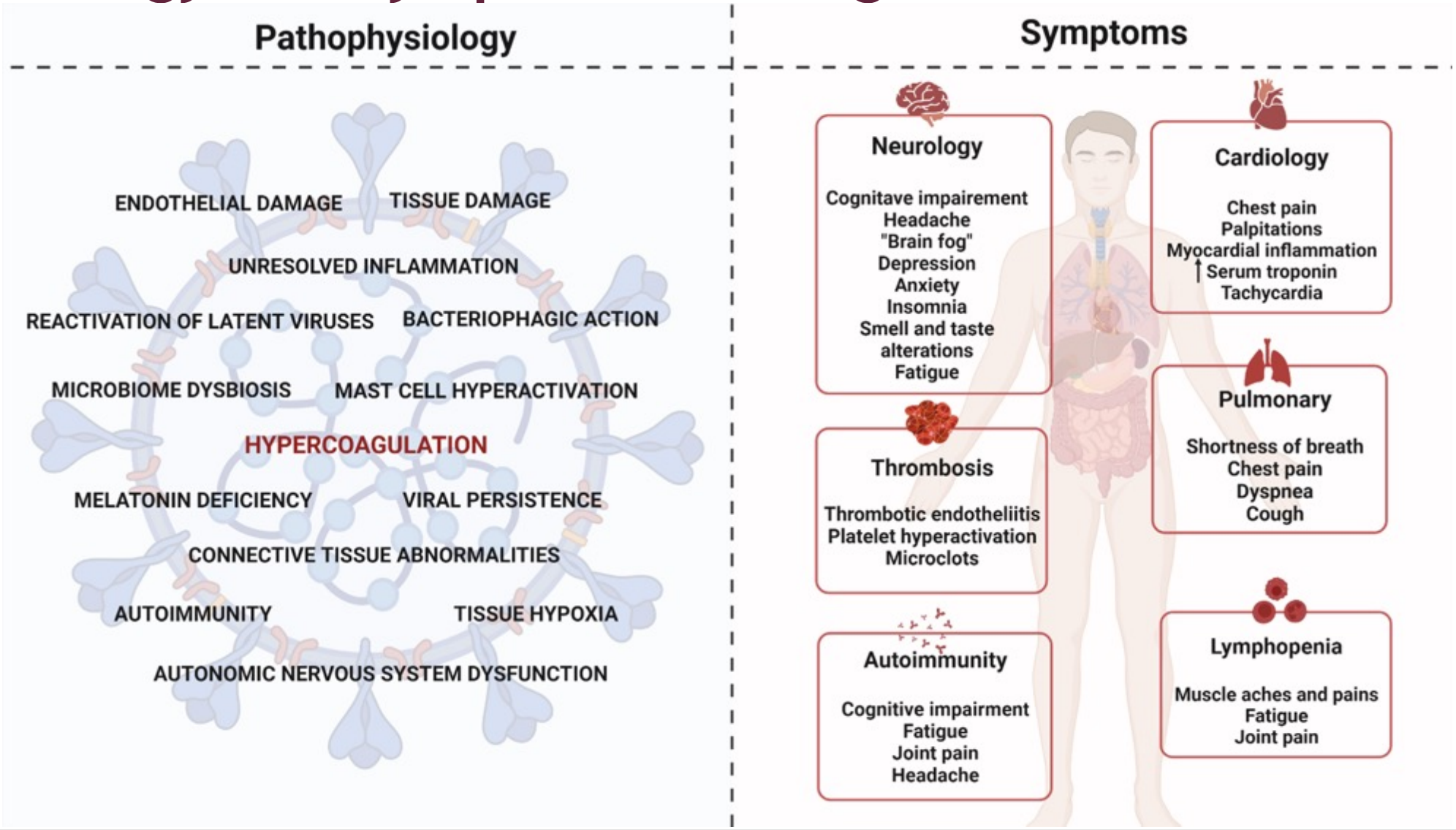
Altmann, D.M., Whettlock, E.M., Liu, S., Arachchillage, D.J., and Boyton, R.J. (2023). The immunology of long COVID. Nature Reviews Immunology *23*, 618-634.

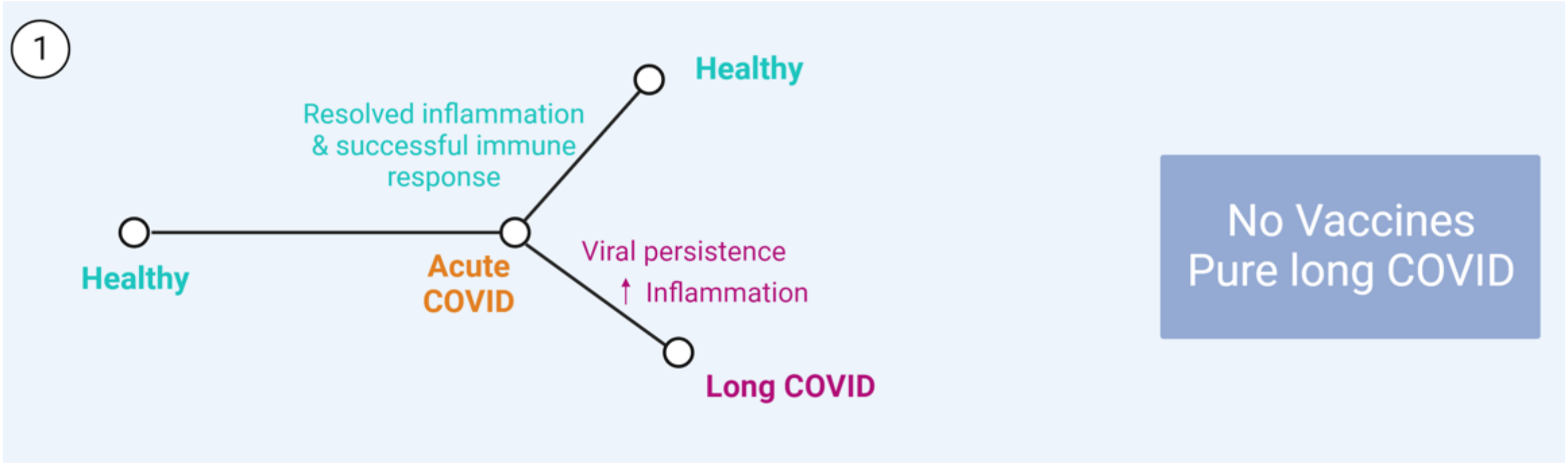


- Lower estimate 65M, upper, 100 of millions
- “Taking a conservative estimate of 5% suffering long term sequelae gets us to 100s of millions”

The most vulnerable individuals: Pathology and Symptoms of Long COVID



Long COVID



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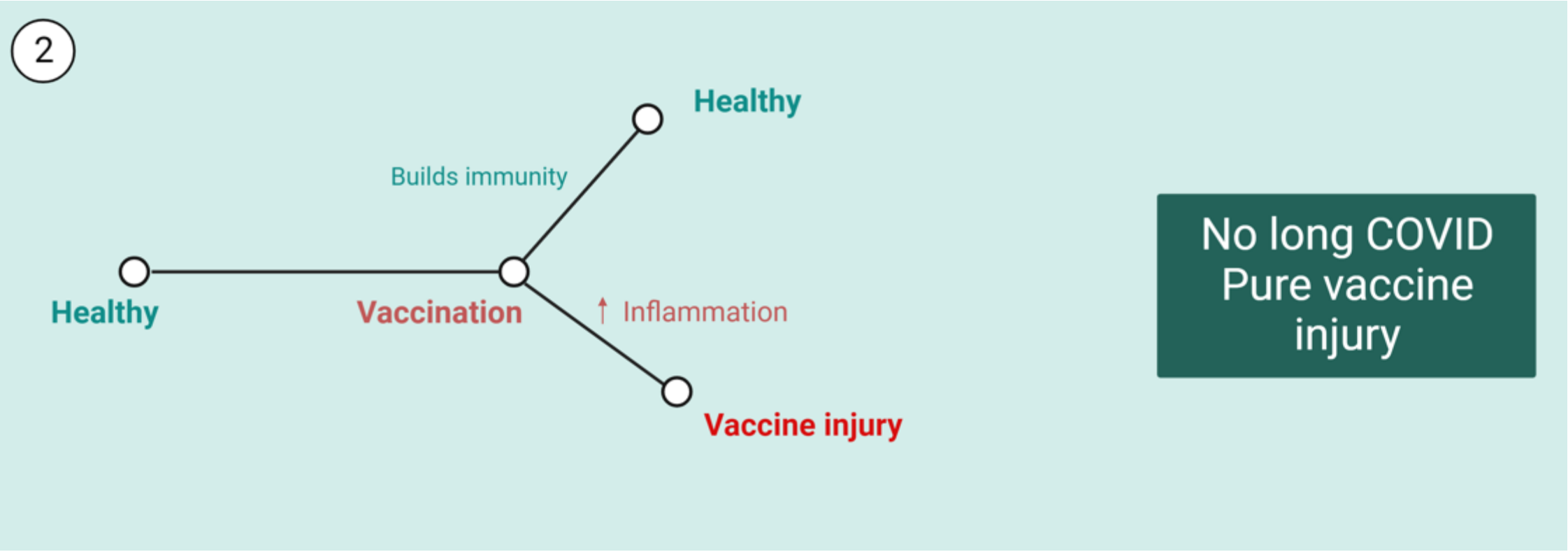
Vaccine Injury



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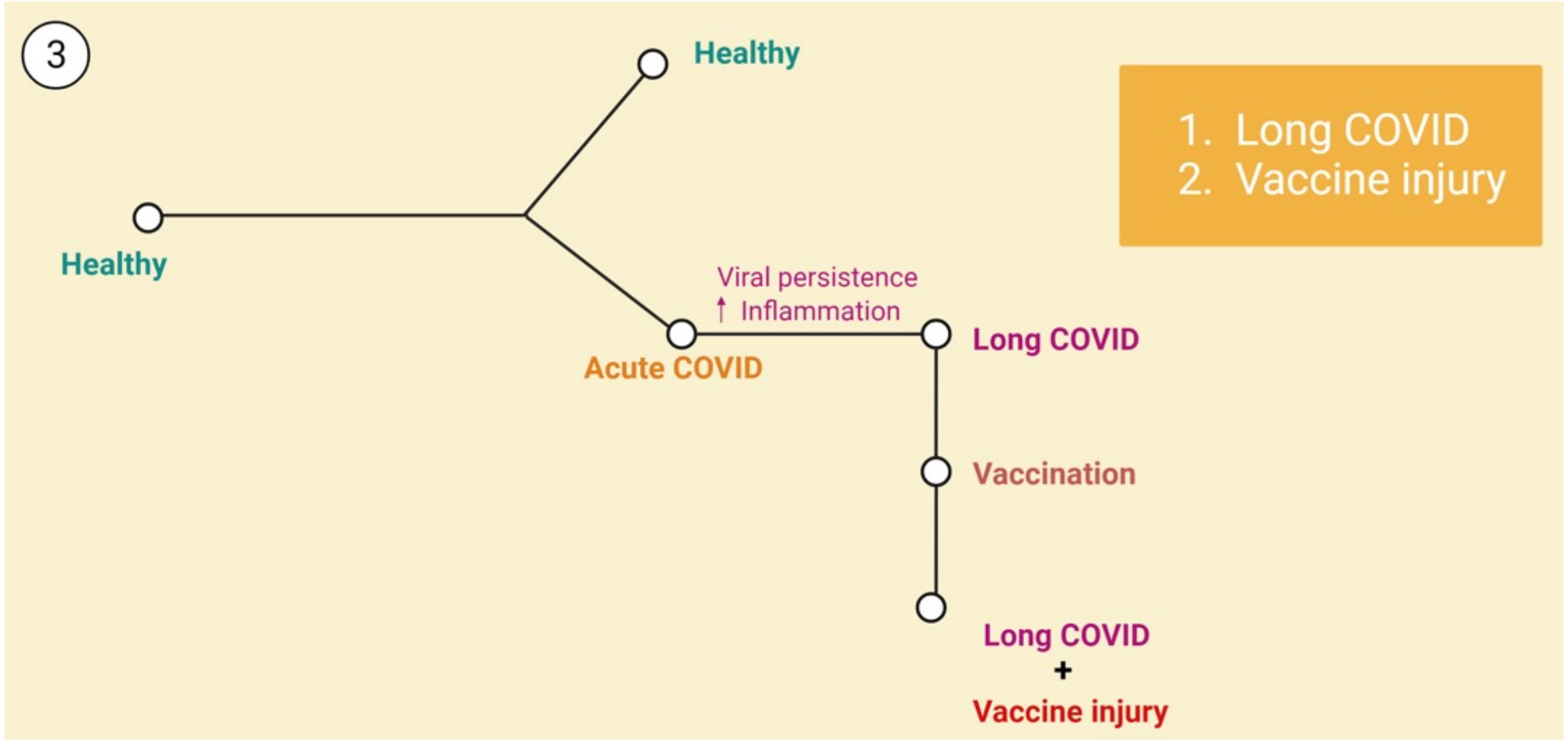
Long COVID + Vaccine Injury



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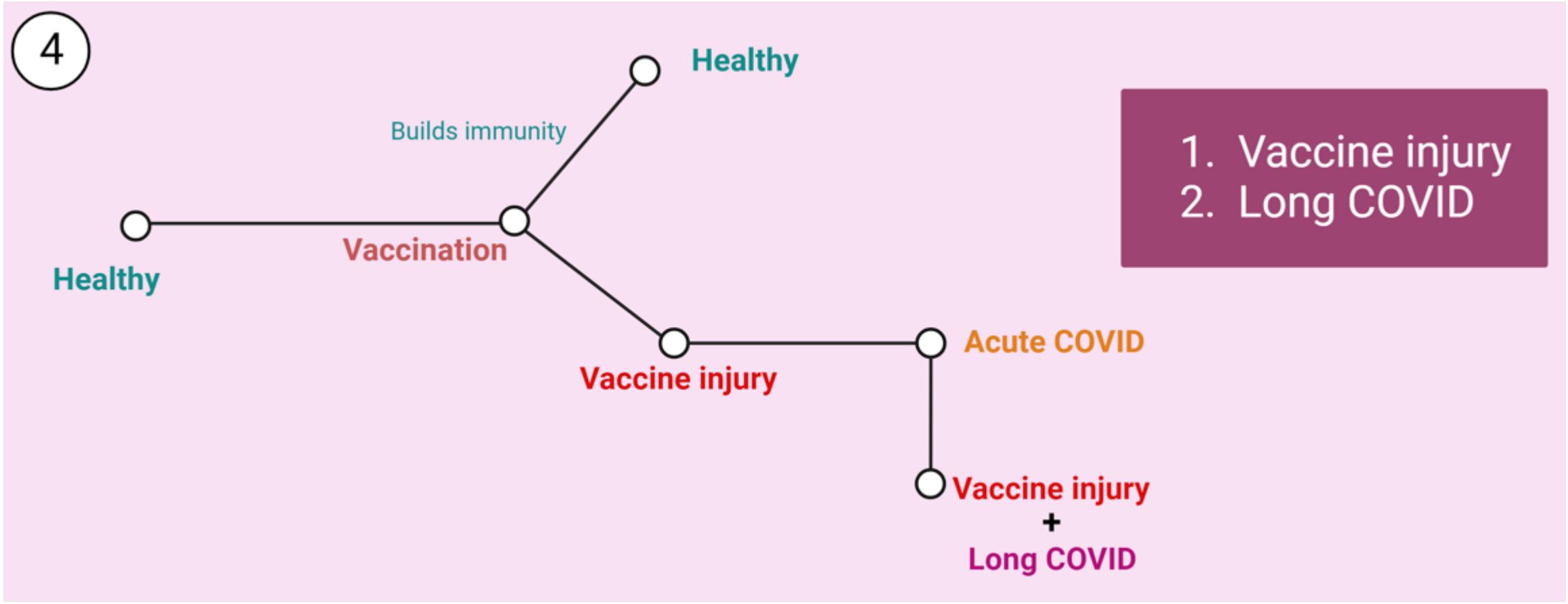
Vaccine Injury + Long COVID



Curtesy Maxine Waters

We need to look at acute COVID to understand the clotting pathologies and thrombotic endothelialitis in Long COVID

Platelet and clotting pathologies in acute COVID-19

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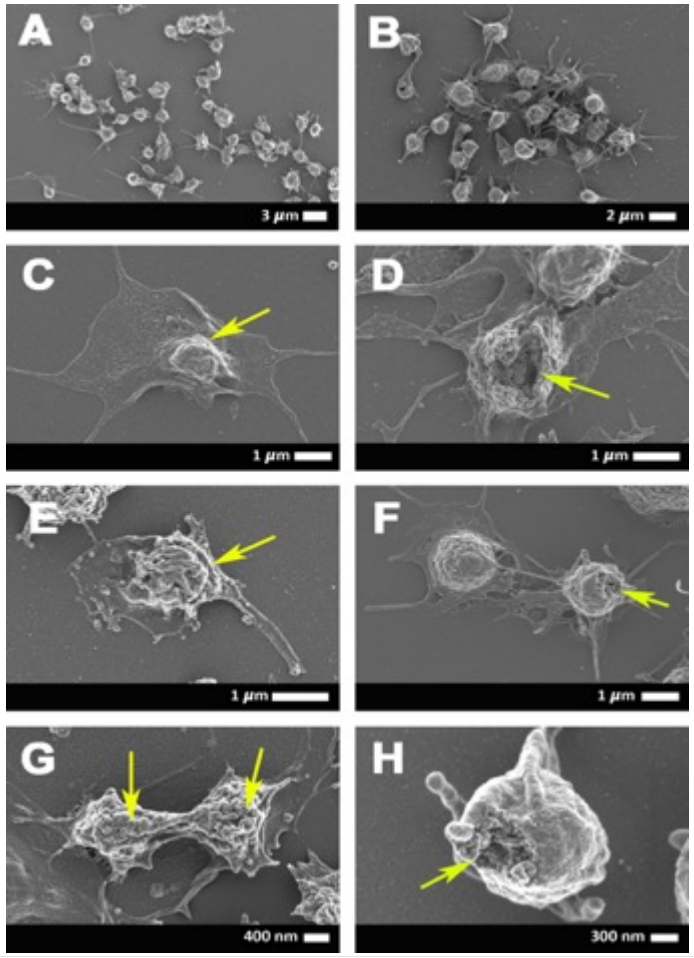
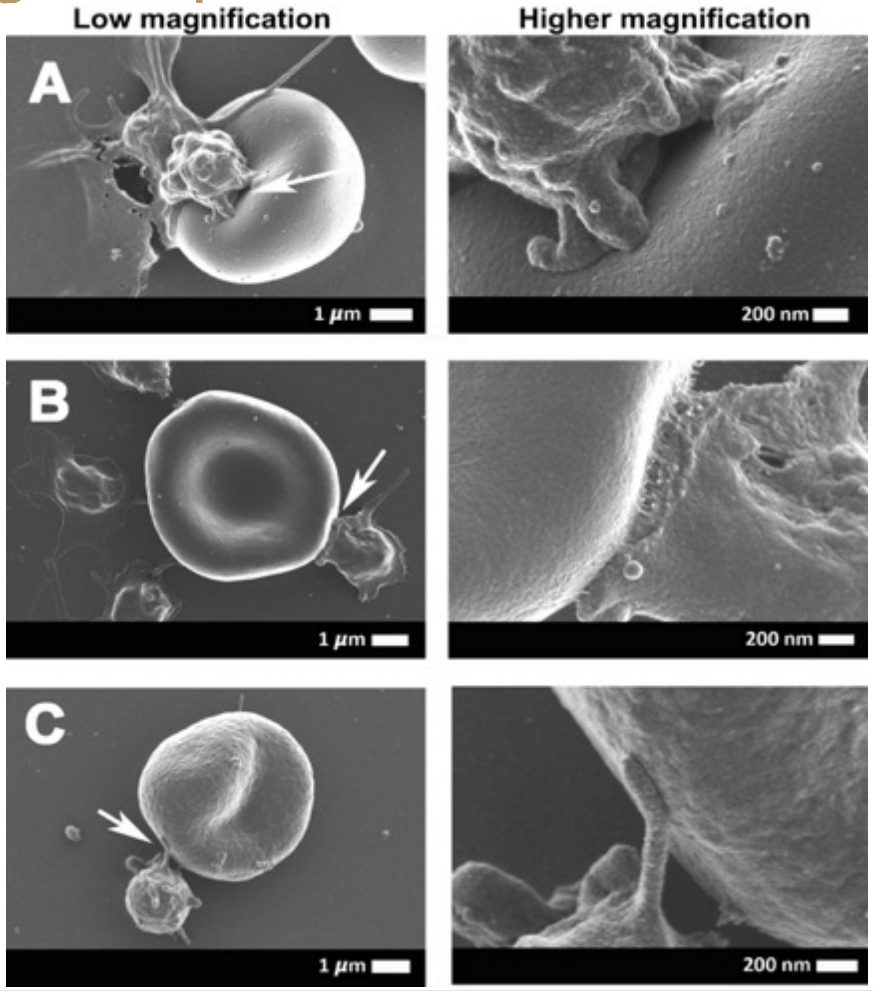
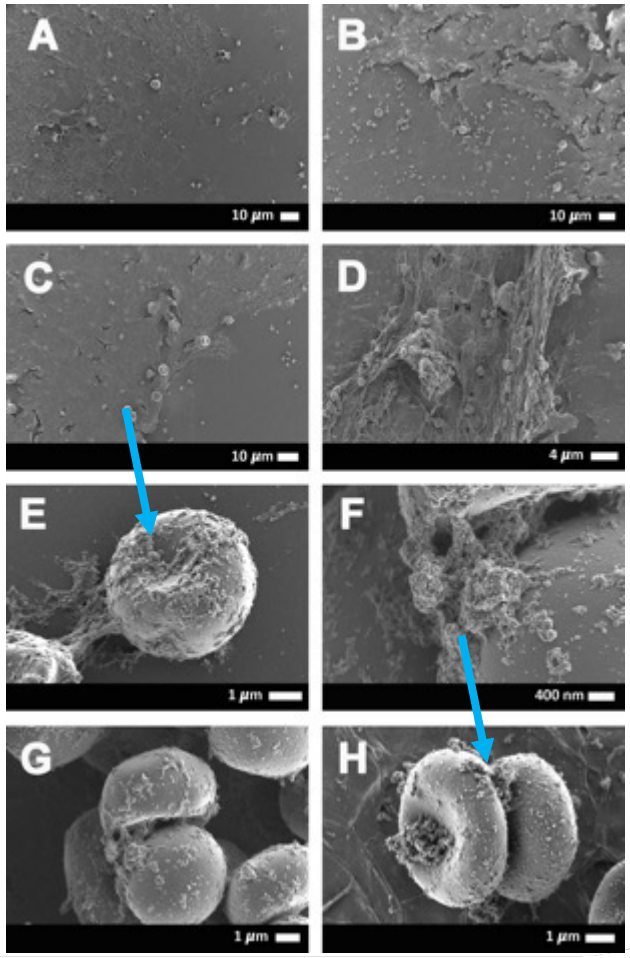
Structural changes in platelets





Microclots



Structural Changes in Fibrin(ogen)

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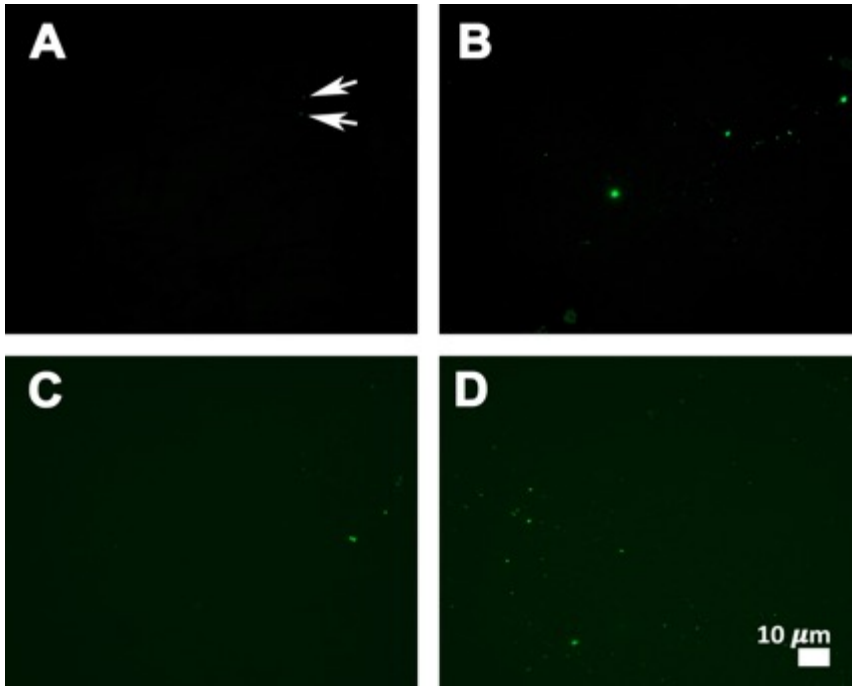
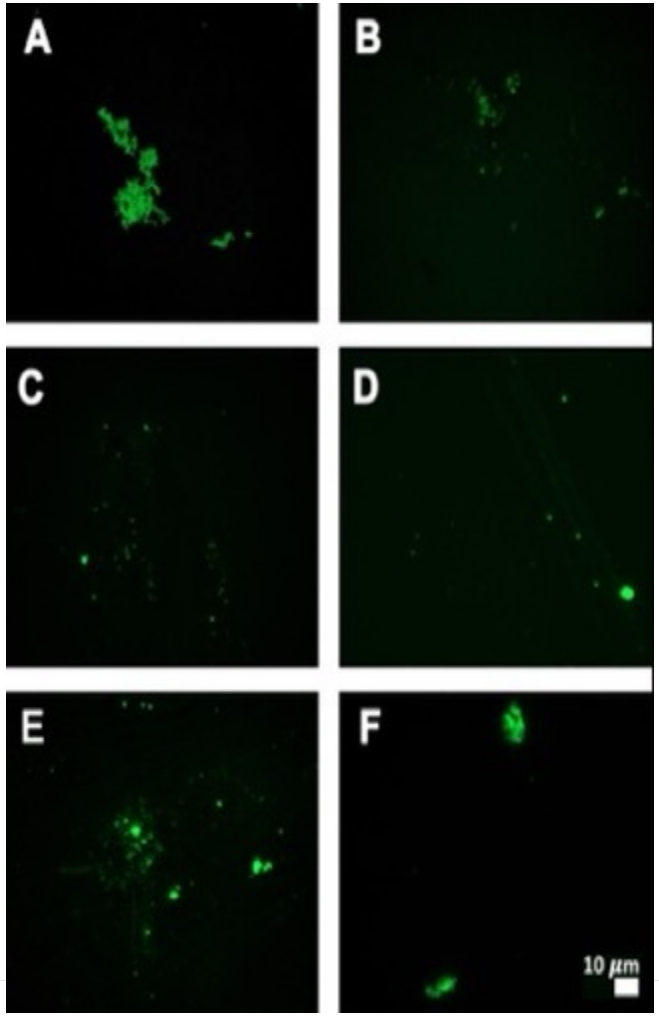
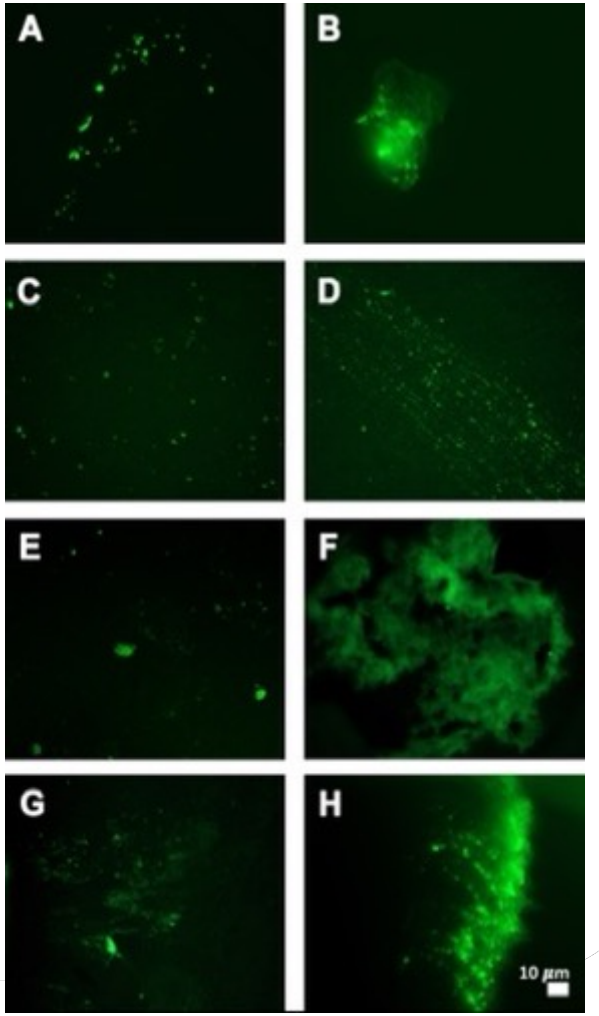
Healthy Plasma



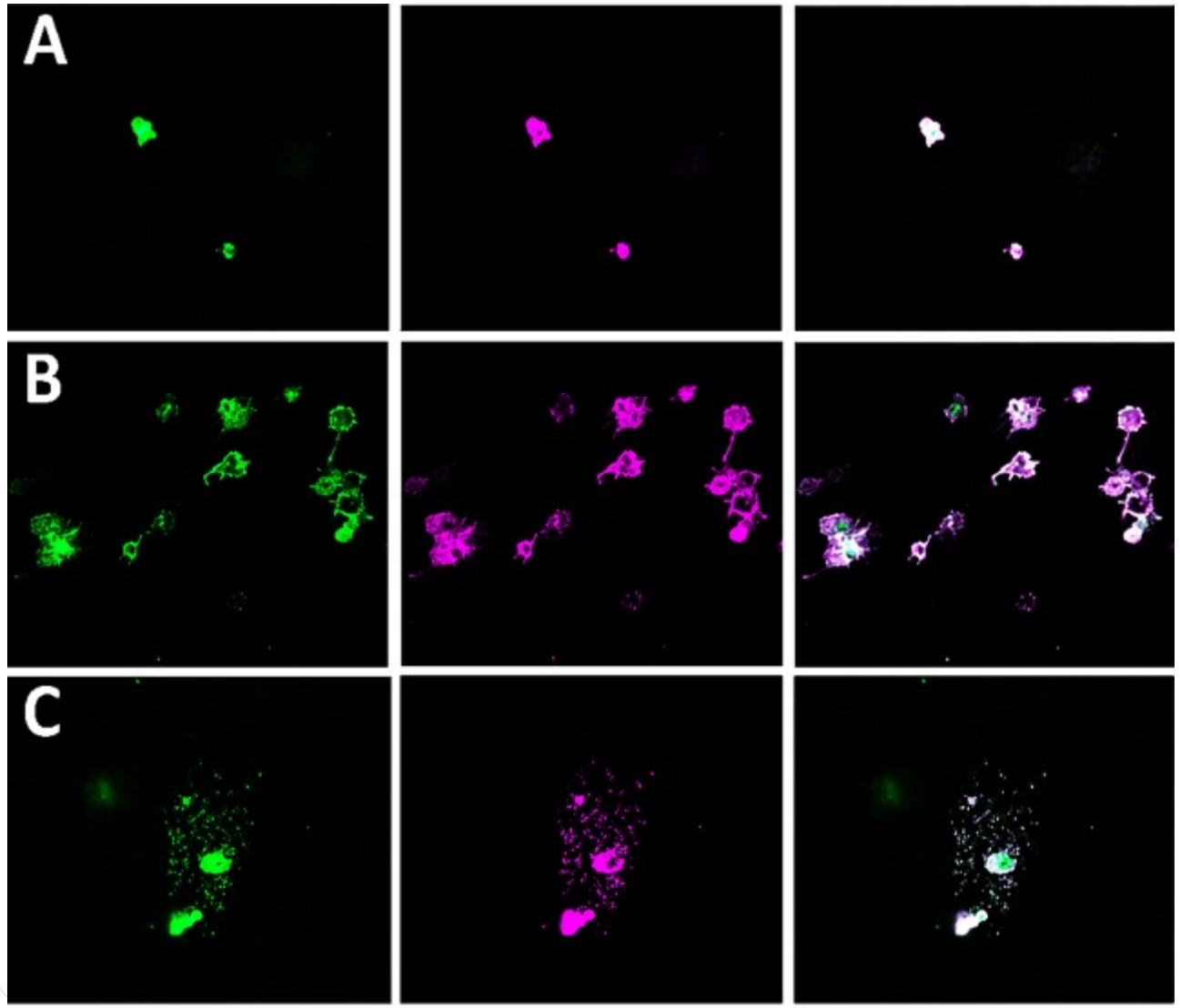
Type 2 Diabetes Plasma

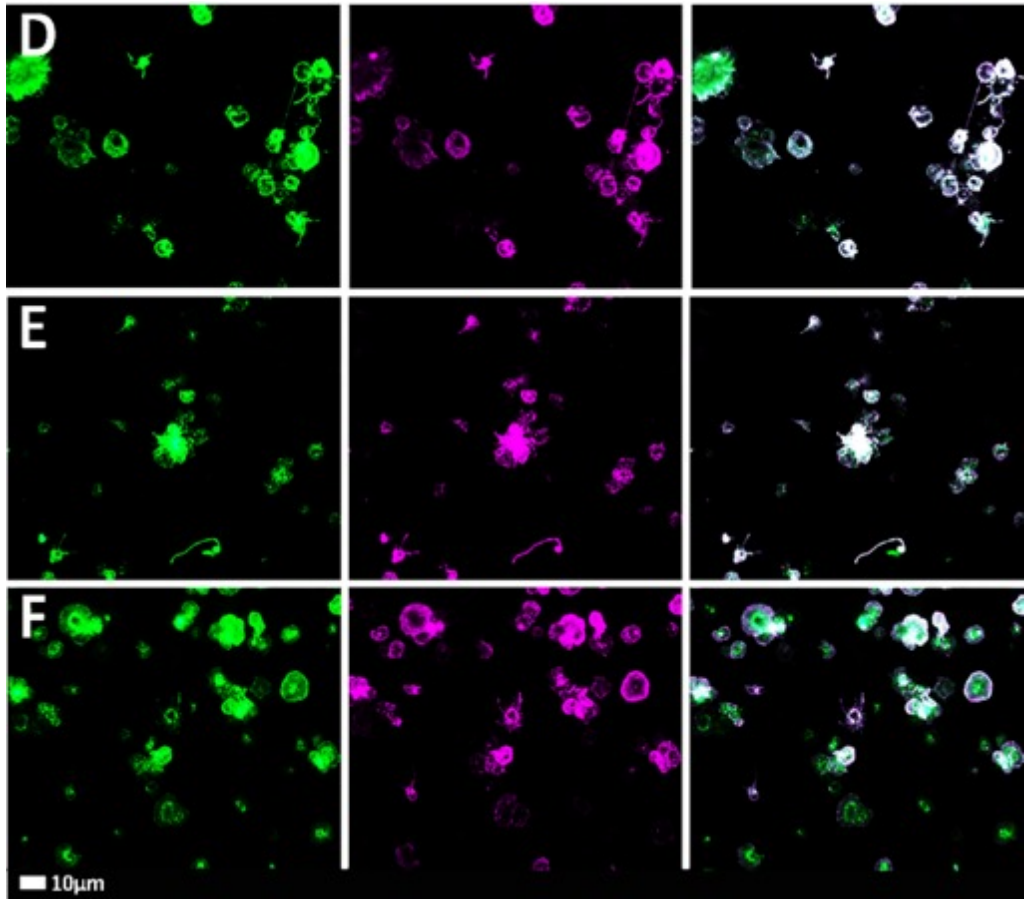


COVID-19 Plasma



Platelets in controls (A) and Acute COVID-19 (B-F)

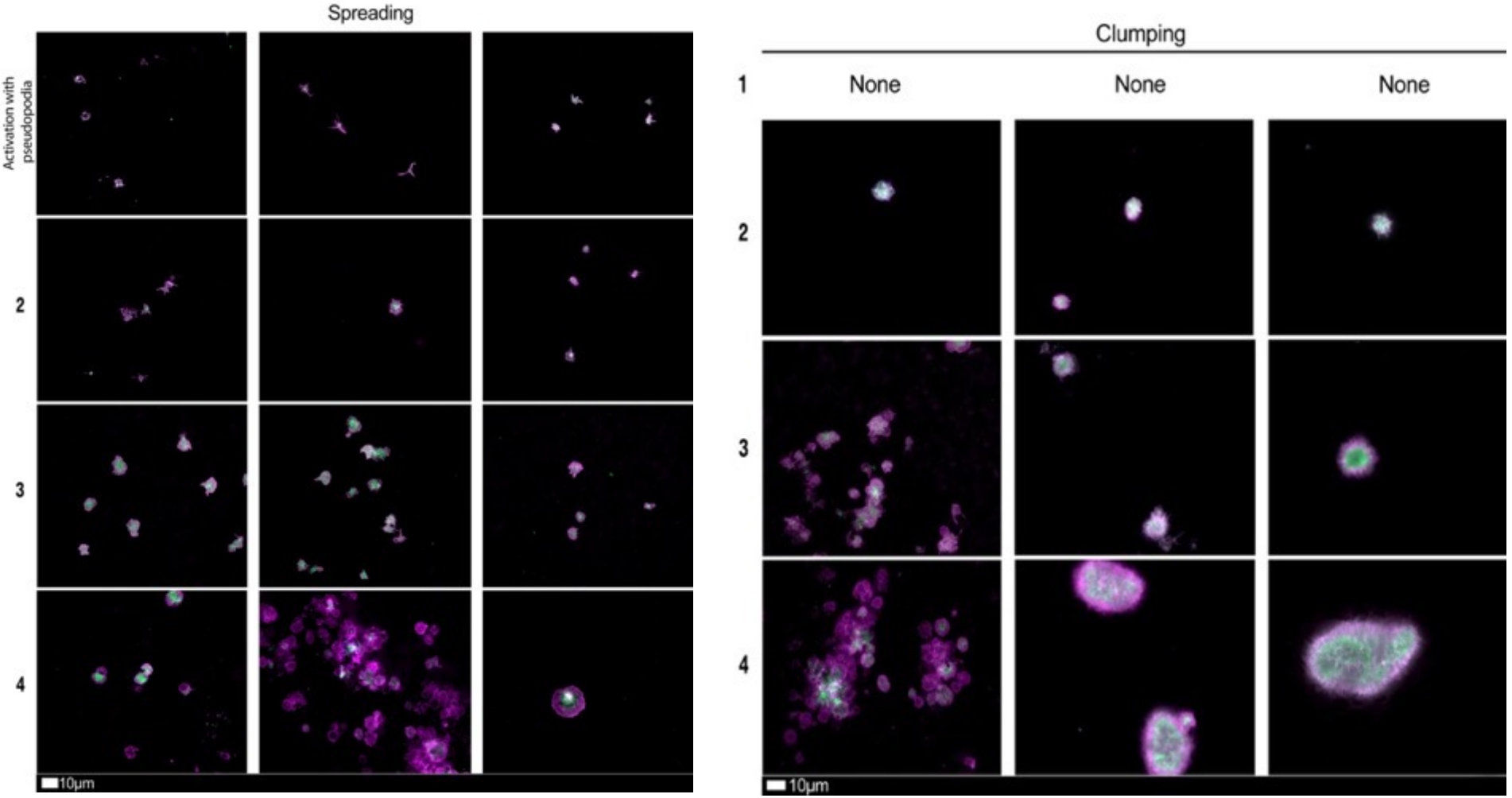




CD62P (PE-conjugated) (Pinkish signal) = P-selectin

PAC-1 (green signal) = glycoprotein IIb/IIIa on the platelet membrane.

A Platelet Grading System



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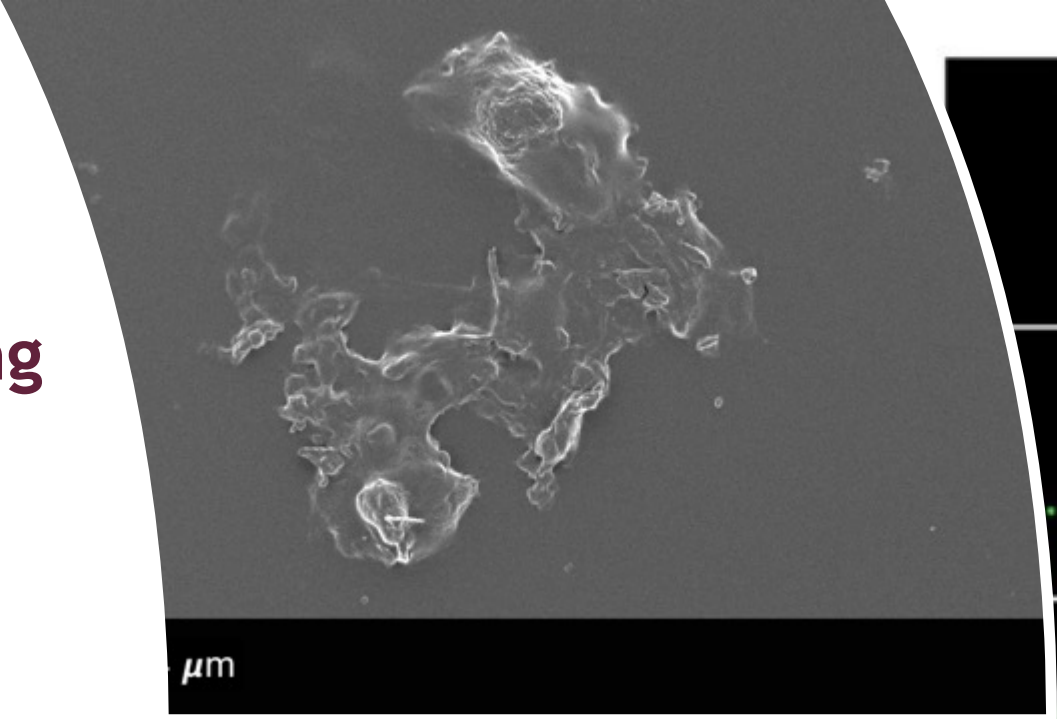
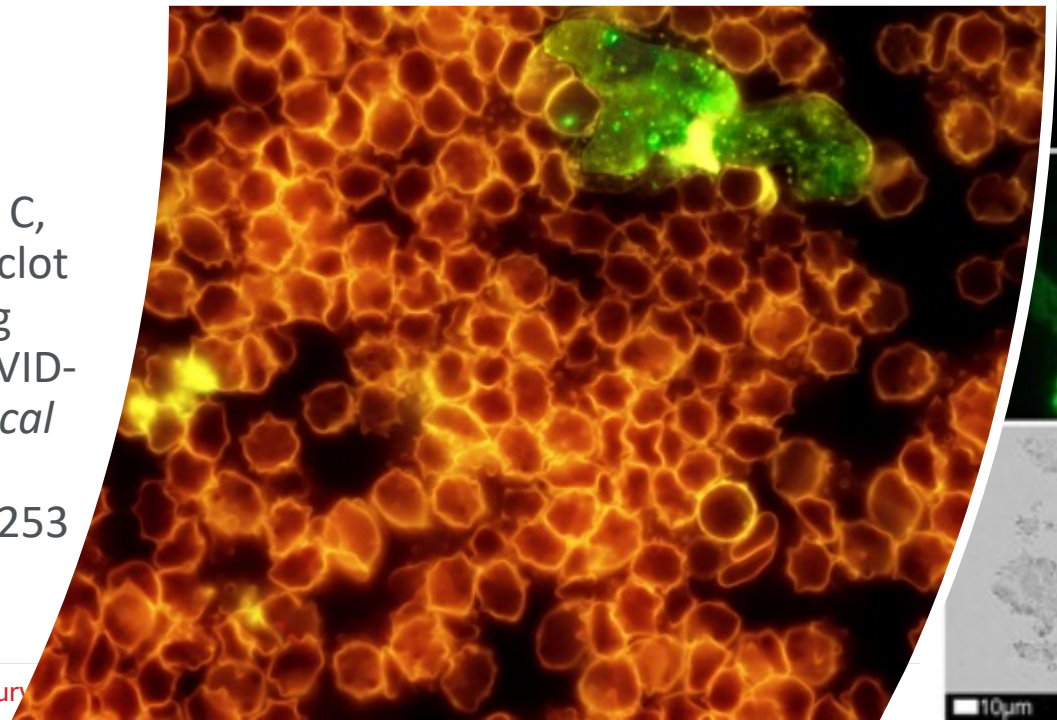
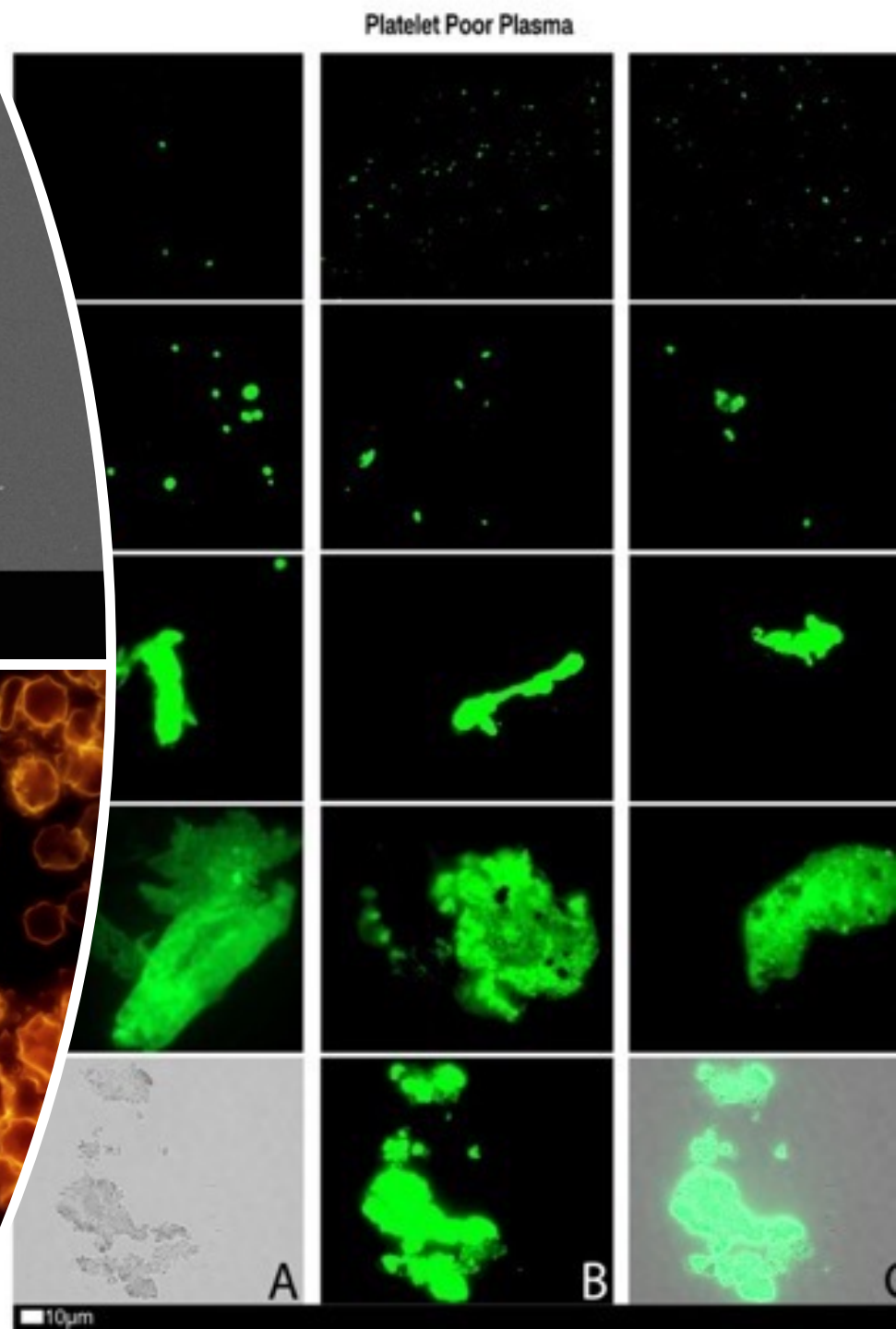
Laubscher GJ, Lourens PJ, Venter C, Kell DB, Pretorius E. TEG®, Microclot and Platelet Mapping for Guiding Early Management of Severe COVID-19 Coagulopathy. *Journal of Clinical Medicine*. 2021;10(22)doi:10.3390/jcm10225381

A microclot grading system: fluorescence microscopy







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SA Long COVID registry data

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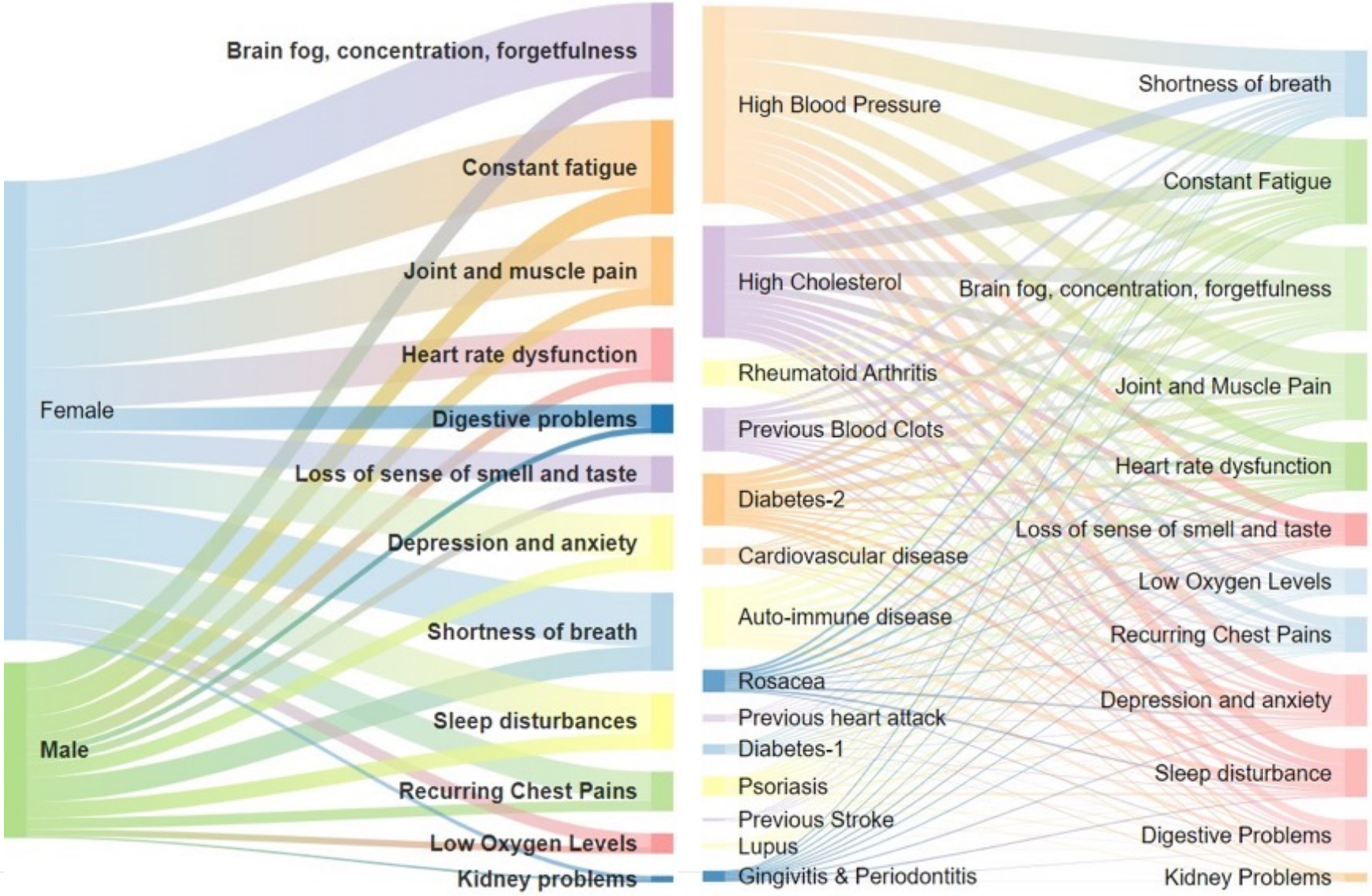
Symptoms

Co-morbidities



Pretorius, E., Venter, C., Laubscher, G.J., Kotze, M.J., Oladejo, S.O., Watson, L.R., Rajaratnam, K., Watson, B.W., and Kell, D.B. (2022). Prevalence of symptoms, comorbidities, fibrin amyloid microclots and platelet pathology in individuals with Long COVID/Post-Acute Sequelae of COVID-19 (PASC). Cardiovascular Diabetology *21*, 148.

Platelet ultrastructure: Healthy versus Long COVID

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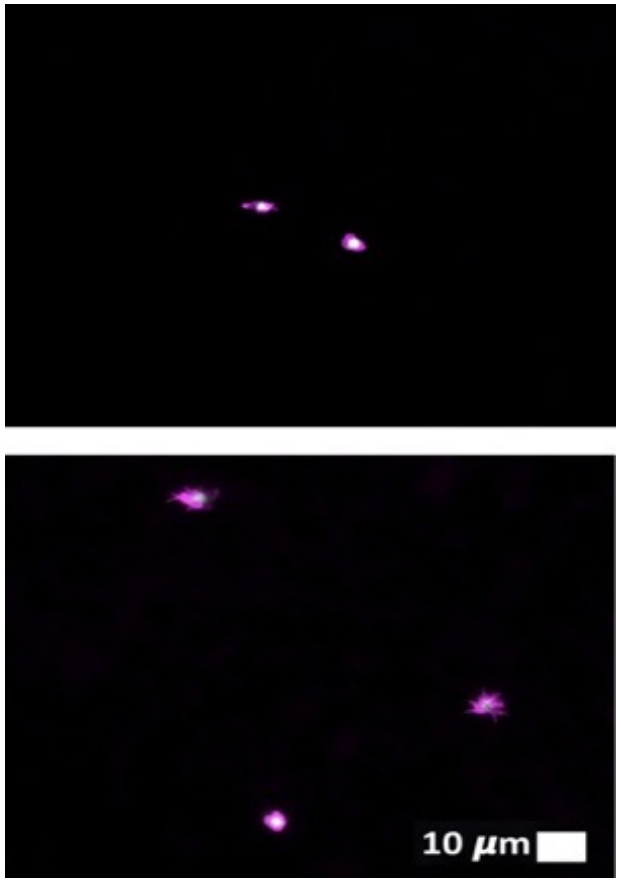
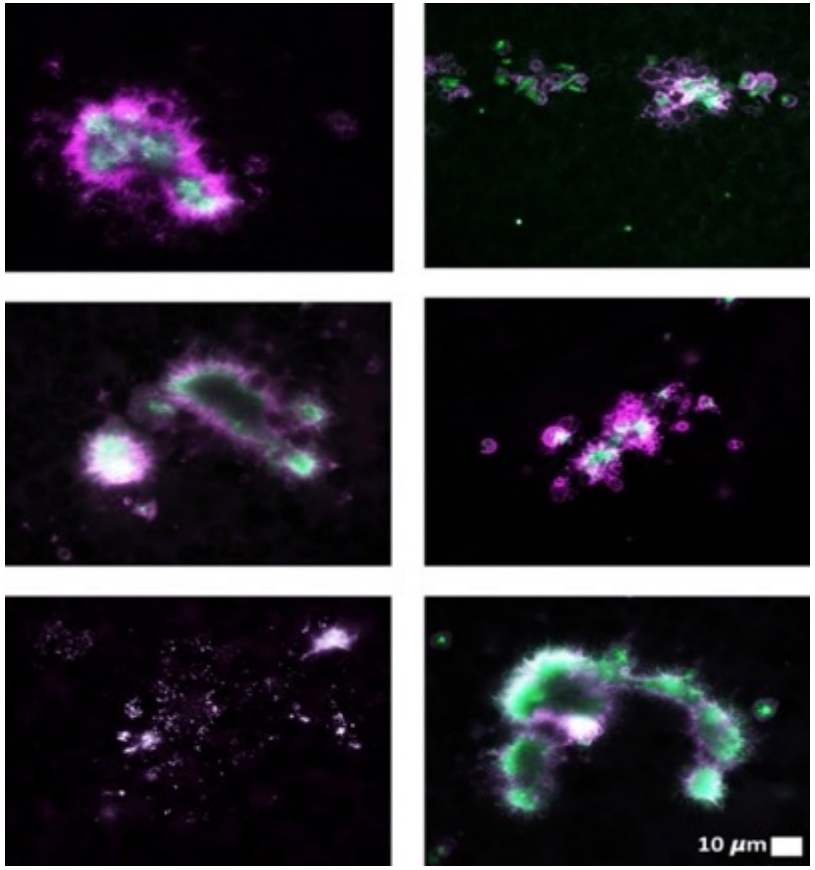
Platelets from healthy individuals



Platelets from individuals with Long COVID



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Platelet ultrastructure: healthy versus Long COVID

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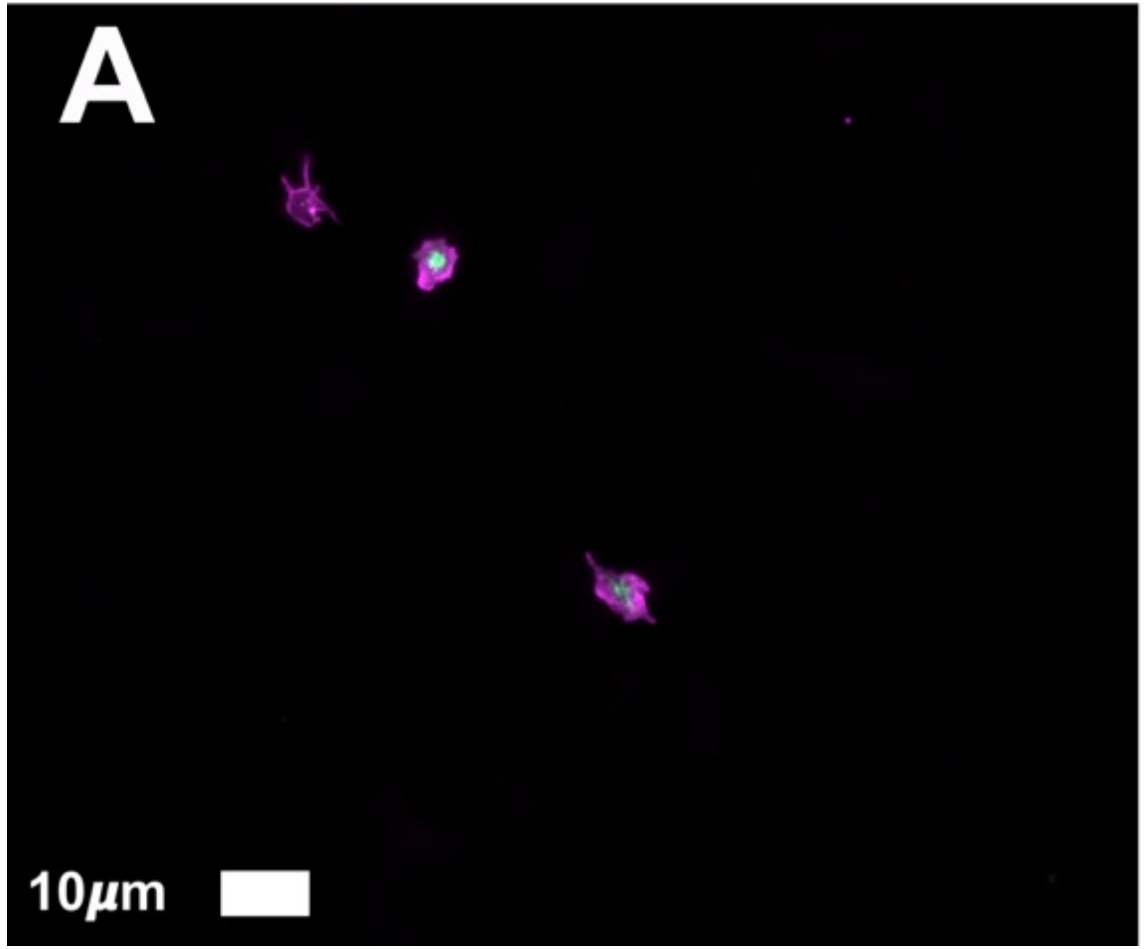
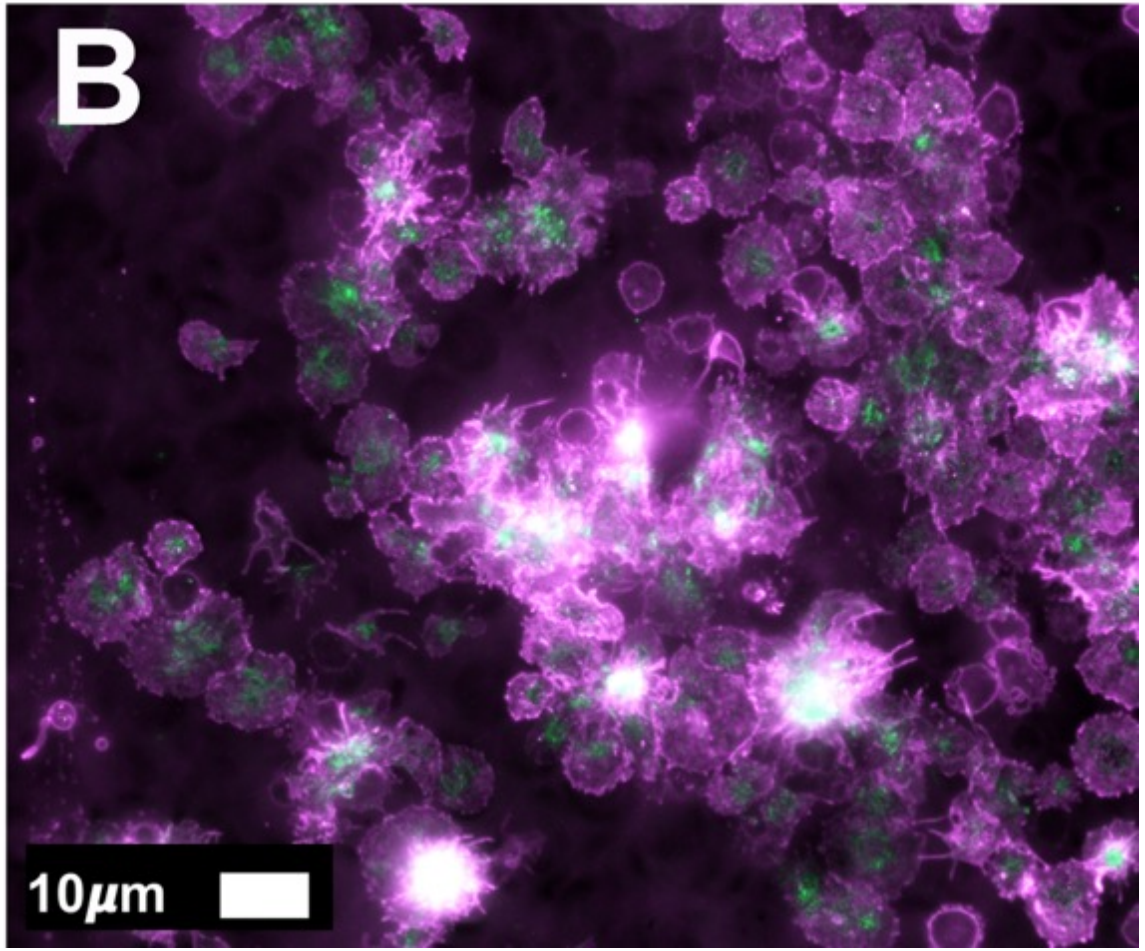
Healthy platelets



Platelets in Long COVID



Platelet ultrastructure: Healthy vs Long COVID

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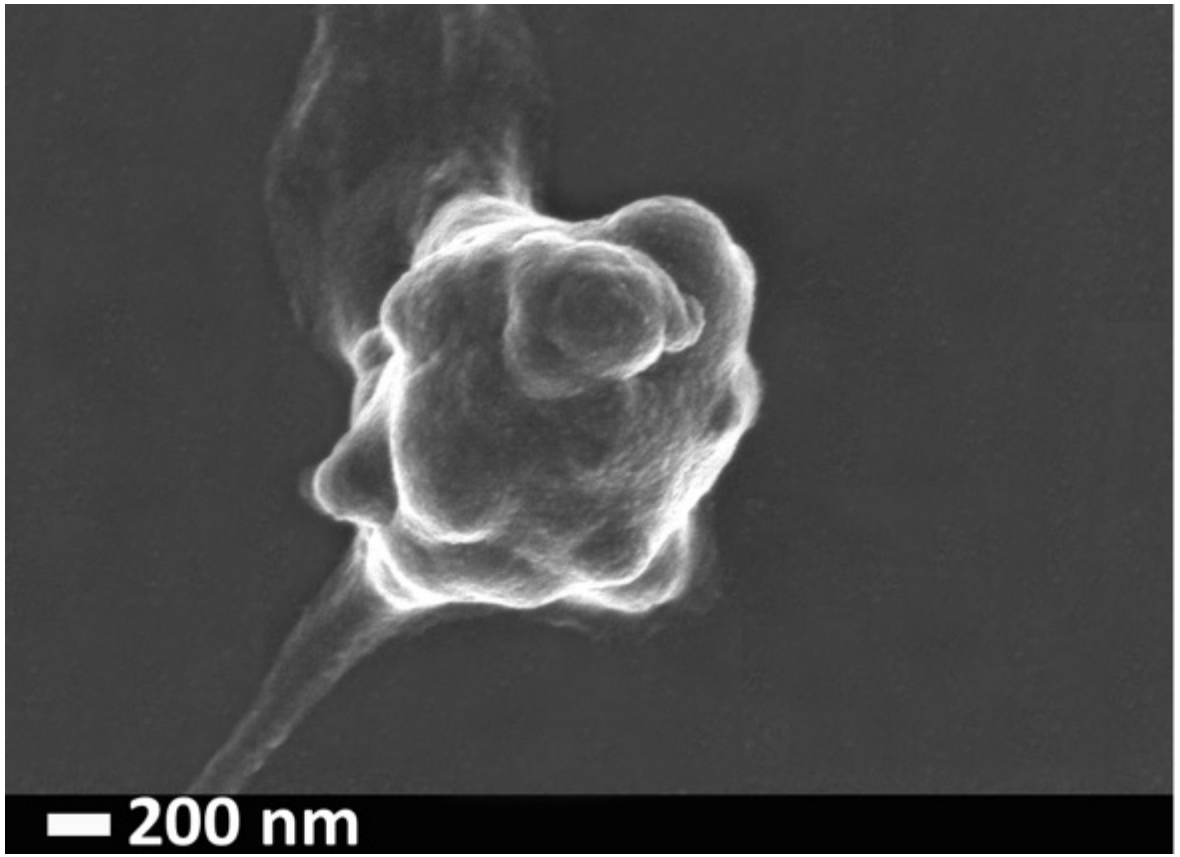
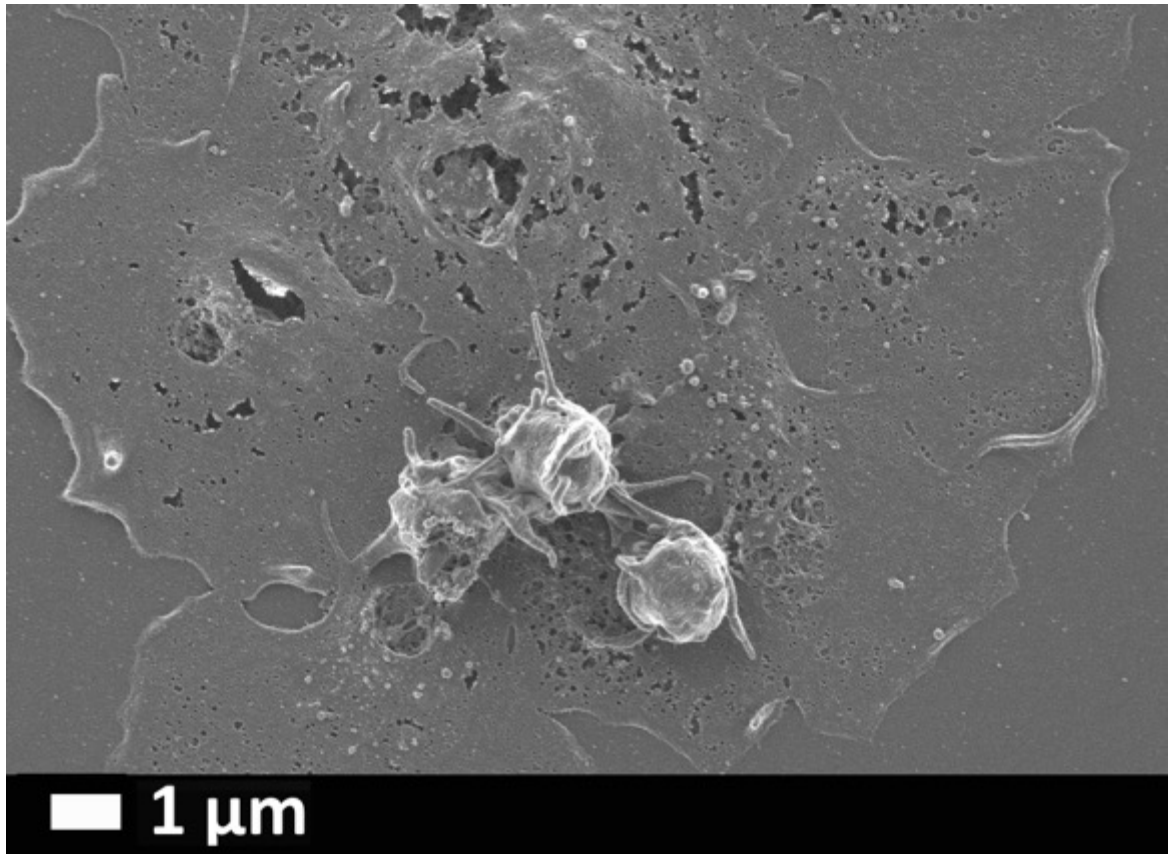
Platelet from healthy individuals



Platelets from individuals with Long COVID



RBCs: Healthy vs Long COVID

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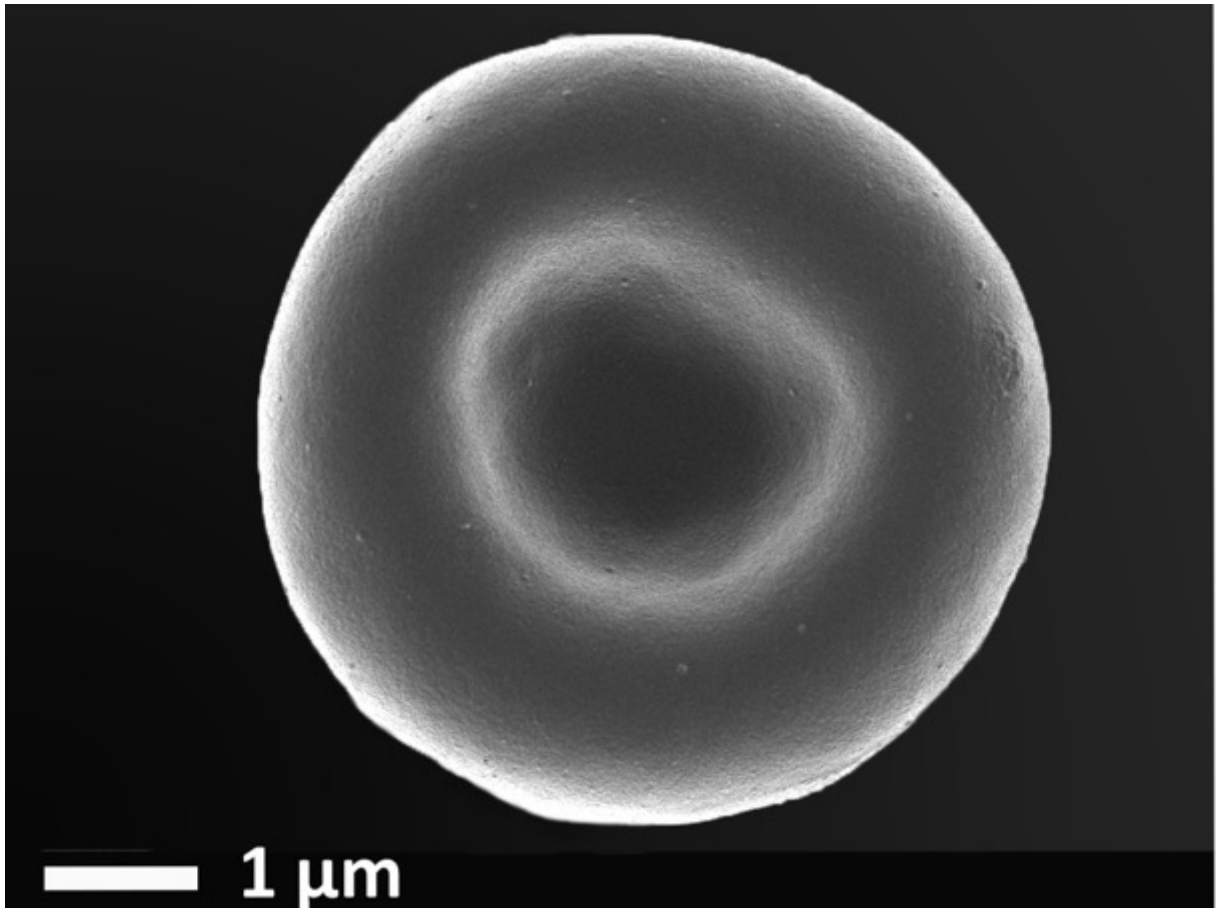
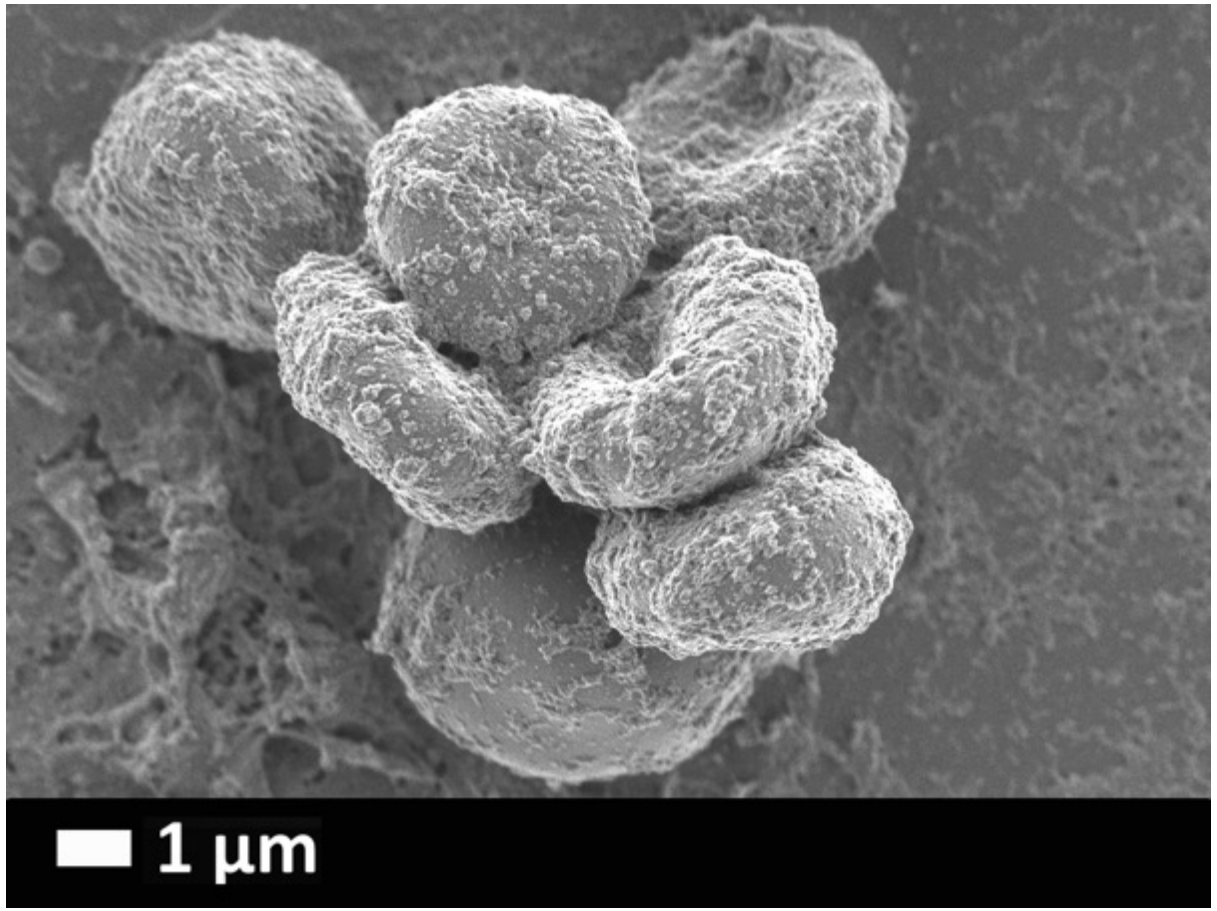
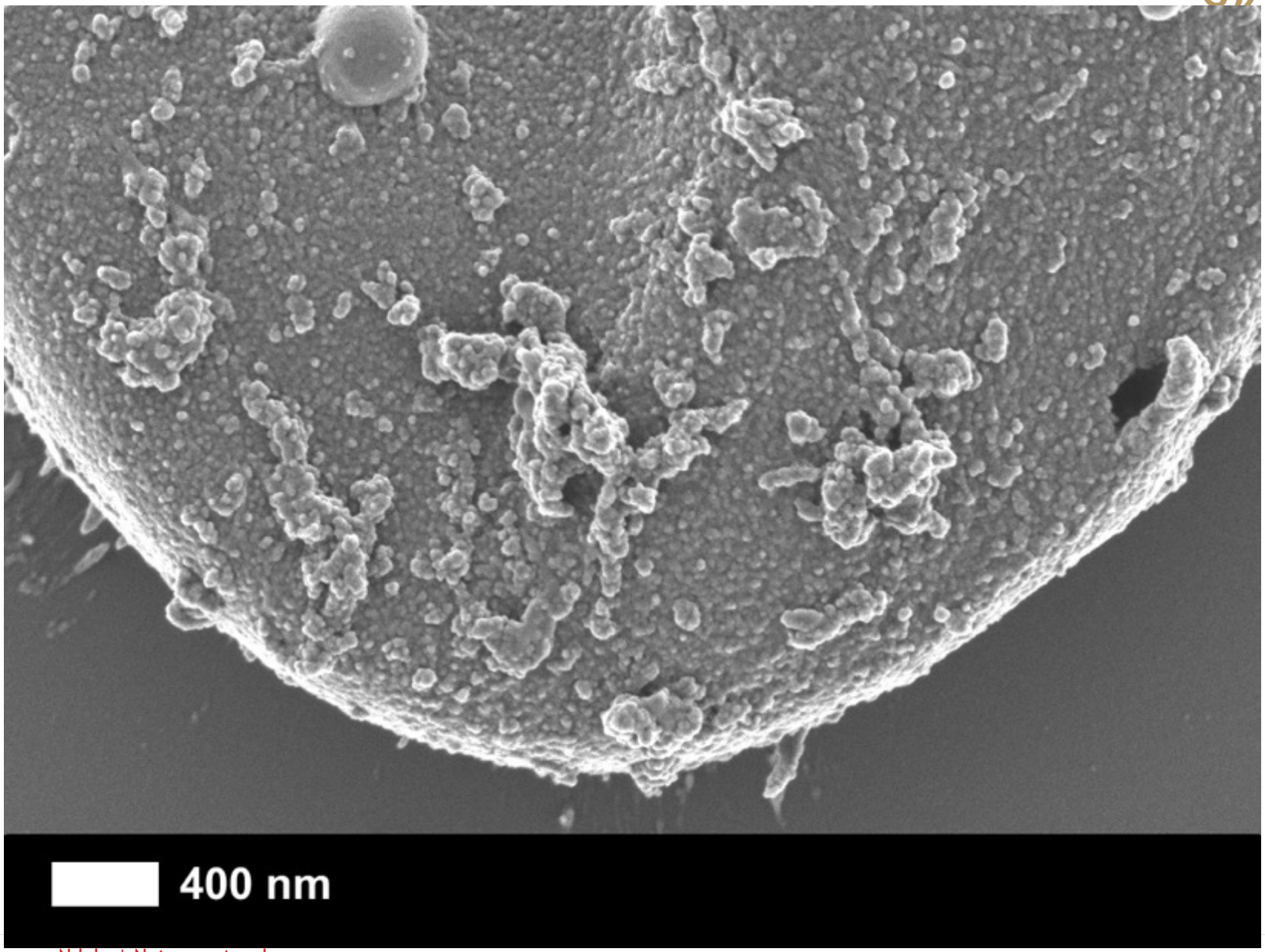
RBC from a healthy individual



RBCs from individual with Long COVID

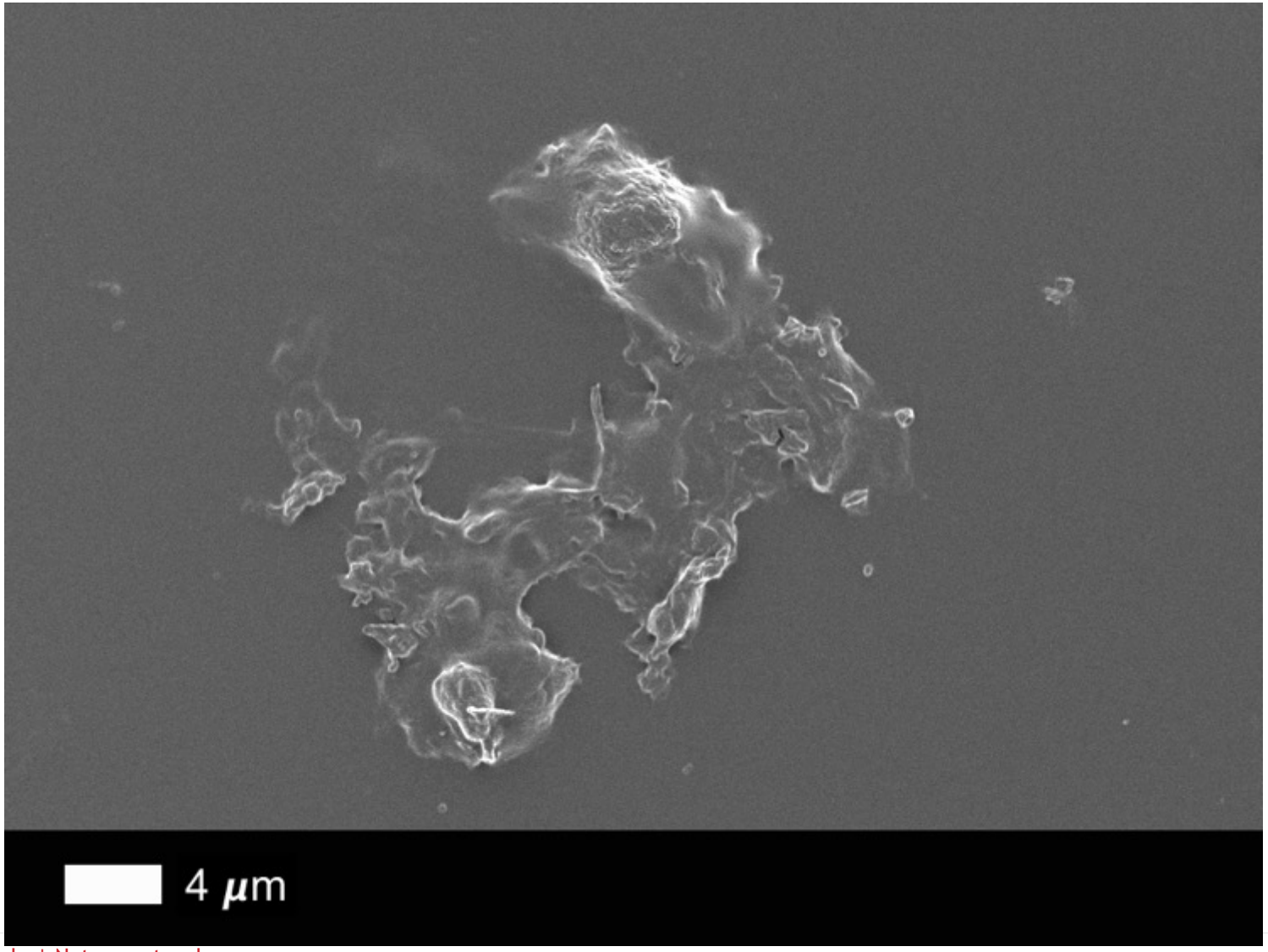


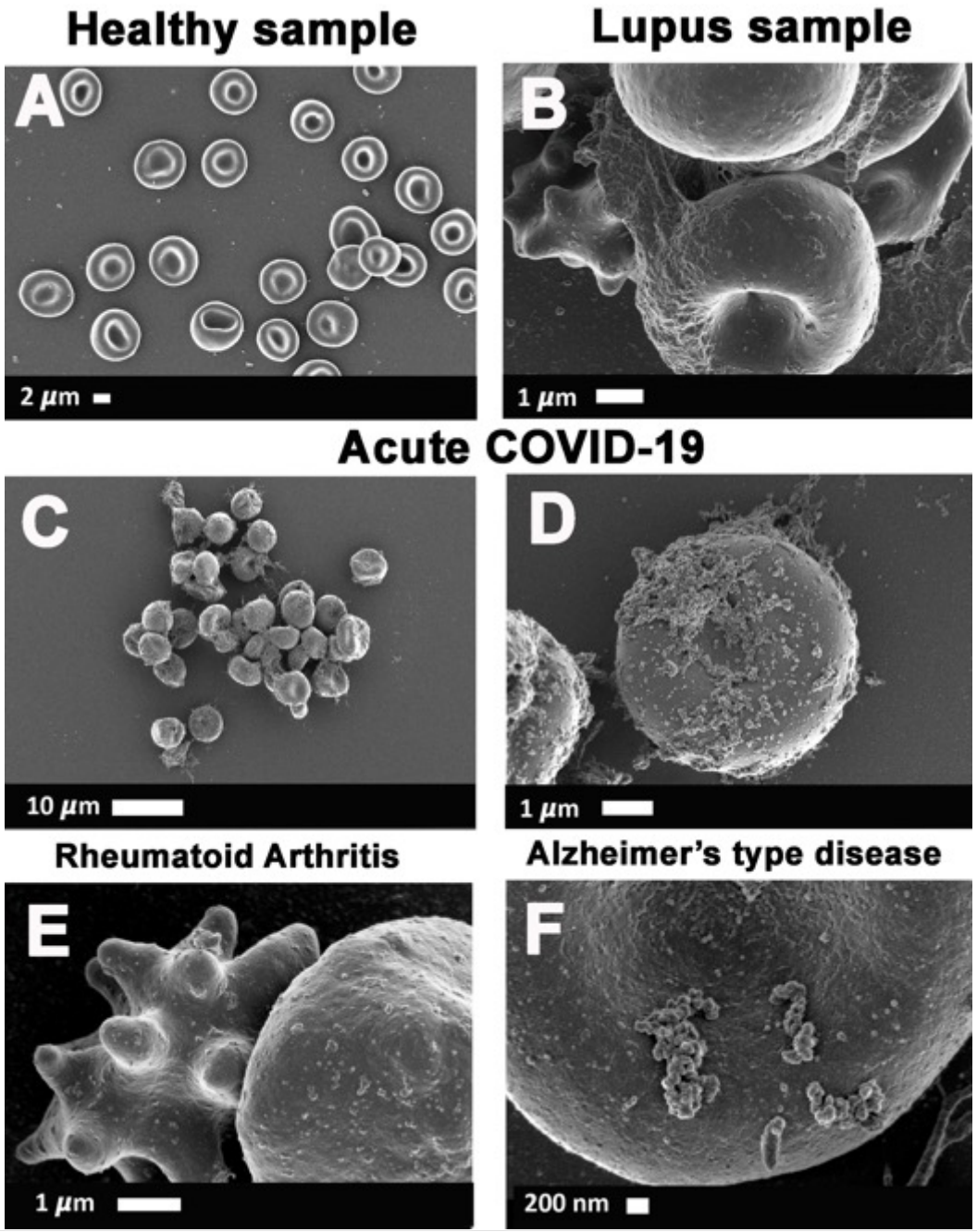
RBC with plasma deposits (microclots): Long COVID



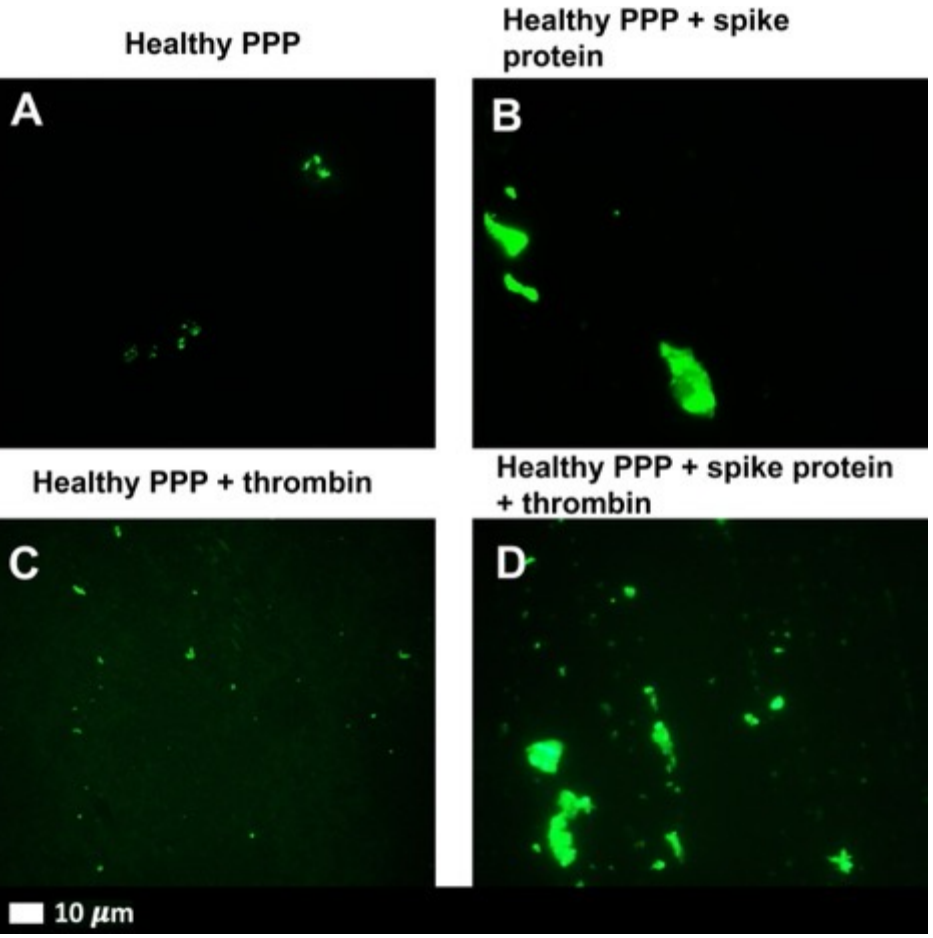
Microclots in whole blood: Long COVID

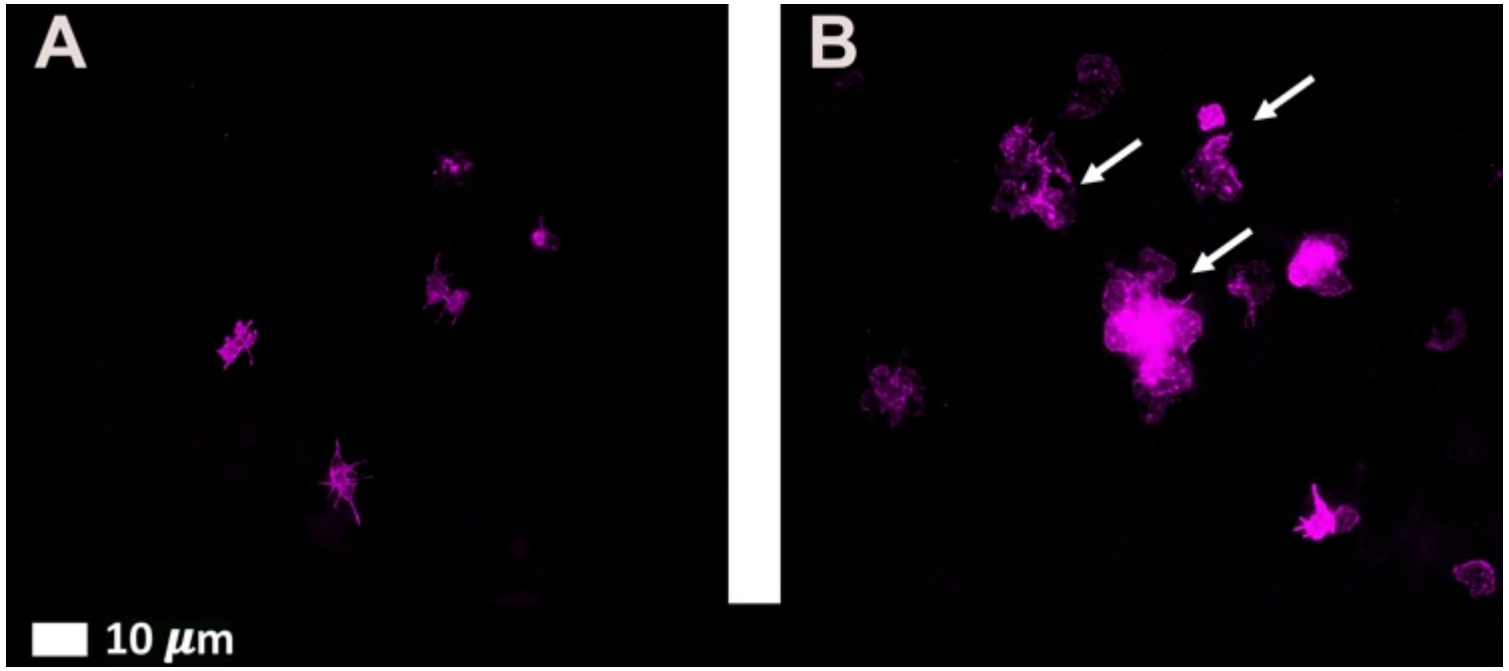
Microcots from an Individual with Long COVID




Healthy sample
Lupus sample
A
2 µm
B
1 µm
Acute COVID-19
C
10 µm
D
1 µm
Rheumatoid Arthritis
Alzheimer's type disease
E
1 µm
F
200 nm

Spike protein S1 can induce fibrinolytic-resistant microclots and platelet hyperactivation





Grobbelaar, L.M., Venter, C., Vlok, M., Ngoepe, M., Laubscher, G.J., Lourens, P.J., Steenkamp, J., Kell, D.B., and Pretorius, E. (2021). SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. Biosci Rep *41*.

Scanning electron Microscopy and microfluidics: Spike protein S1

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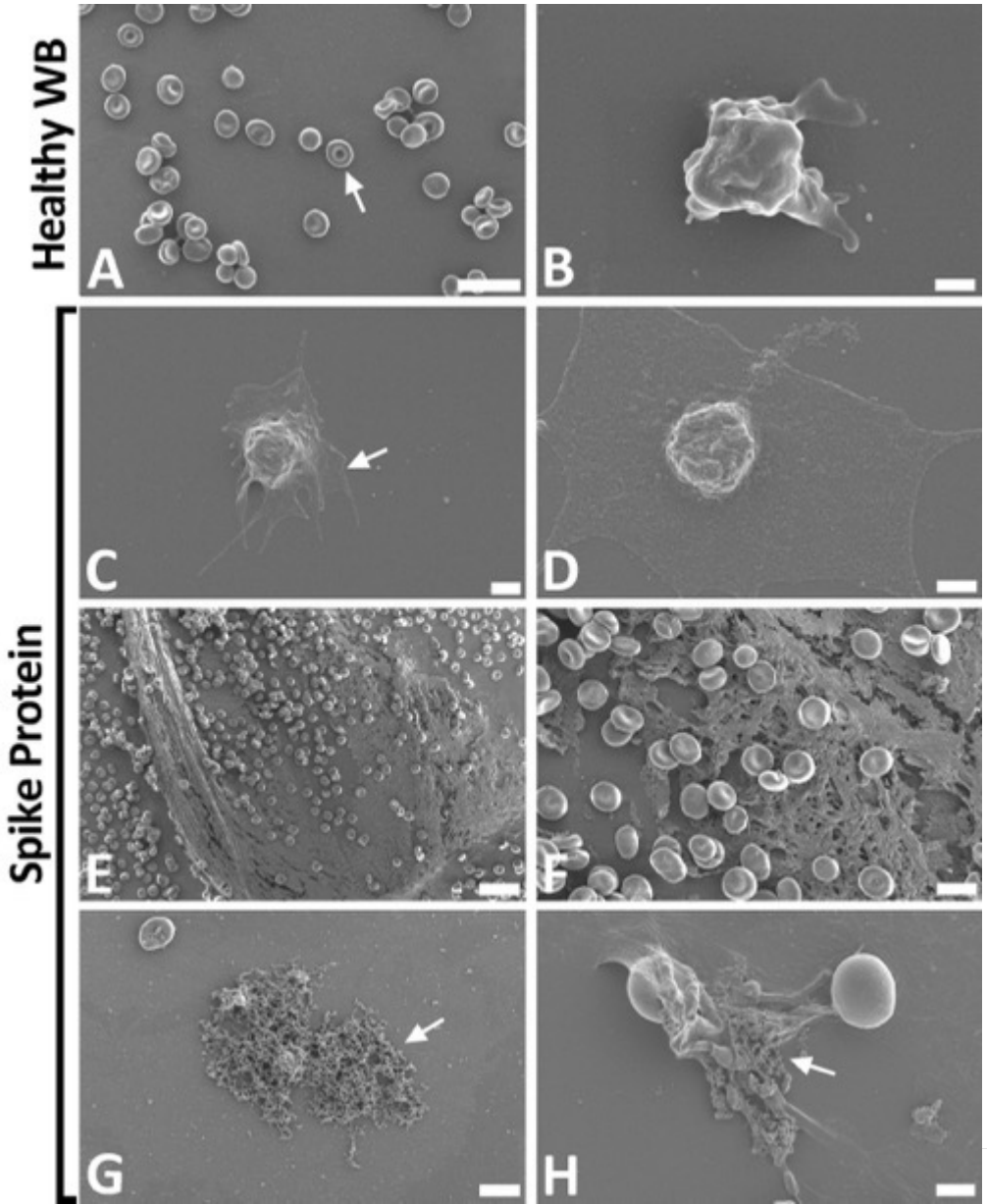
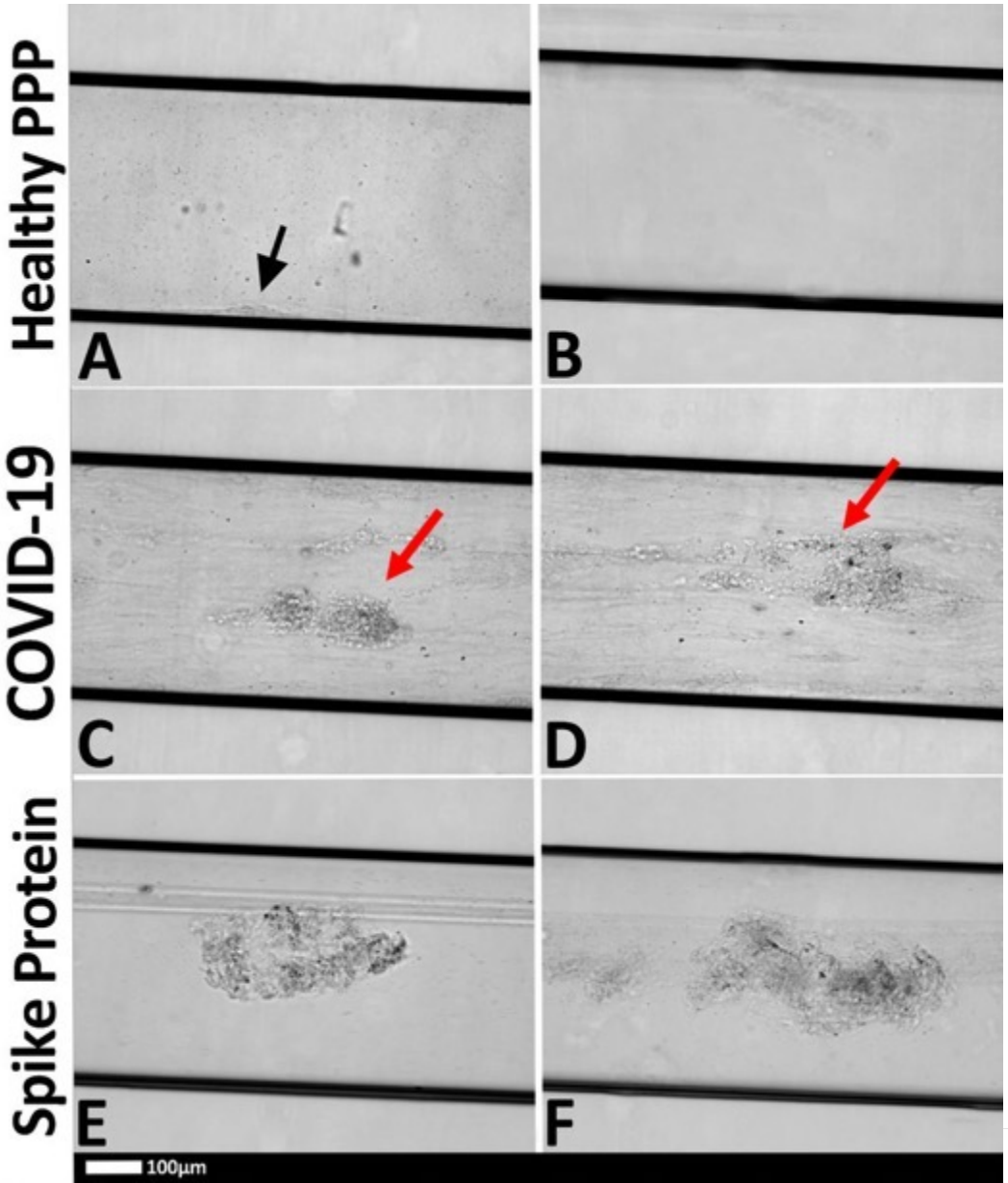
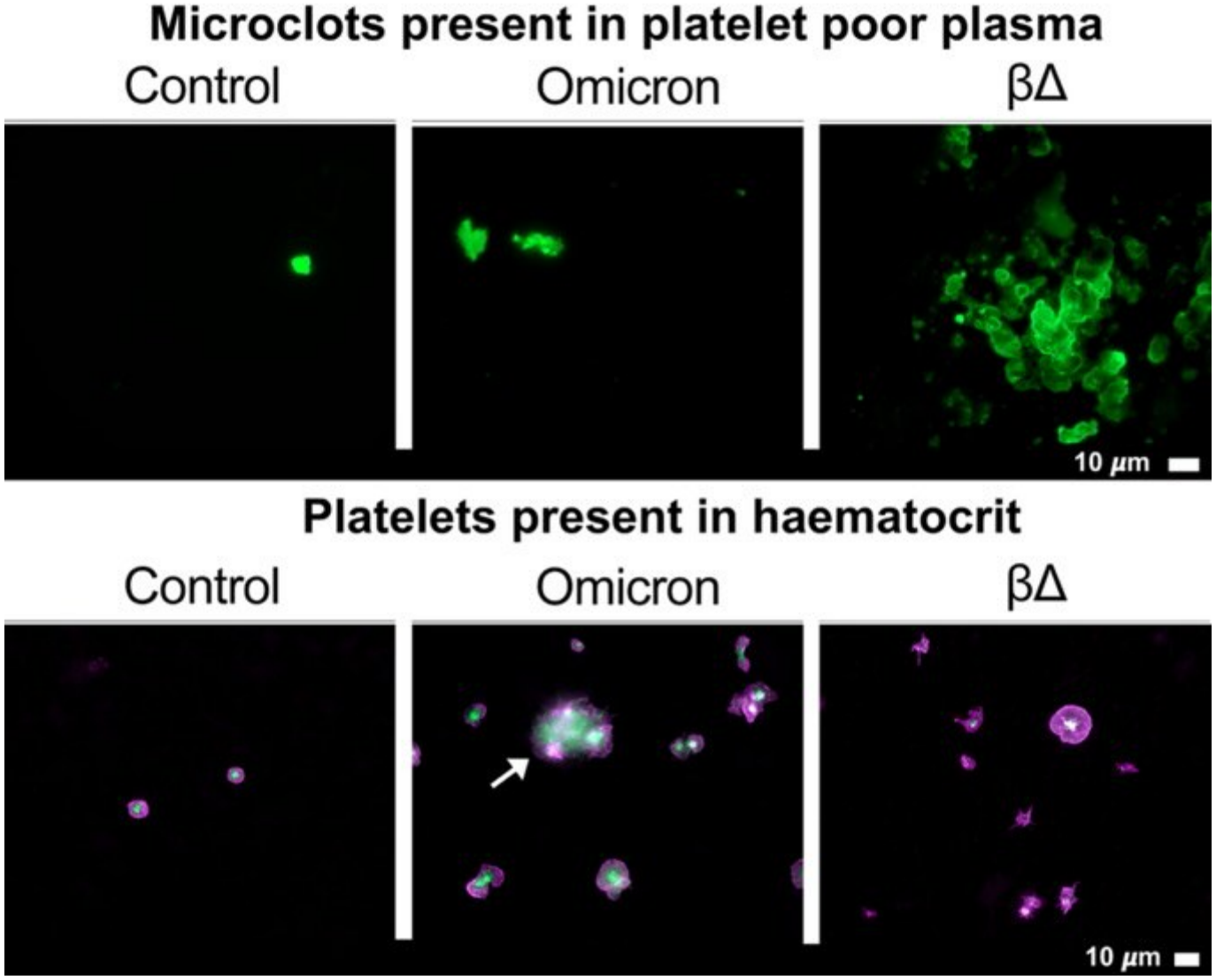


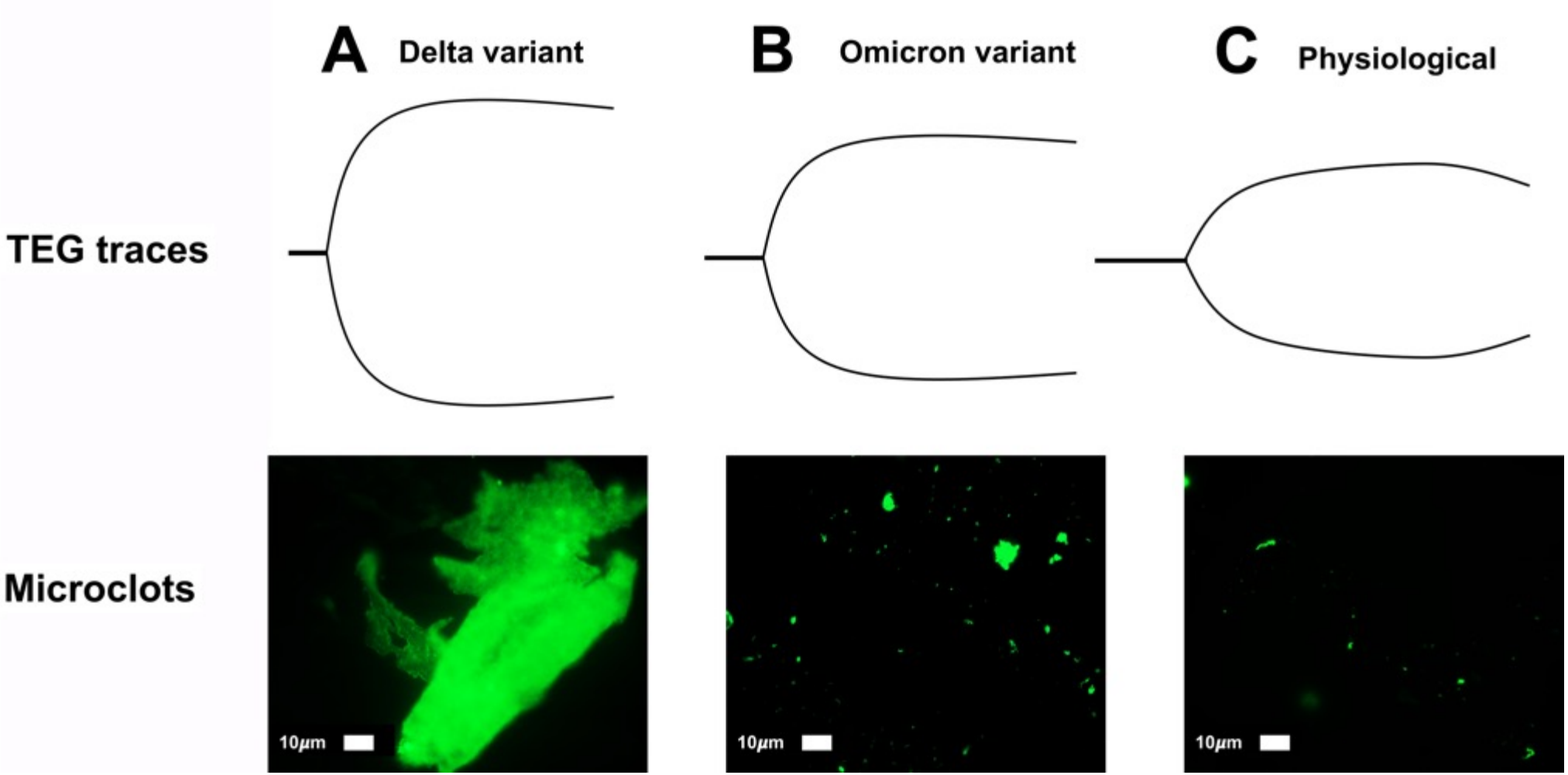


Healthy plasma versus older beta/delta vs Omicron

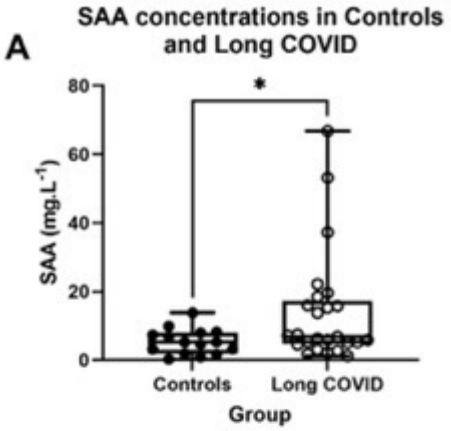


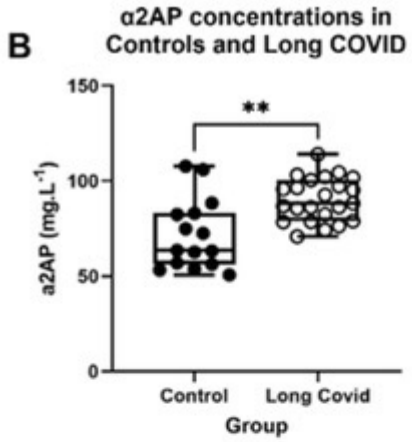
Grobbelaar, L.M., Kruger, A., Venter, C., Burger, E.M., Laubscher, G.J., Maponga, T.G., Kotze, M.J., Kwaan, H.C., Miller, J.B., Fulkerson, D., *et al.* (2022). Relative Hypercoagulopathy of the SARS-CoV-2 Beta and Delta Variants when Compared to the Less Severe Omicron Variants Is Related to TEG Parameters, the Extent of Fibrin Amyloid Microclots, and the Severity of Clinical Illness. Semin Thromb Hemost *48*, 858-868.

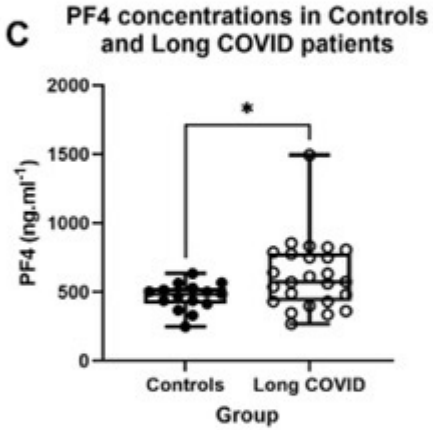
Another paper from the collaboration

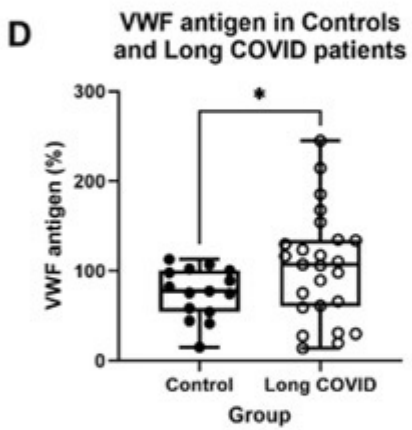


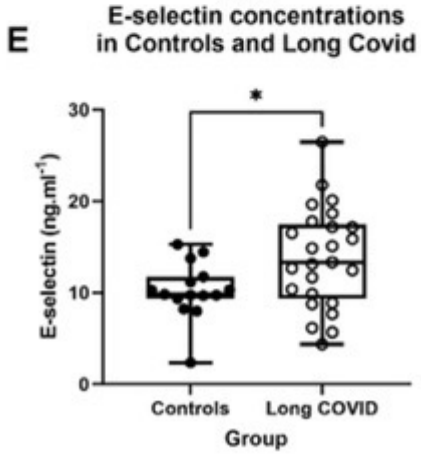
Bunch, C.M., Moore, E.E., Moore, H.B., Neal, M.D., Thomas, A.V., Zackariya, N., Zhao, J., Zackariya, S., Brenner, T.J., Berquist, M., *et al.* (2022). Immuno-Thrombotic Complications of COVID-19: Implications for Timing of Surgery and Anticoagulation. Frontiers in Surgery

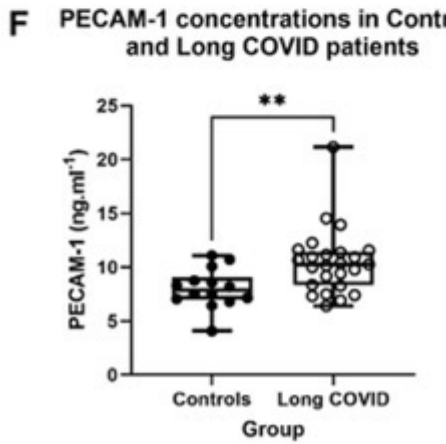






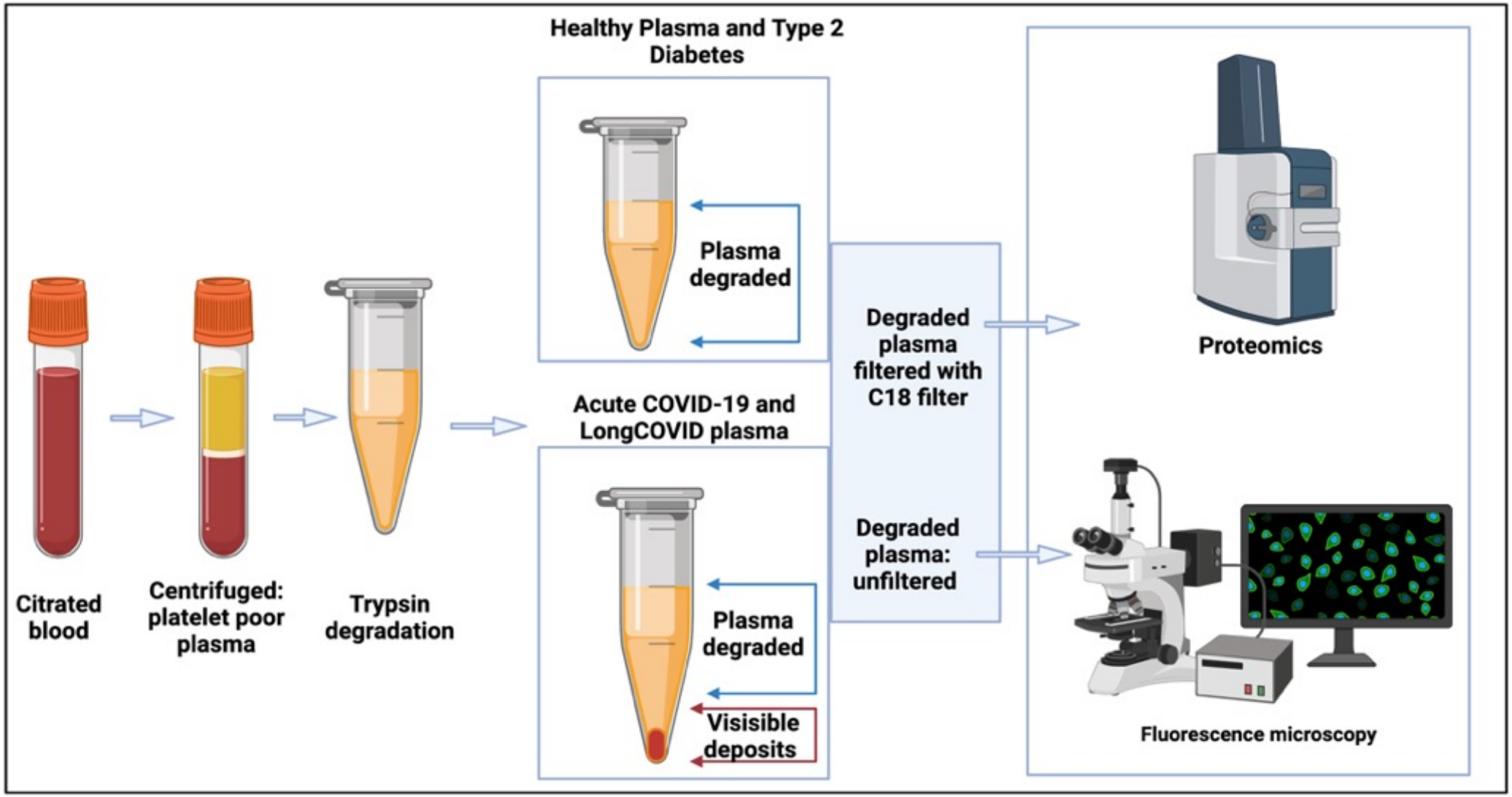




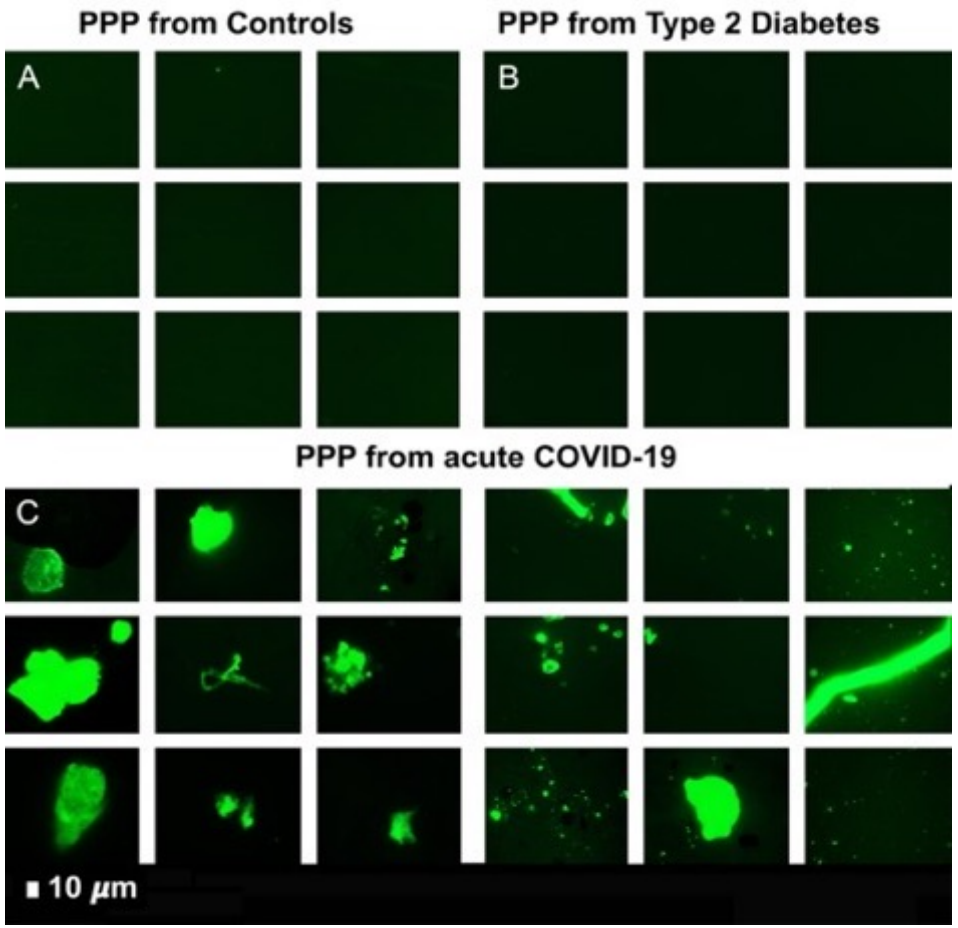


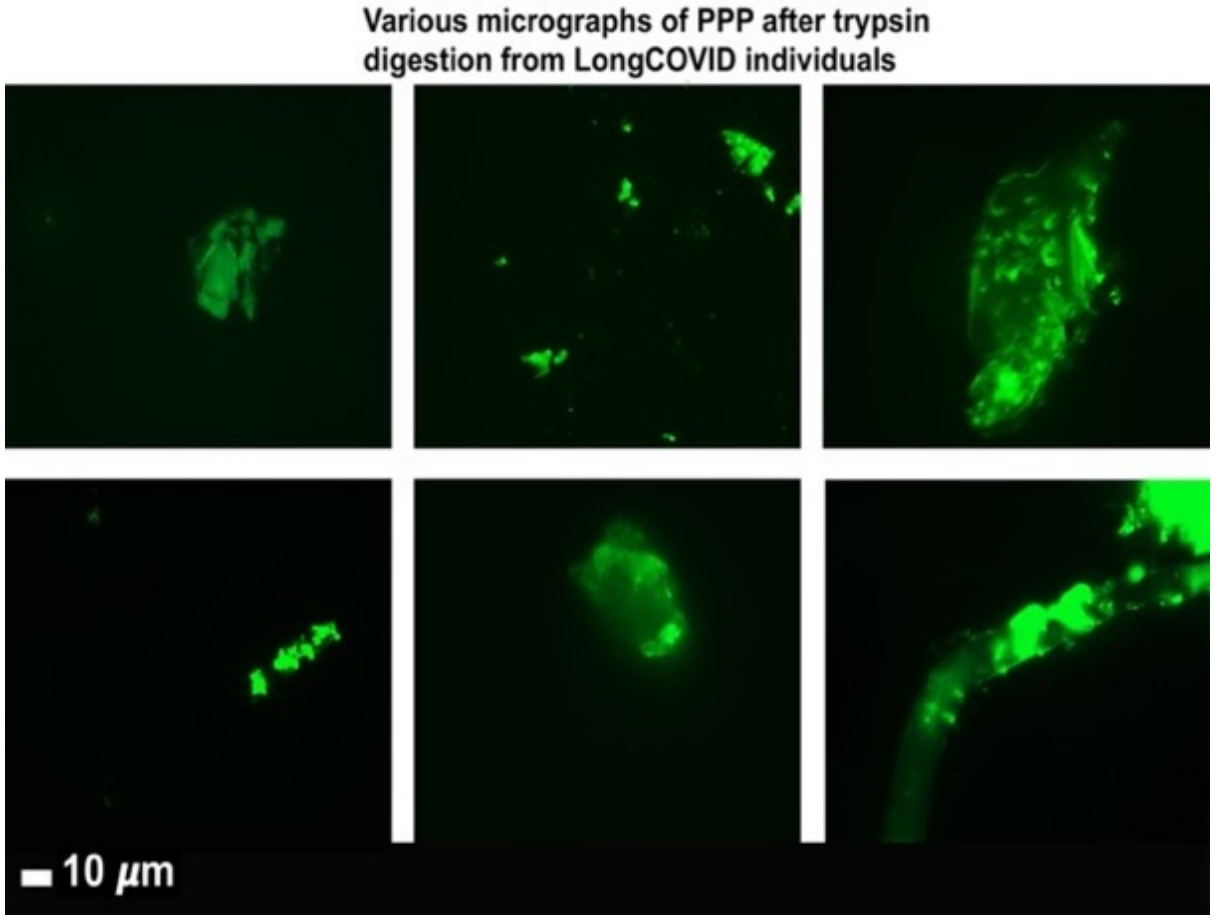
Inflammatory molecule	Reference range	Controls (n=15): Mean (SD) OR Median (Q1-Q3)	Long COVID(n=25): Mean (SD) OR Median (Q1-Q3)	Unit	P-value
SAA	0-10	5.3(1.9-8.0)	6.9(4.8-17.25)	$mg.L^{-1}$	*p<0.05
α-2AP	60-80	71.73(±18.48)	90.28(±11.31)	$mg.L^{-1}$	**p<0.01
PF4	197-1390	484.2(412.8-526.8)	572.4(430.6-779.9)	$ng.ml^{-1}$	*p<0.05
VWF	55.9 - 161.6	76.6(±28.03)	104.8(±60.1)	%	*p<0.05
E-selectin	8.5-26	10.26(±3.07)	13.86(±5.4)	$ng.ml^{-1}$	**p<0.01
PECAM-1	5.3-15	8.19(7.06-10.08)	10.27(8.32-11.45)	$ng.ml^{-1}$	*p<0.05

Proteomics of Plasma from Healthy, Diabetic, Acute COVID-19 and Long COVID



Microclots remaining in Acute COVID-19 and Long COVID after 1st digestion step





Pretorius E, Vlok M, Venter C, et al. 2021 Persistent clotting protein pathology in Long COVID/ Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovascular Diabetology*

2021 Proteomics Analysis

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Digested pellet deposits (microclots) from acute COVID-19 samples vs digested plasma from Control samples These proteins are present in both sample types; and a fold change value more than 1 = the protein that more prevalent inside the digested pellet deposits from COVID-19 samples. These proteins were concentrated inside the digested pellet deposits.		
Protein name	Fold change	P-value
von Willebrand Factor	4.5	0.02
Complement component C4b	4.1	0.05
C-reactive protein	18.7	0.003
Digested pellet deposits from Long COVID/PASC microclots samples vs digested plasma from Control samples These proteins are present in both sample types; and a fold change value more than 1 = the protein that more prevalent inside the digested pellet deposits from Long COVID/PASC samples. These proteins were concentrated inside the digested pellet deposits.		
Coagulation factor XIII A chain	6.9	0.001
Plasminogen	3	0.001
Fibrinogen alpha chain	4.1	0.0001
α2 antiplasmin (α2AP)	**7.9**	**0.0002**
von Willebrand Factor	10.2	0.001
C-reactive protein	11.2	0.007
Serum Amyloid A (SAA4)	17.5	0.01
Complement component C7	20	0.0002
Digested pellet deposits from Long COVID/PASC microclots samples vs digested pellet deposits (microclots) from acute COVID-19 samples These proteins are present in both sample types; and a fold change value more than 1 = the protein that more prevalent inside the digested pellet deposits from Long COVID/PASC samples. These proteins were concentrated inside the digested pellet deposits.		
Plasminogen	2.3	0.0007
Fibrinogen β chain	2.8	0.007
Coagulation factor XIII B	2.7	0.01
Fibrinogen α chain	3.1	0.0002
Complement component C6	7.5	0.01
α2 antiplasmin (α2AP)	**9.2**	**0.0003**
Complement factor 1	25	0.0009

Long COVID and trapped inflammatory molecules

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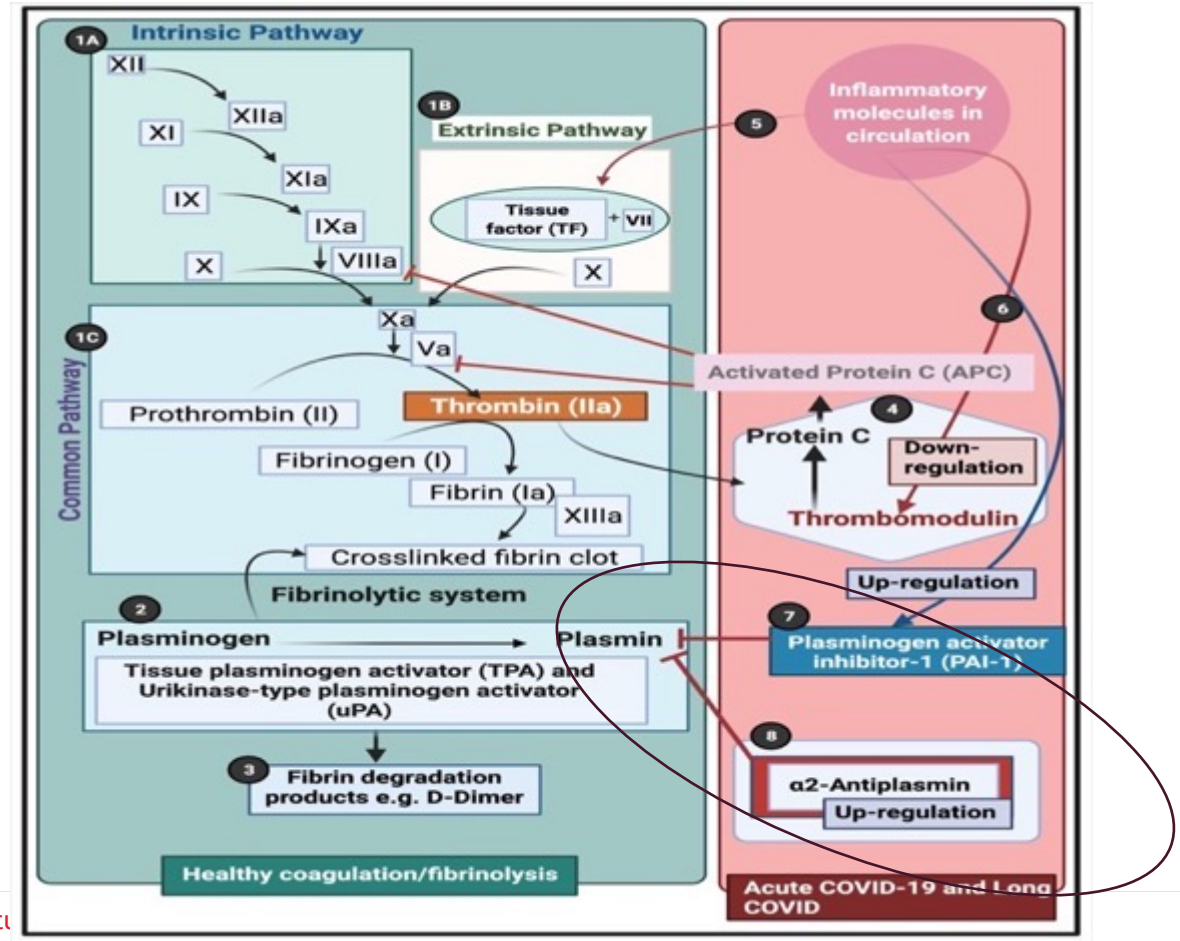
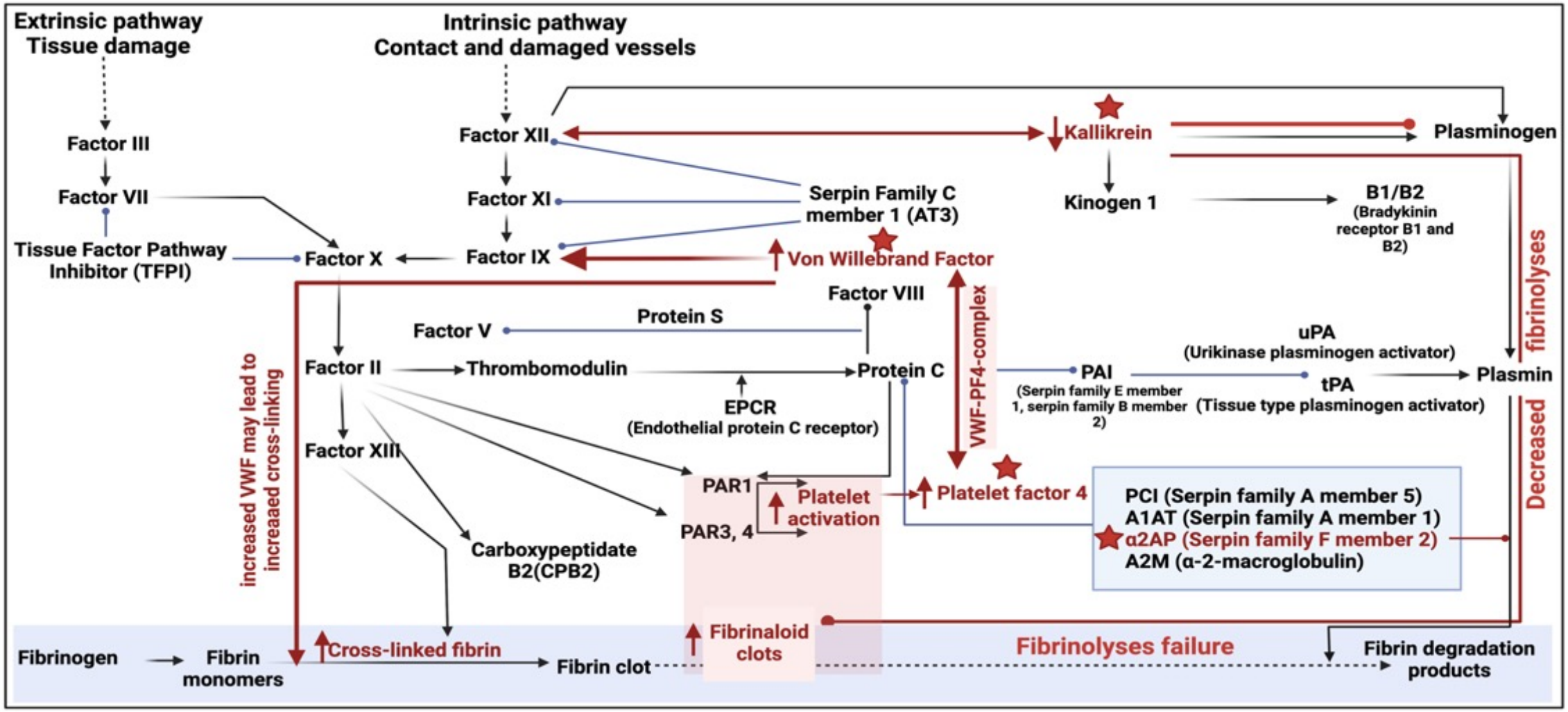
α2-antiplasmin (α2AP) inhibit plasmin and ultimately will prevent sufficient fibrinolysis to happen

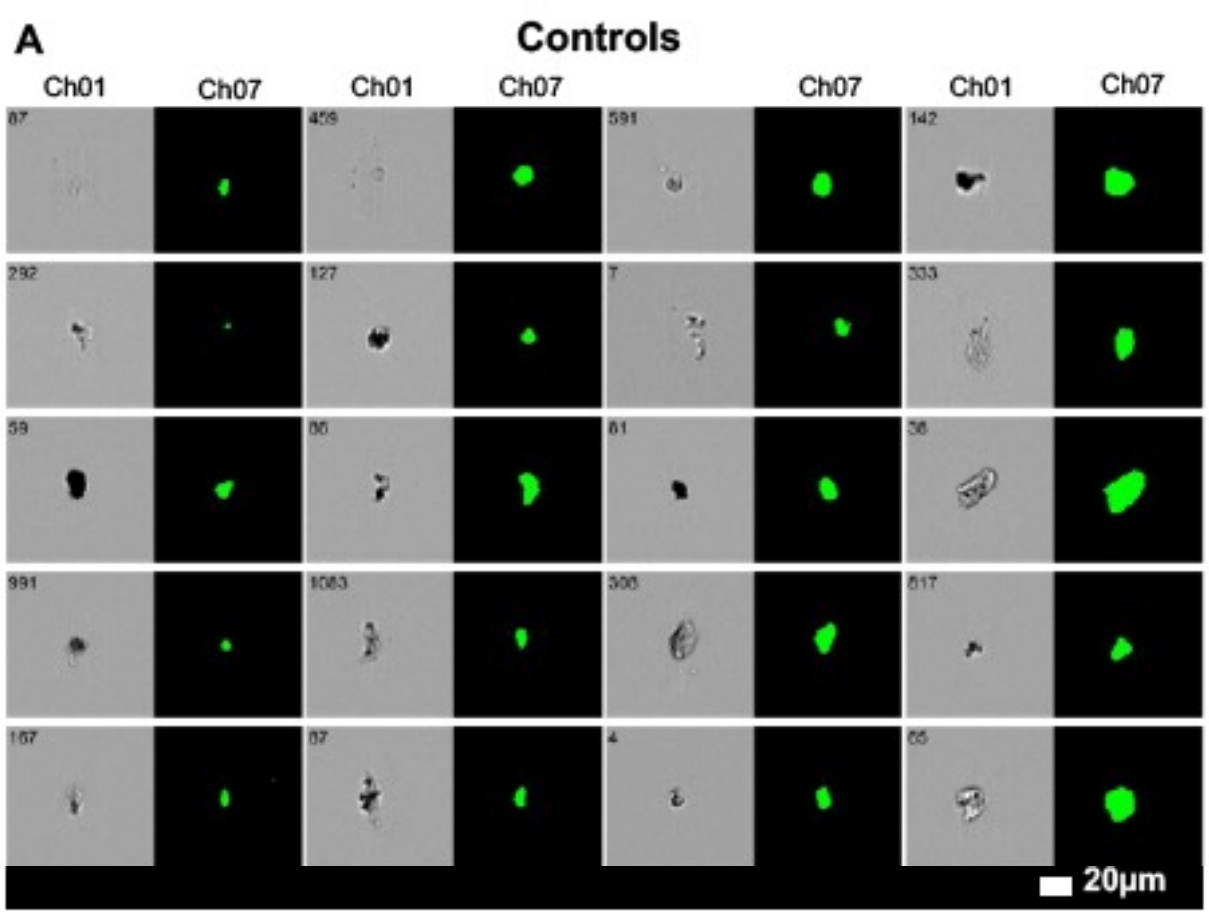


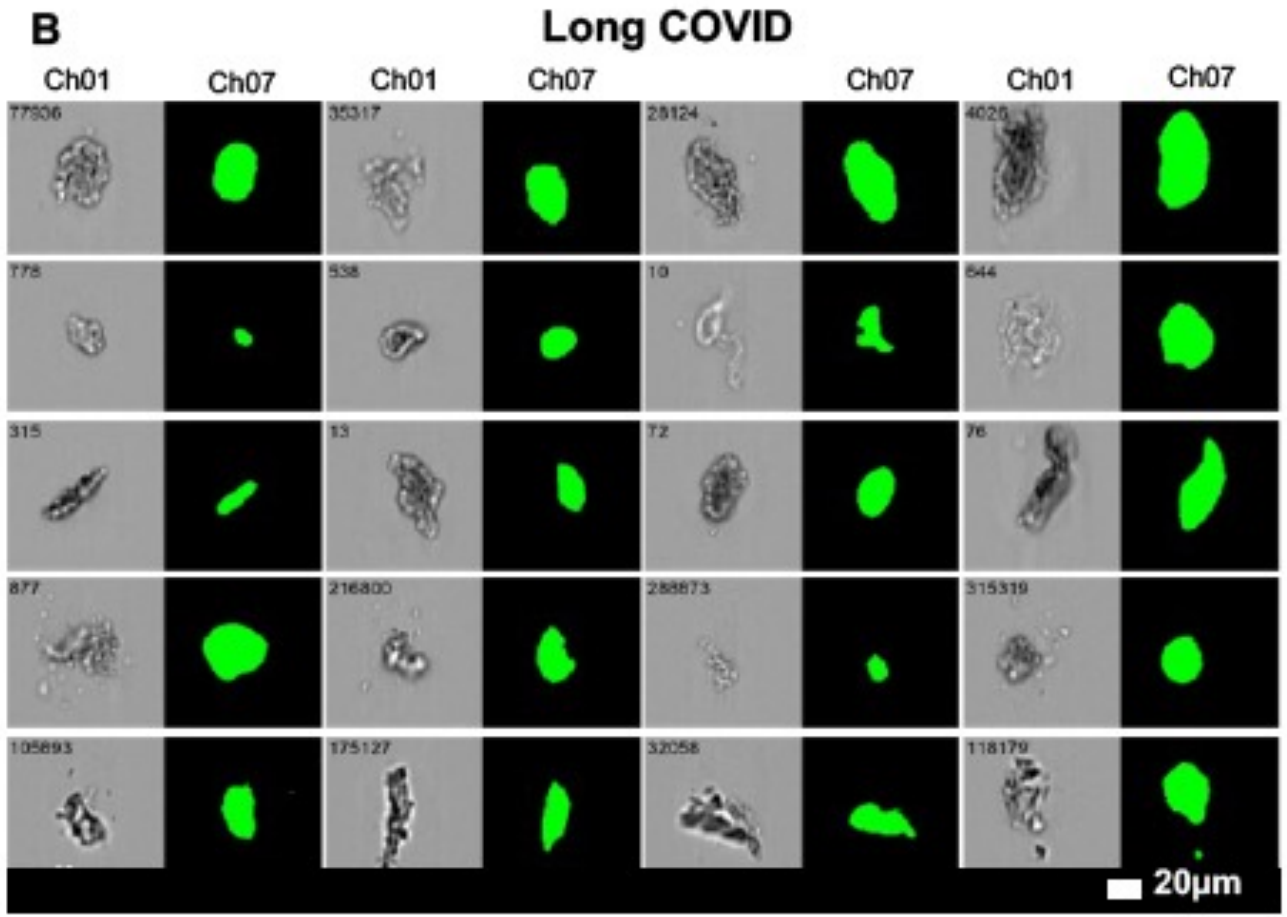
2022 Proteomics Analysis



Kruger, A., Vlok, M., Turner, S., Venter, C., Laubscher, G.J., Kell, D.B., and Pretorius, E. (2022). Proteomics of fibrin amyloid microclots in long COVID/post-acute sequelae of COVID-19 (PASC) shows many entrapped pro-inflammatory molecules that may also contribute to a failed fibrinolytic system. Cardiovasc Diabetol *21*, 190.

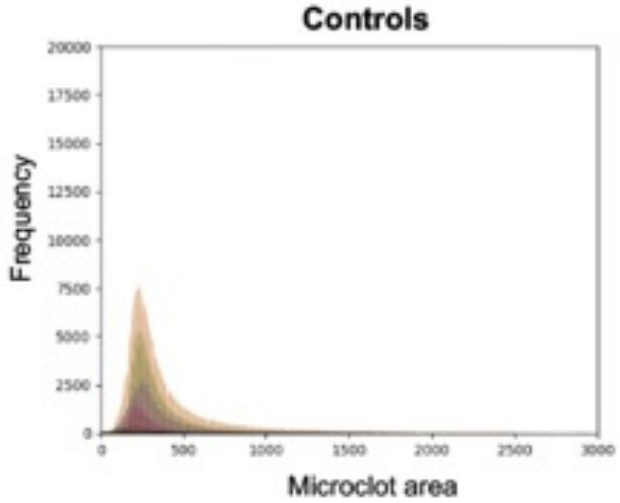
A place for flow cytometry? (Balvi Foundation, funded together with KERNLS and Polybio Research Foundation)

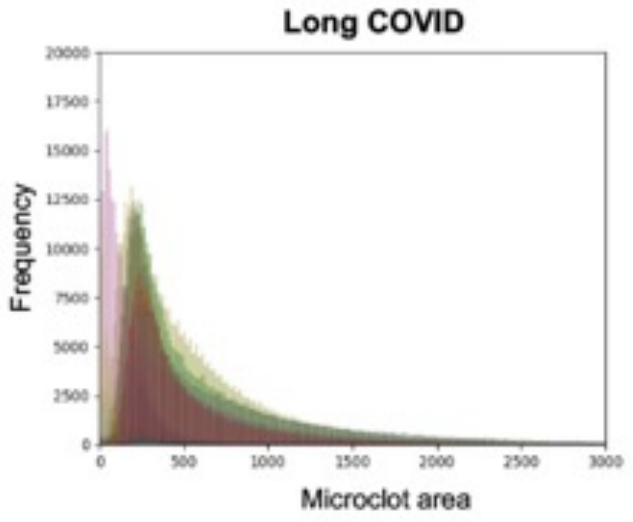


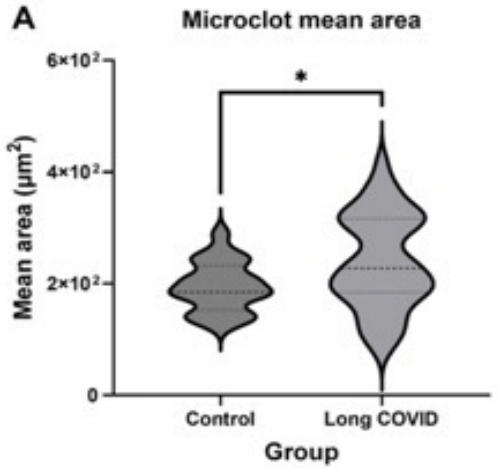


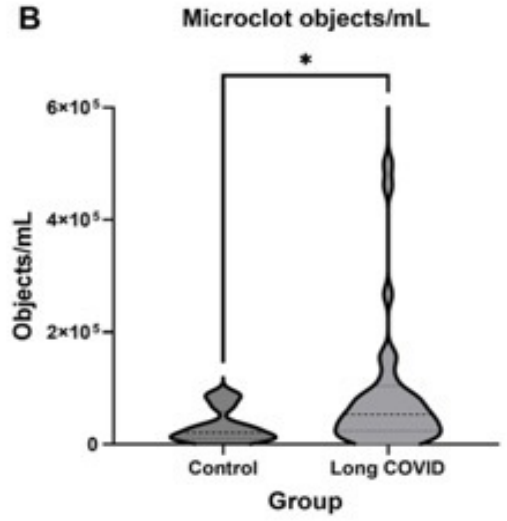
Turner, S., Laubsher, HG.J., Khan, M.A., Kell, D.B., and Pretorius, E. (2023). Accelerating Discovery: A Novel Flow Cytometric Method for Detecting Fibrin(ogen) Amyloid Microclots Using Long COVID as a Model. Heliyon *9* e19605.

A place for flow cytometry?



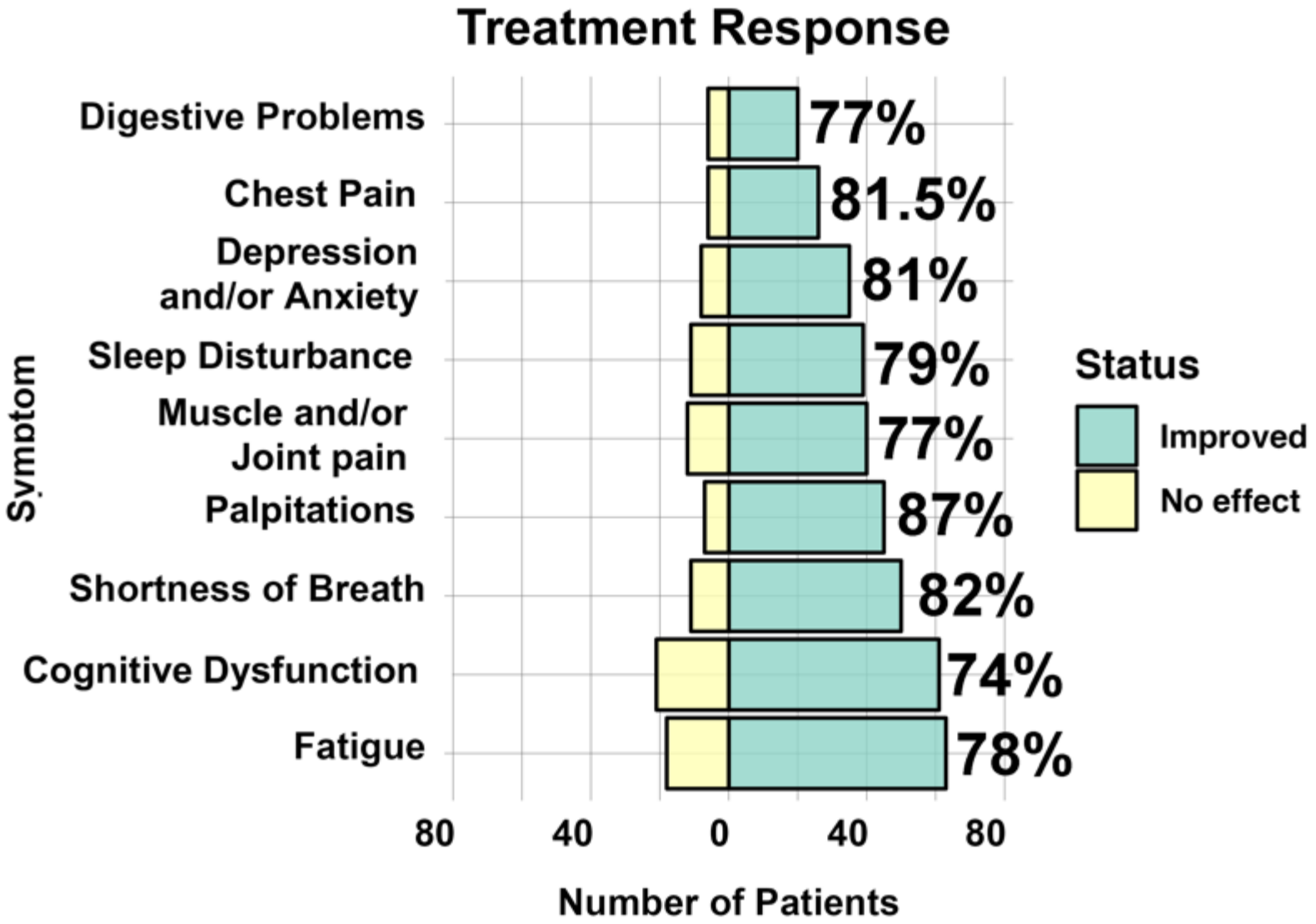






Turner, S., Laubsher, HG.J., Khan, M.A., Kell, D.B., and Pretorius, E. (2023). Accelerating Discovery: A Novel Flow Cytometric Method for Detecting Fibrin(ogen) Amyloid Microclots Using Long COVID as a Model. Heliyon *9* e19605.

A Place for treating microclots and platelet hyperactivation?



A Place for treating microclots and platelet hyperactivation?

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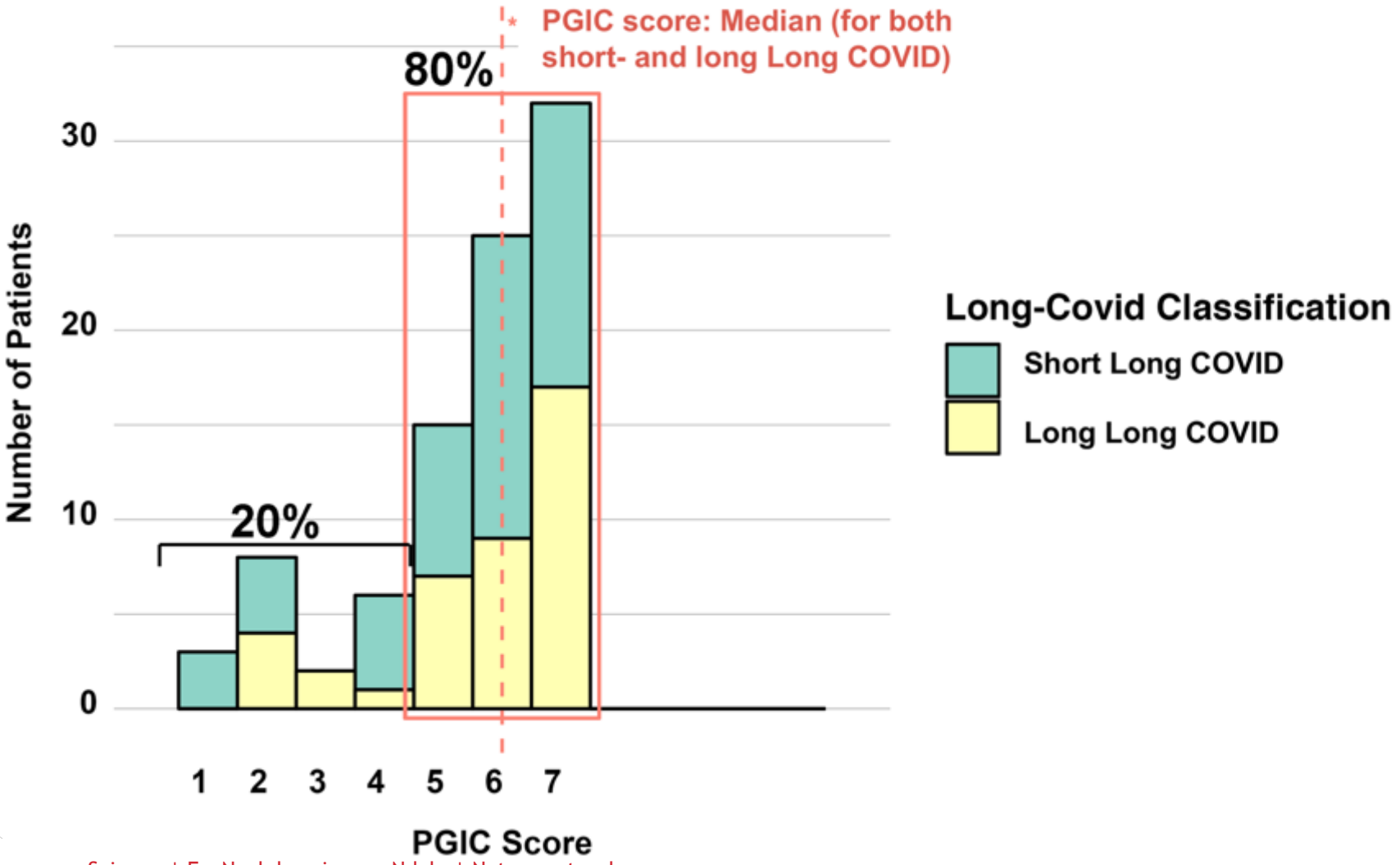
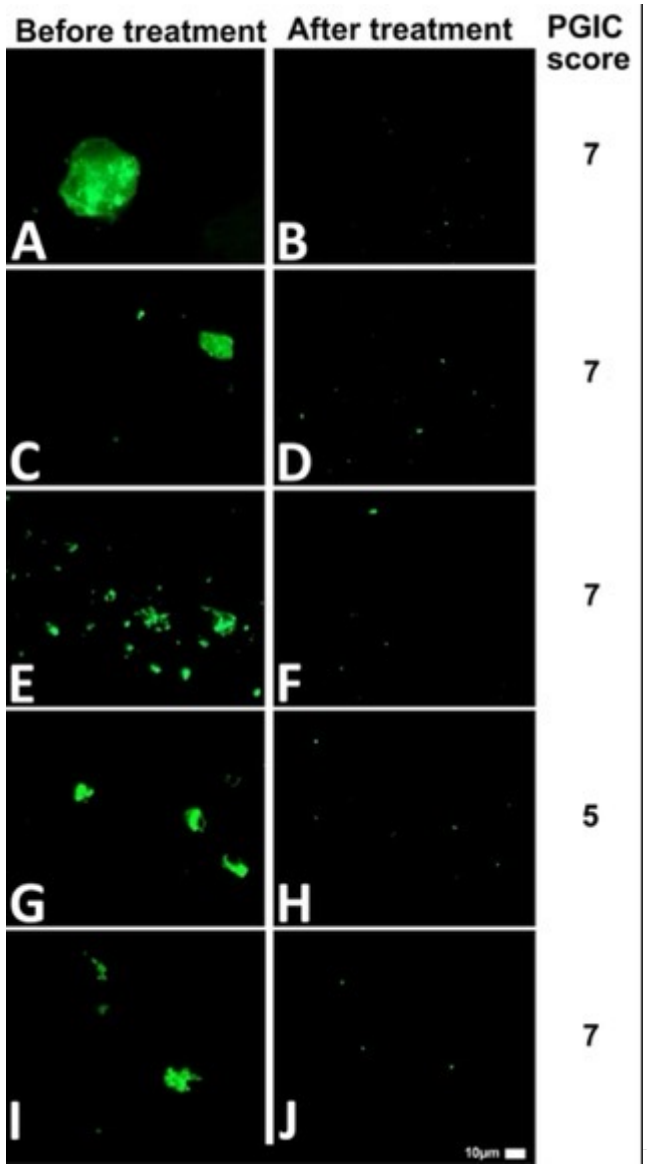
Histogram of PGIC Scores of Patients





ME/CFS (Myalgic encephalomyelitis/chronic fatigue) syndrome (Polybio funded)

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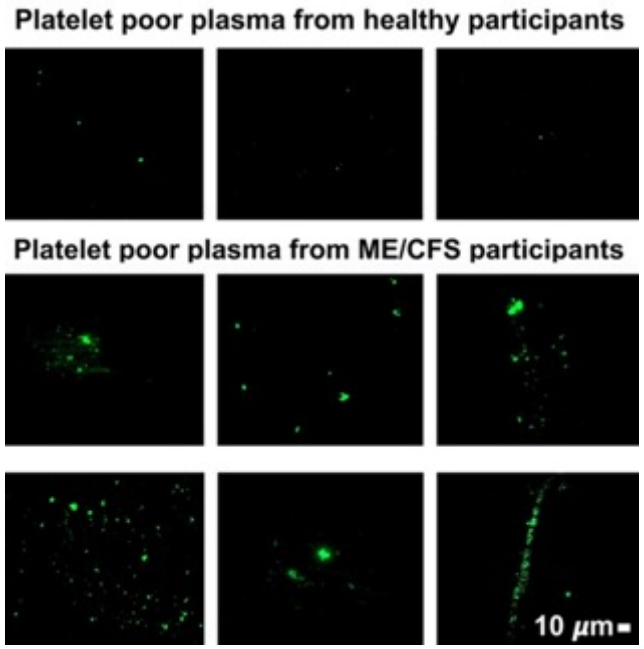

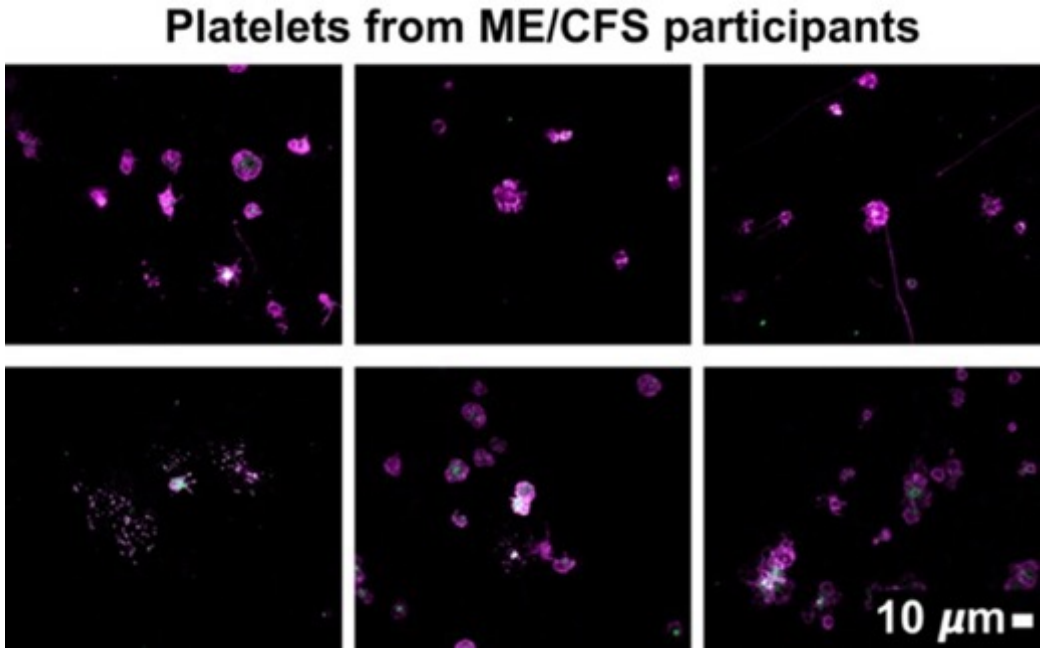

Nunes, J.M., Kruger, A., Proal, A., Kell, D.B., and Pretorius, E. (2022). The Occurrence of Hyperactivated Platelets and Fibrinaloid Microclots in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Pharmaceuticals *15*, 931.

- Imaging Flow Cytometry (ThT)
- Fluorescence microscopy using e-selectin
- Imaging Flow cytometry using e-selectin

Endothelial debris in platelet poor plasma: ME/CFS and Long COVID? (Polybio funded)

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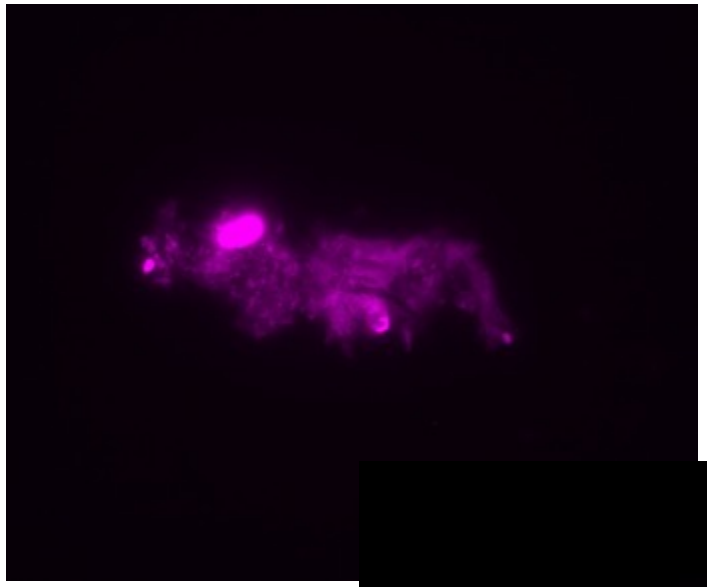
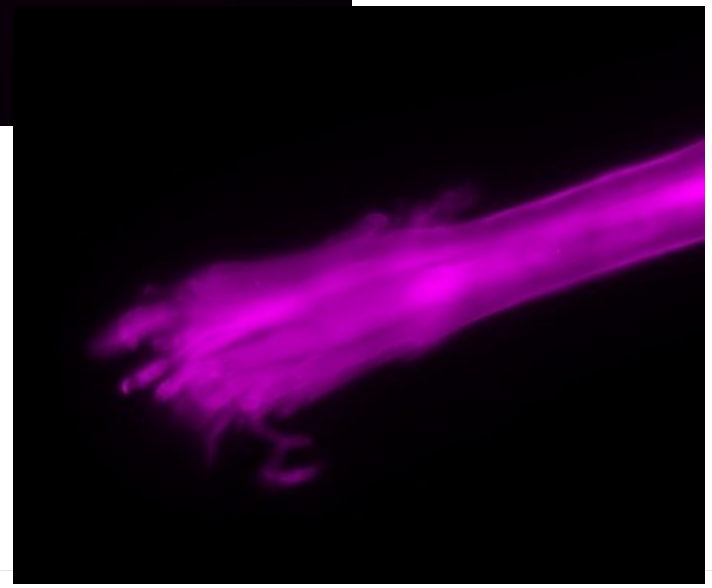
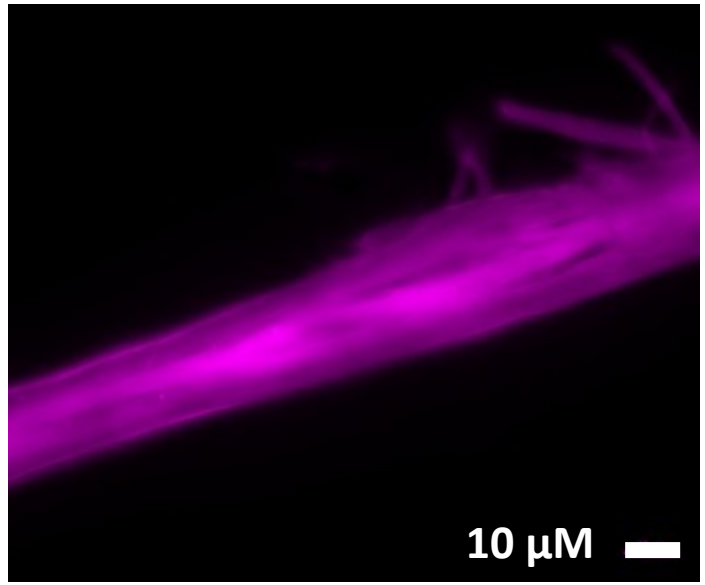
Control

Long COVID





ME/CFS



E-selectin: CD62-E: BD Pharmingen™

Long COVID Biofilm Research (Polybio funded)

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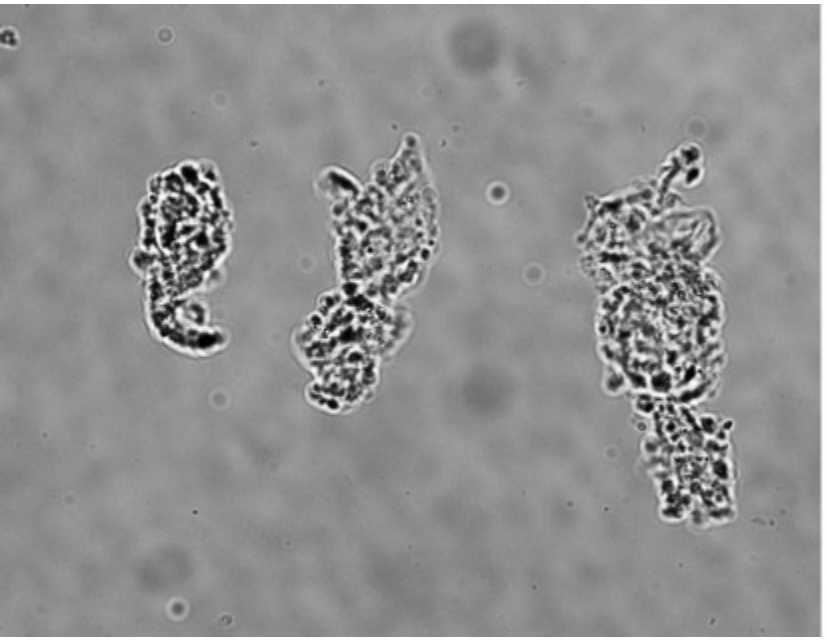
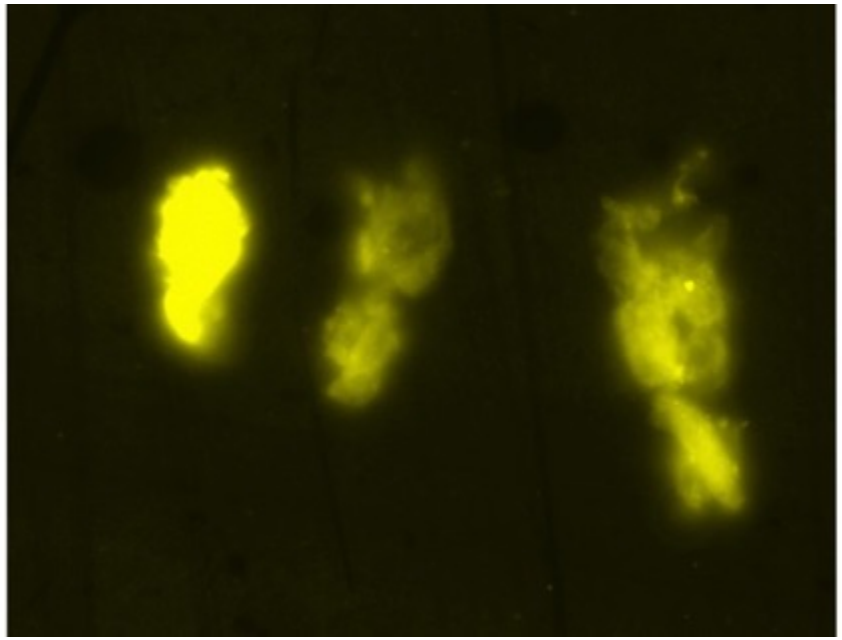
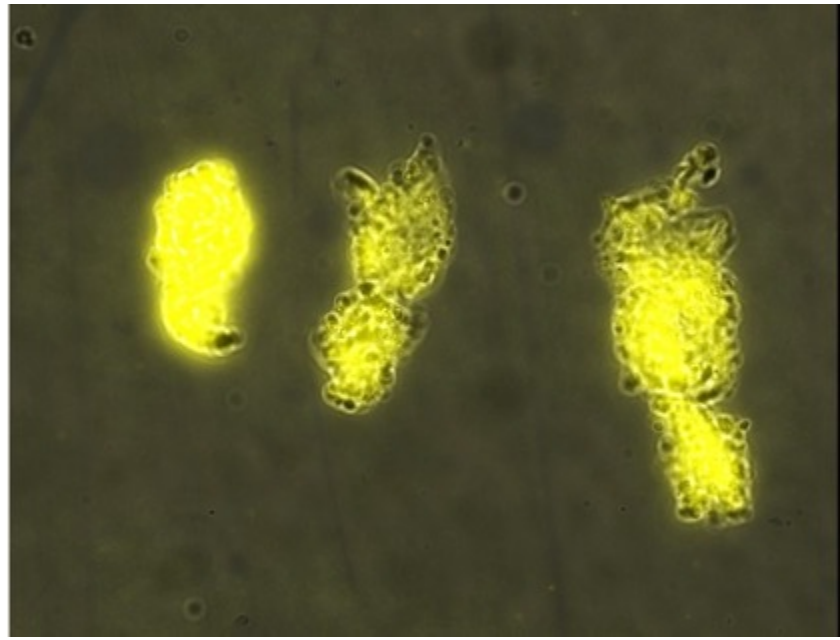
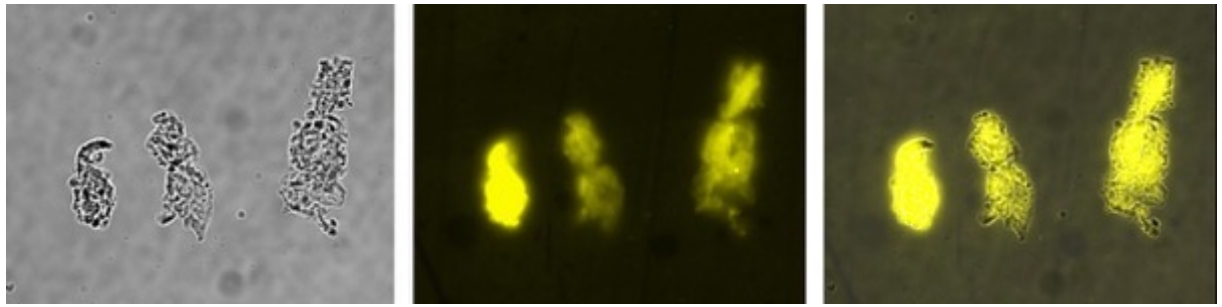


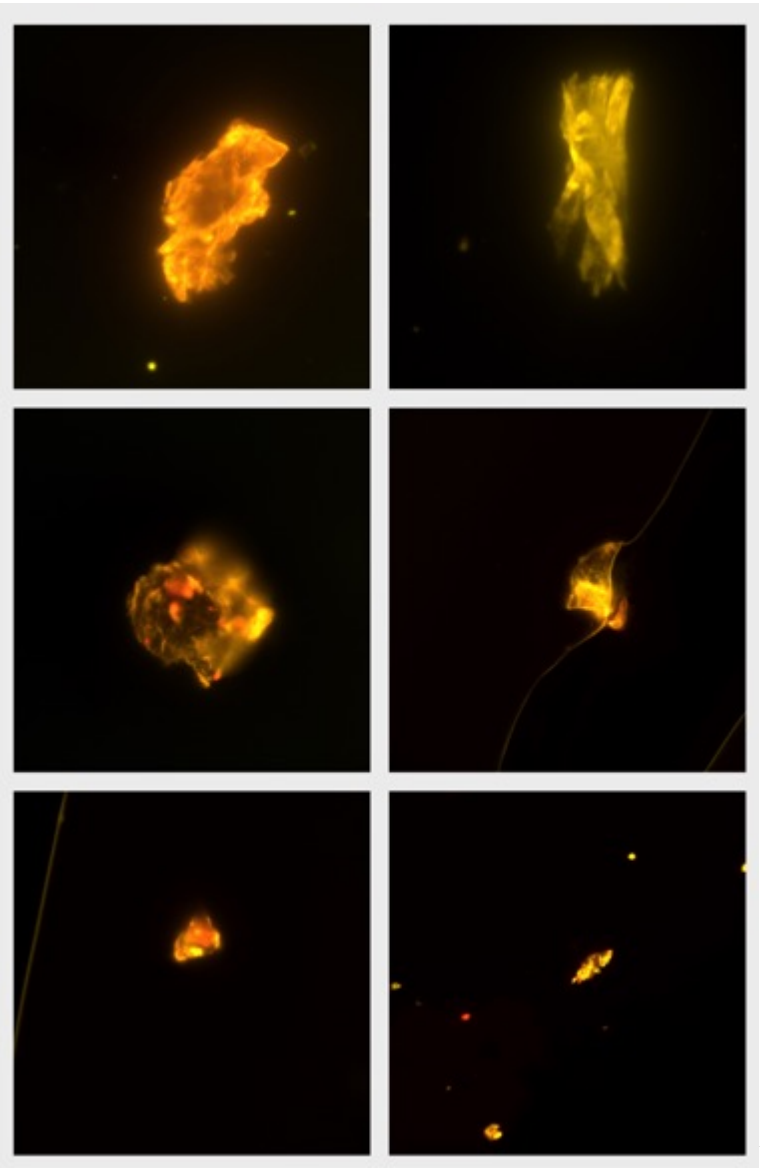




FilmTracer™ FM® 1-43 Green Biofilm Cell Stain

Long COVID Biofilm Tracer and Amytracker (Polybio funded)

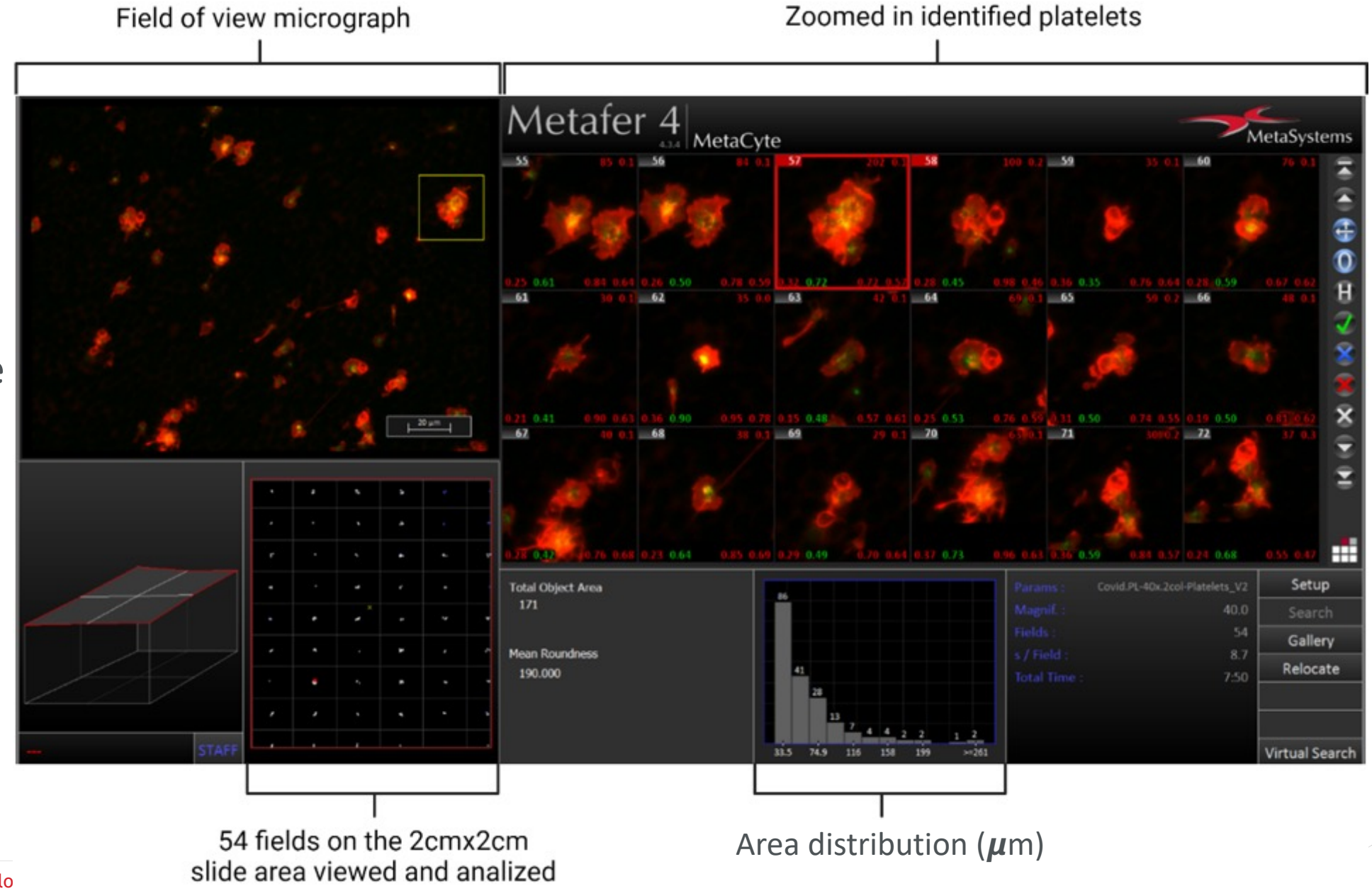




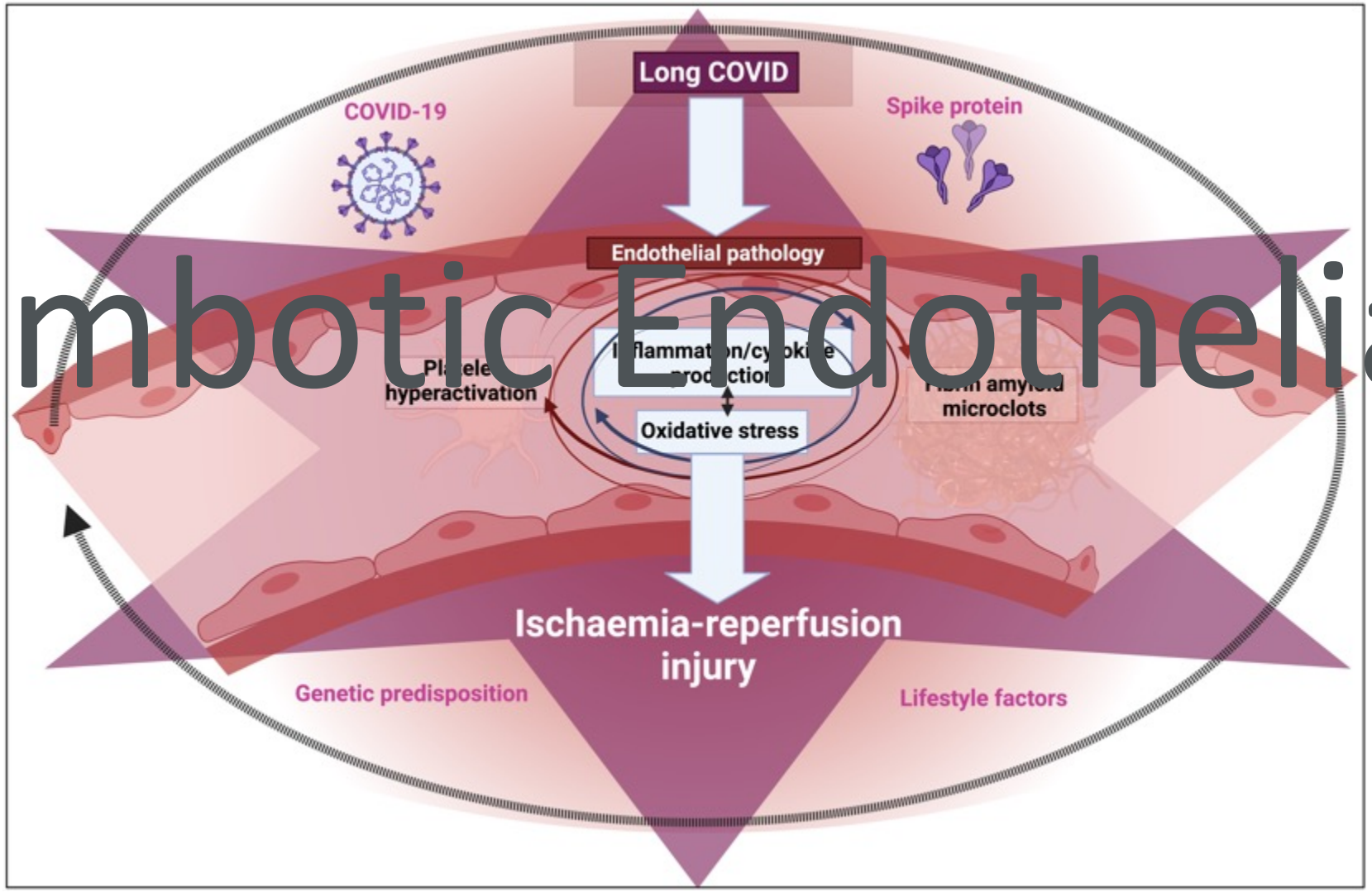
- Fluorescence microscopy using Biofilm Tracer and Amytracker
- Imaging Flow Cytometry (Biofilm Tracer)
- (50 controls and 50 Long COVID)

Metasystems Automated Analysis of Platelets

- PAC-1: Green fluorescence
- CD62 PE: Orange fluorescence



Ischaemia–reperfusion (I–R) injury



Thrombotic Endothelialitis

Kell, D.B., and Pretorius, E. (2022). The potential role of ischaemia-reperfusion injury in chronic, relapsing diseases such as rheumatoid arthritis, Long COVID, and ME/CFS: evidence, mechanisms, and therapeutic implications. Biochem J *479*, 1653-1708.

Bigger Picture: Microclot presence

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Fibrinaloid microclots are **widely present in all chronic, inflammatory diseases** studied to date and ALSO in healthy individuals
These diseases include Alzheimer's, Parkinson's rheumatoid arthritis, and infection with SARS-CoV-2 leading to acute or long COVID-19
They can be **stained with fluorogenic dyes** such as thioflavin T or the Amytracker® dyes.
They are commonly in the size range 2-200 μm
They might be **resistant to the normal processes of fibrinolysis**; some are even resistant to trypsin – depending on their molecular content
They **can be induced *in vitro*** (in both whole blood and platelet poor plasma) with a variety of substances, including bacterial lipopolysaccharide, lipoteichoic acid, 17-b-oestradiol, and SARS-CoV-2 S1 spike protein
They can **exhibit considerable structural (and even spectral) heterogeneity**, *reflecting the molecules that were bound to the fibrinogen before polymerisation*

Pretorius, E., and Kell, D.B. (2023). A Perspective on How Fibrinaloid Microclots and Platelet Pathology May be Applied in Clinical Investigations. Semin Thromb Hemost.

Microclotting and endothelial damage point to heterogenous and complex disease pathology

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- It is **not only (simply) the size/numbers of microclots** present in healthy vs Long COVID (or any other disease with circulating inflammatory molecules), but their:
  - *content*
  - *activity*
  - *biochemical characteristics*
- We cannot ignore the role of hyperactivated platelets
- Ultimately these 2 pathologies are driving thrombotic endothelialitis

Kell, D.B., and Pretorius, E. (2022). The potential role of ischaemia-reperfusion injury in chronic, relapsing diseases such as rheumatoid arthritis, Long COVID, and ME/CFS: evidence, mechanisms, and therapeutic implications. Biochem J *479*, 1653-1708.

Turner, S., Khan, M.A., Putrino, D., Woodcock, A., Kell, D.B., and Pretorius, E. (2023). Long COVID: pathophysiological factors and abnormalities of coagulation. Trends Endocrinol Metab *34*, 321-344.

Pretorius, E., and Kell, D.B. (2023). A Perspective on How Fibrinaloid Microclots and Platelet Pathology May be Applied in Clinical Investigations. Semin Thromb Hemost.

Next Steps

Research, Commercial and Collaborator Endeavours

- Commercial endeavours: Imaging flow cytometry and microscopy microclot testing
- Ranges of microclot presence: other inflammatory diseases
- 3rd Proteomics analysis underway
- Identifying more novel markers: e.g. endothelial debris markers, biofilm presence
- Clinical trials in the US/UK: anticoagulation
- Collaborator grant applications

Prof Melanie Walker, Clinical Professor Department of Neurological Surgery, UW Medicine
Dr Mike van Elzakker, Harvard
Dr Caroline Dalton, Sheffield University
Dr Mark Walsh, Department of Internal Medicine, Indiana University School of Medicine
Dr Akiko Iwasaki, Yale University
Paul Glynne MD, Consultant physician at UCLH
Vanya Gant MD, Divisional Clinical Director for Infection, UCLH
Ashely Woodcock MD, Professor of respiratory medicine University of Manchester
Martin Kräter, Max Planck Institute
Ilene Ruhoy, MD, Neurologist Mount Sinai South Nassau Hospital
David Lee, MD, Emergency Physician, NYU Grossman School of Medicine
Amy Proal, PhD, Microbiologist, PolyBio Research Foundation
Anna Brooks, Cellular Immunologist, University of Auckland
Dr David Putrino, Director of Rehabilitation Innovation Mt Sinai Health System
Inus Laboratories, Switzerland
Dr Graham Lloyd-Jones, Director of Radiology and Consultant Radiologist Salisbury District Hospital

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Thank you
Enkosi
Dankie