Microclots as a common indicator of chronic disease

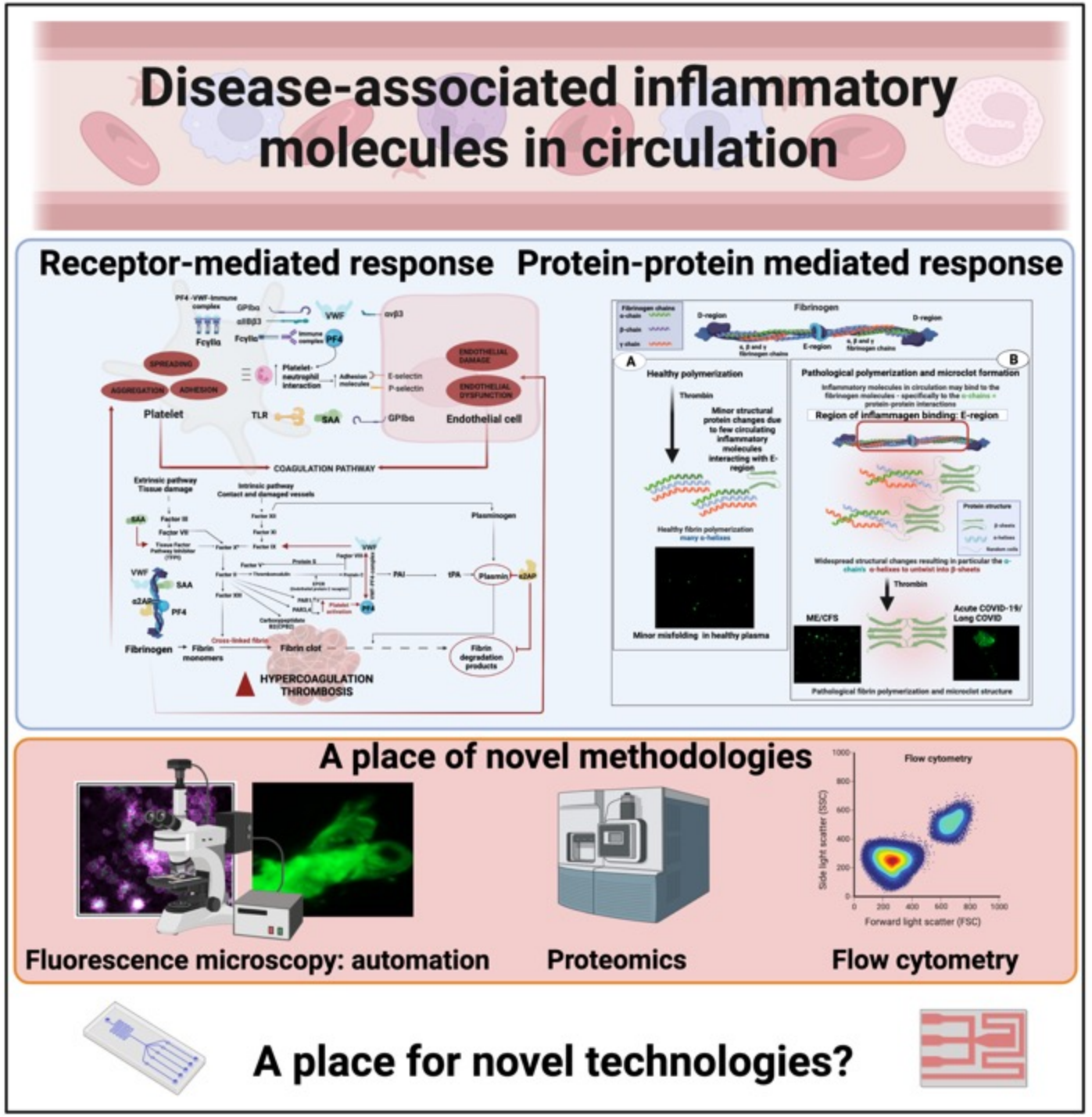
Science | EyeNzululwazi ngezeNdalo | Natuurwetenskappe

Acknowledgements

- Prof Douglas B Kell: University of Liverpool (UK): Systems Biologist
- Clinicians: Dr Jaco Laubscher: Mediclinic Stellenbosch (MMed: Internal Medicine); Dr Arneaux Kruger (Private practice clinician)
- Dr Mare Vlok: Stellenbosch University (SA): Biochemist and Proteomics lab
- Massimo Nunes: PhD student
- Tom Usher: MSc student
- Simone Turner: PhD student
- Dr Chantelle Venter: Stellenbosch University (SA): Physiologist
- *Mediclinic (private hospital group): approved sample collection at their facility*

Focus of this talk

- Relevance of receptor-inflammatory marker interaction in driving disease pathologies
- Relevance of direct protein-protein interactions in clotting pathologies
- The place of novel methods in diagnosing well-known and new diseases



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

Platelets, circulating inflammatory molecules and abnormal blood clotting

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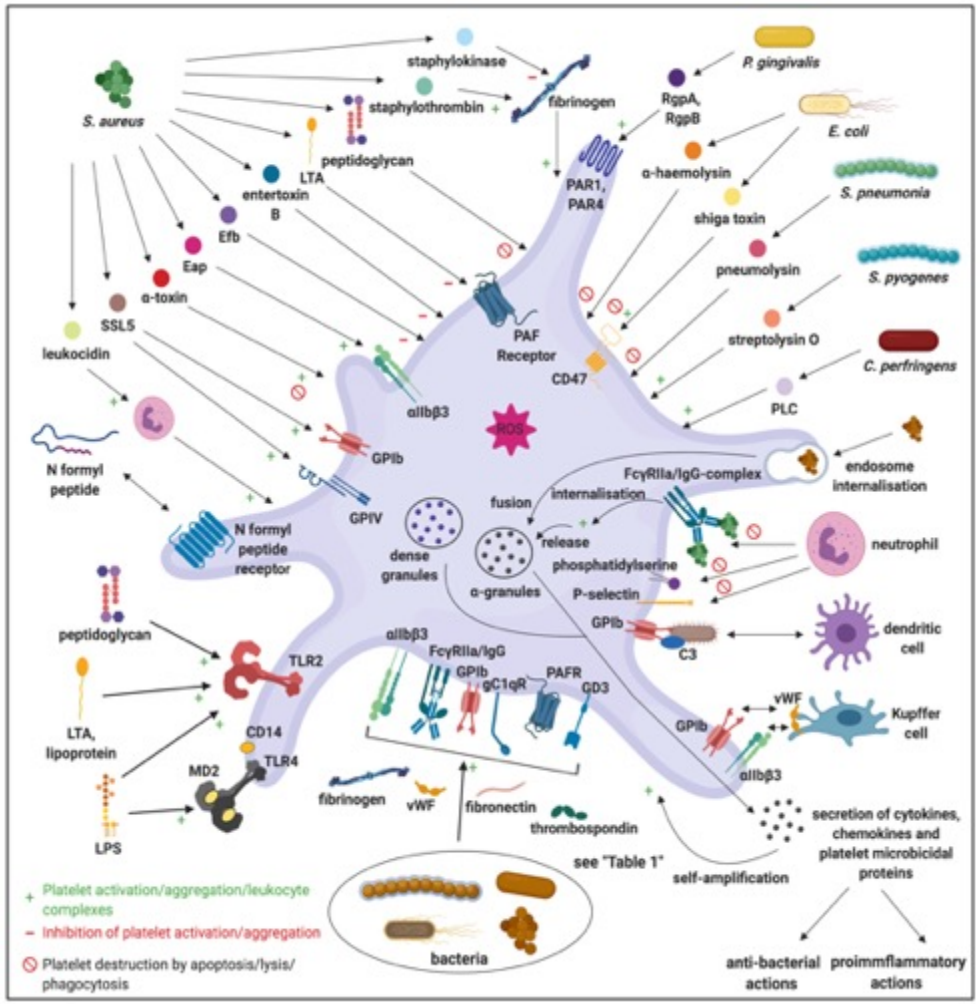
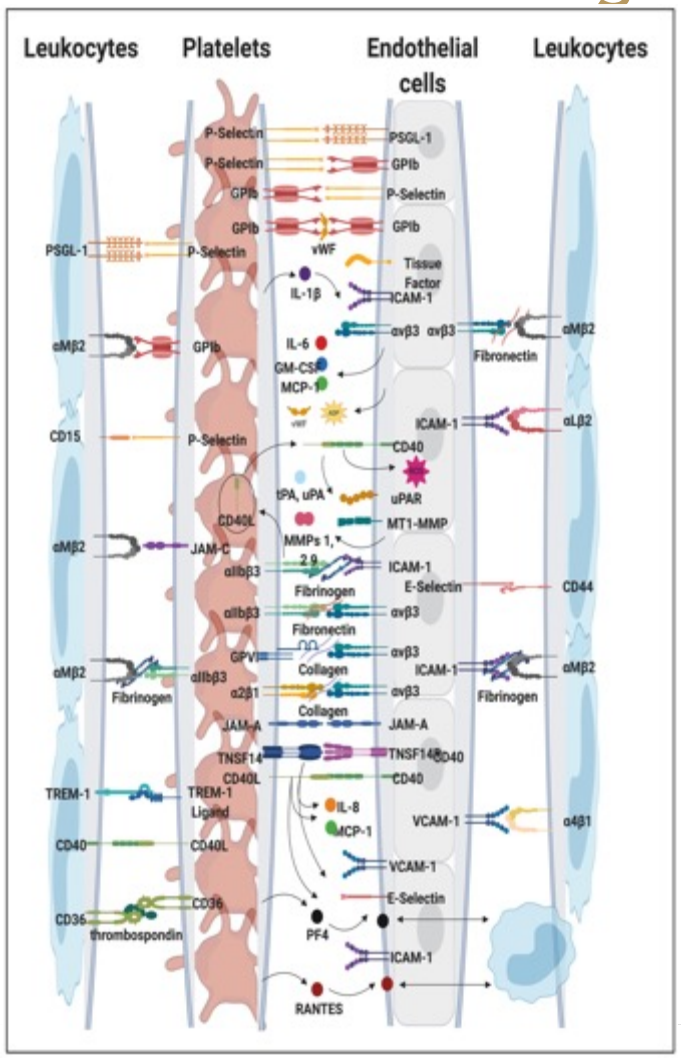
Platelet receptors

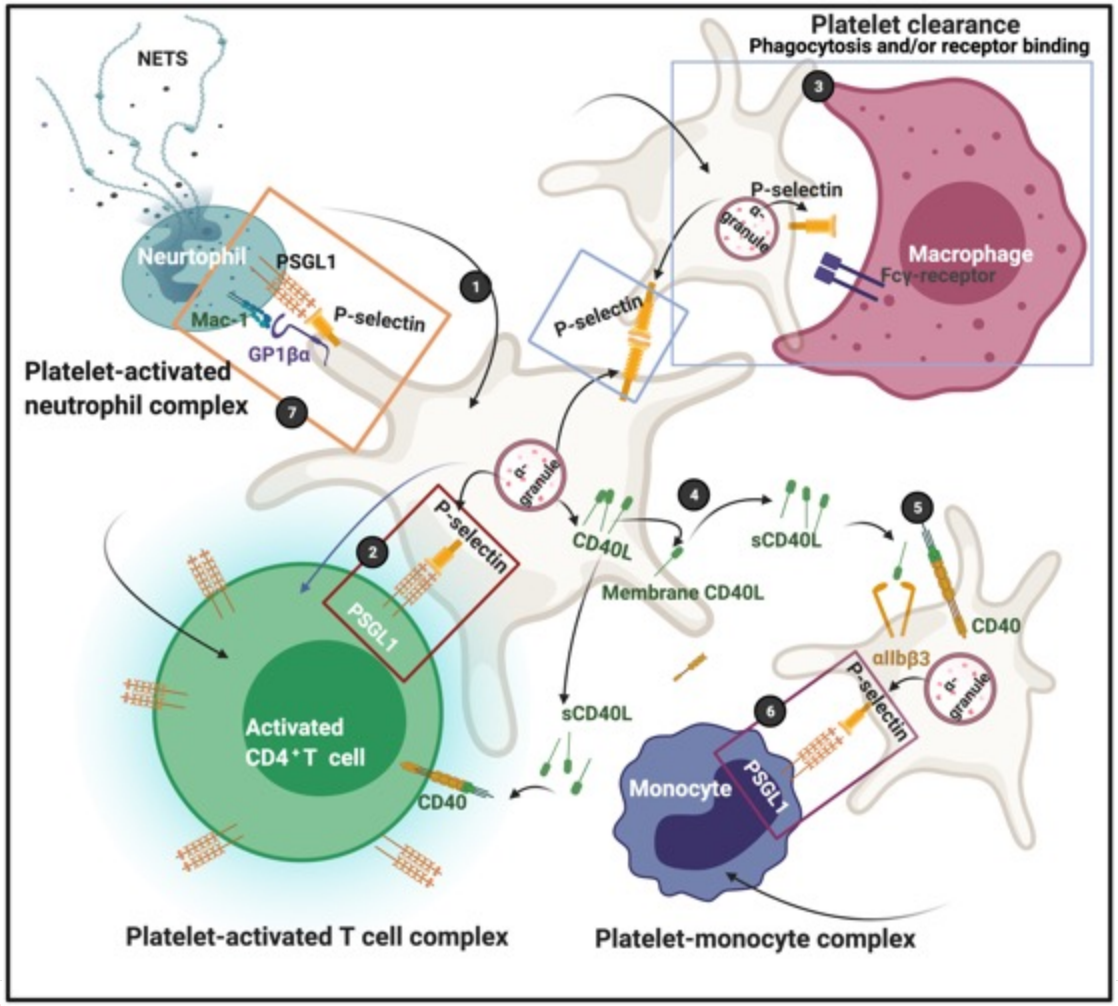


Platelet/endothelial cell signalling

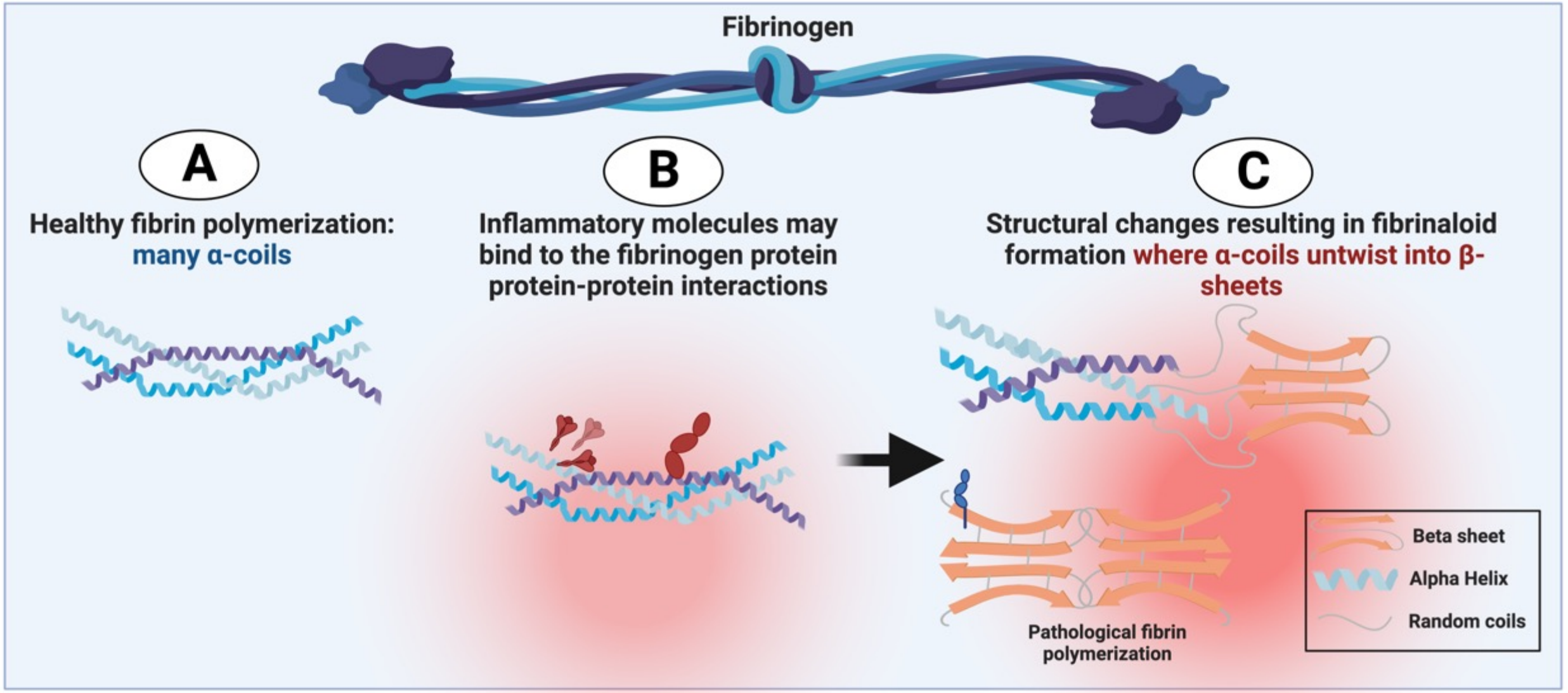


Platelets Interactions

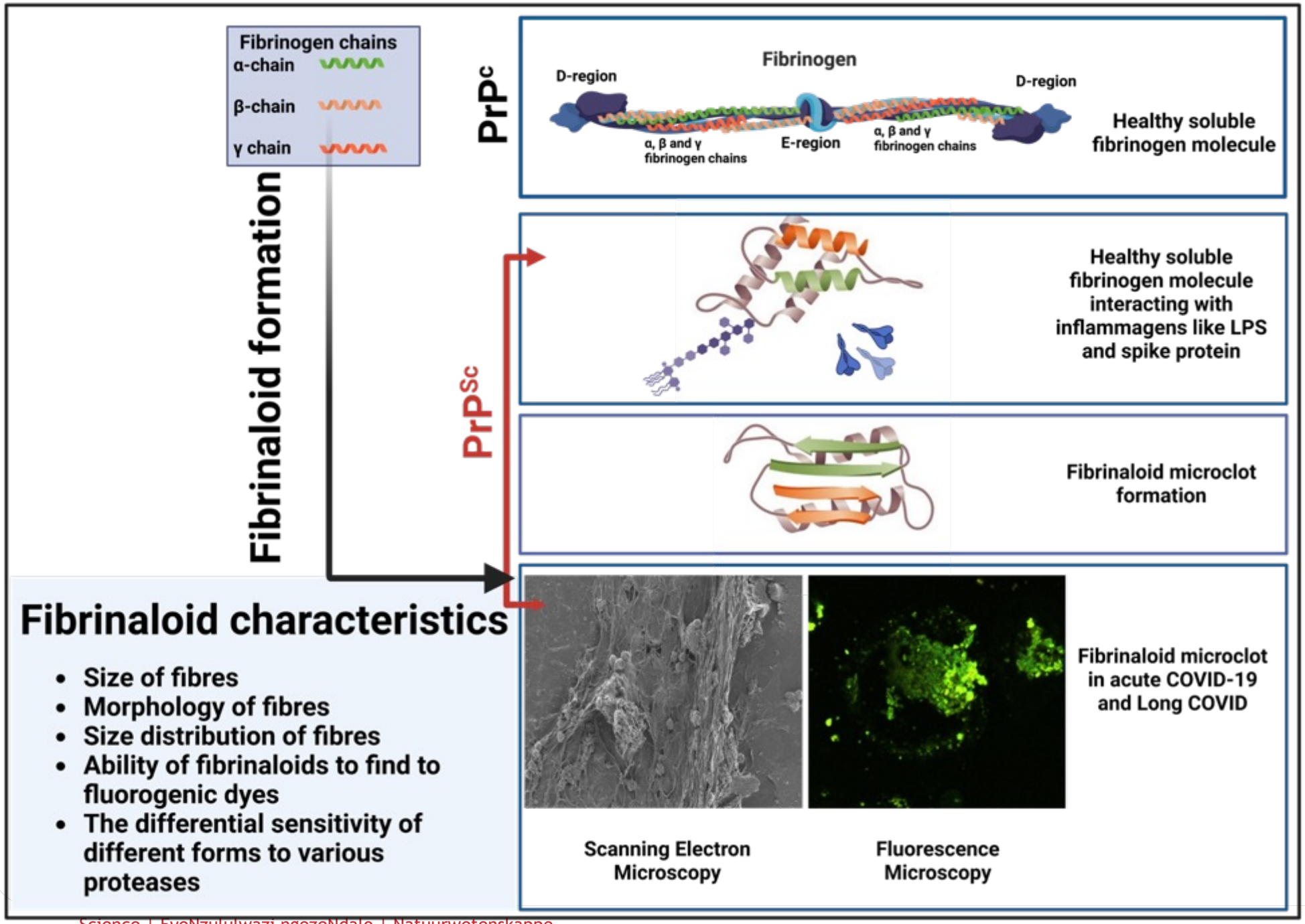
Platelet-Immune Cell Complexes



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.



Fibrinaloid characteristics

- Size of fibres
- Morphology of fibres
- Size distribution of fibres
- Ability of fibrinaloids to find to fluorogenic dyes
- The differential sensitivity of different forms to various proteases

Platelet and clotting pathologies in acute COVID-19

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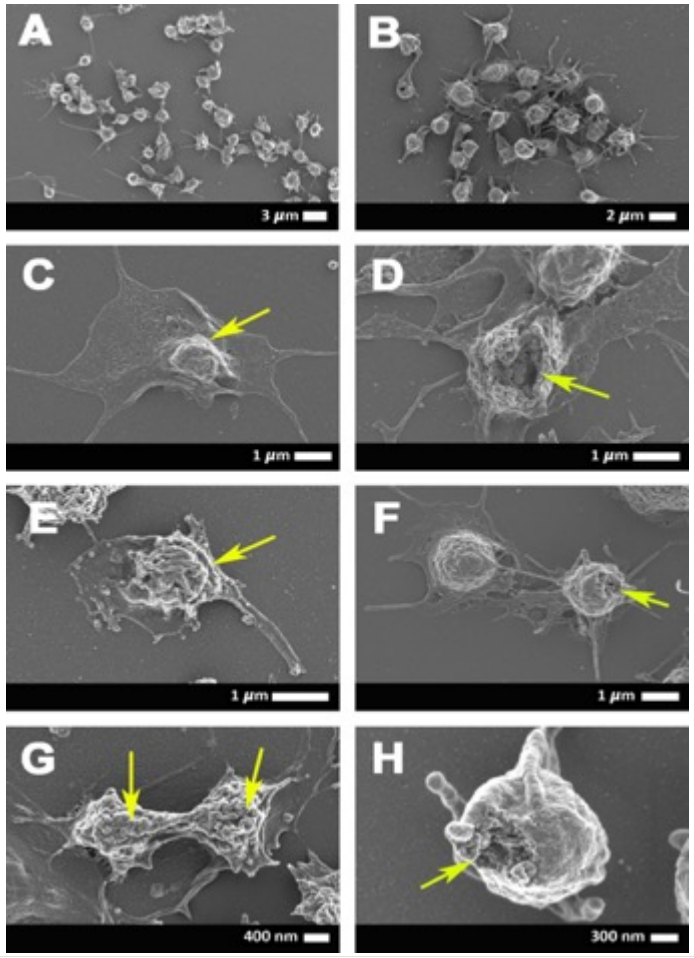
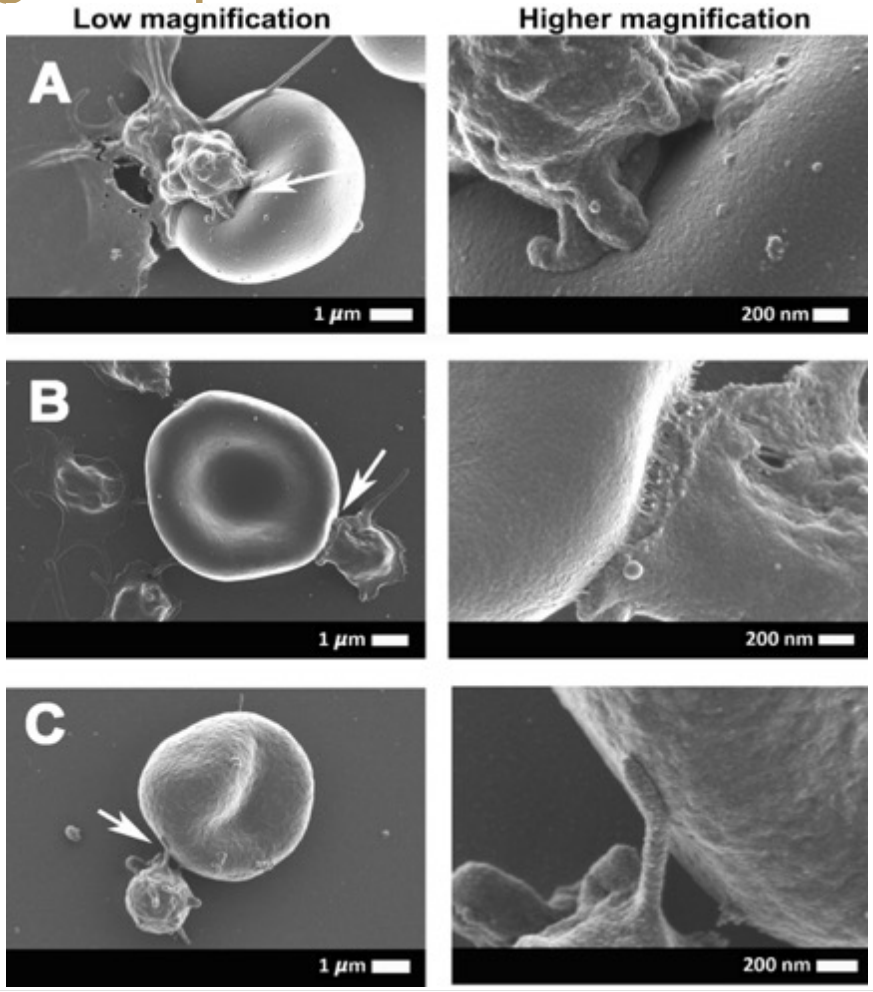
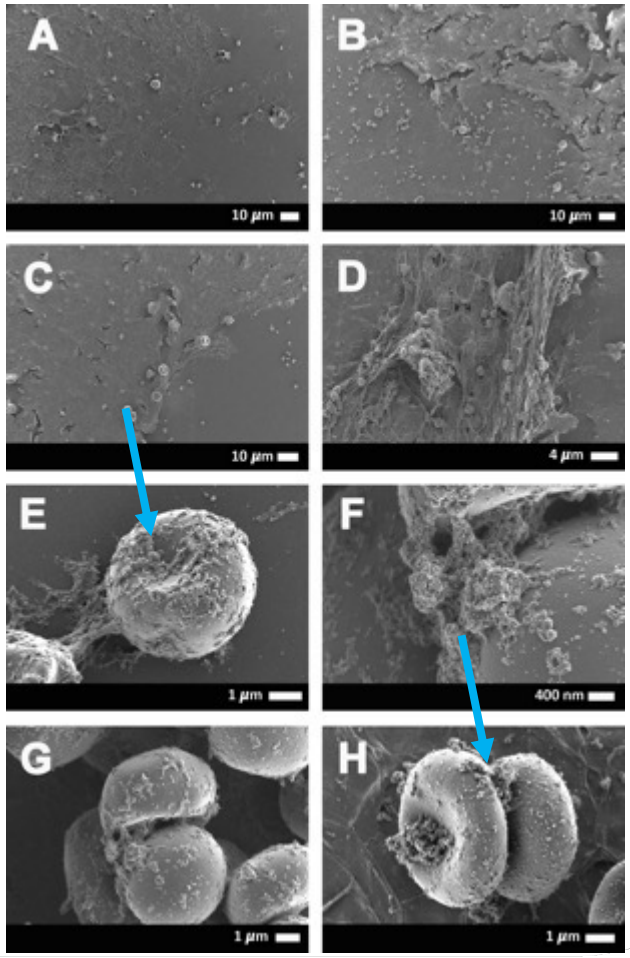
Structural changes in platelets





Microclots



Structural Changes in Fibrin(ogen)

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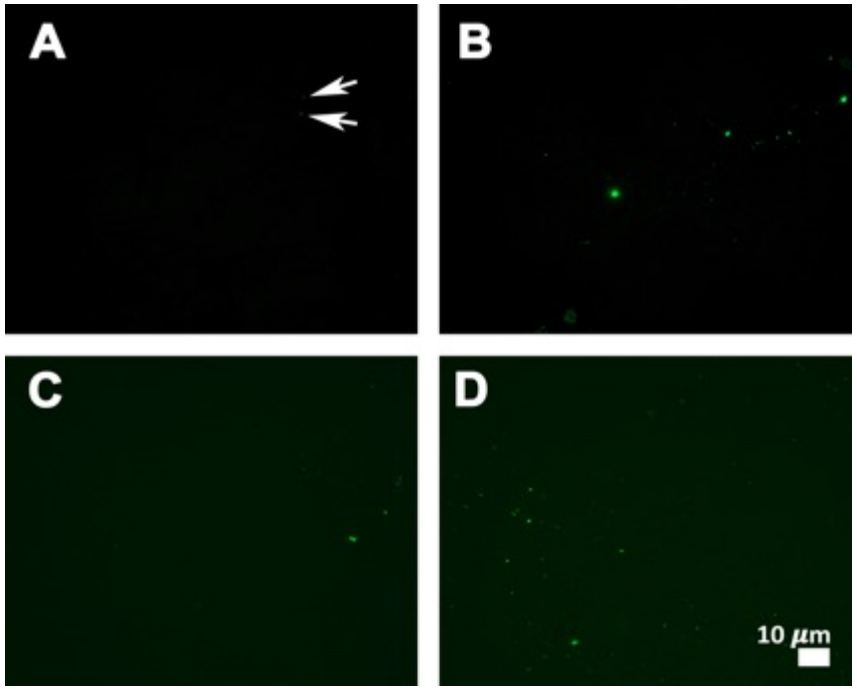
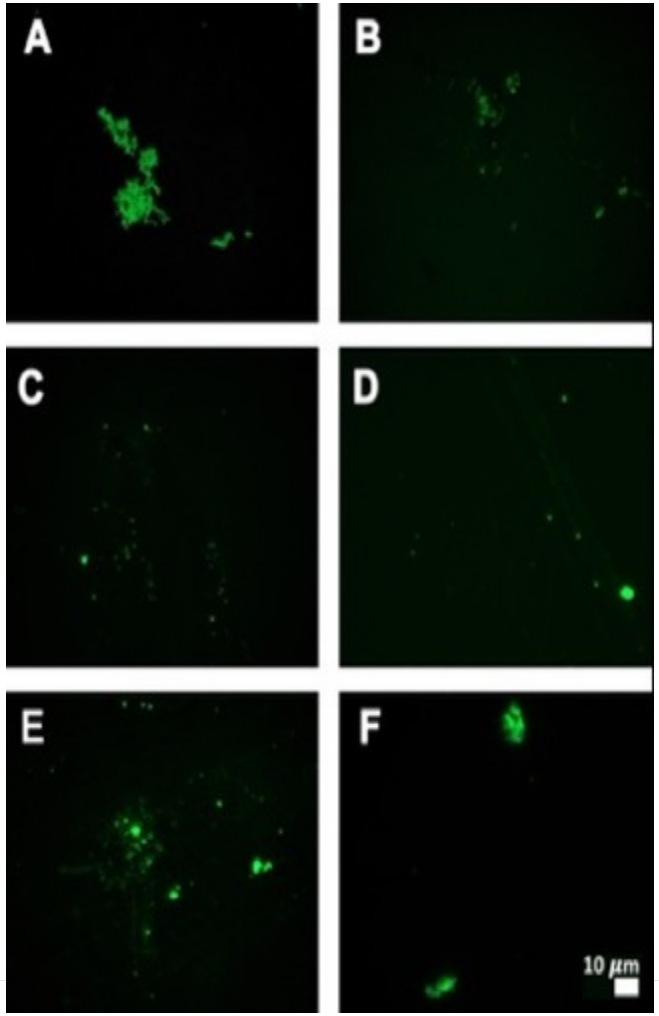
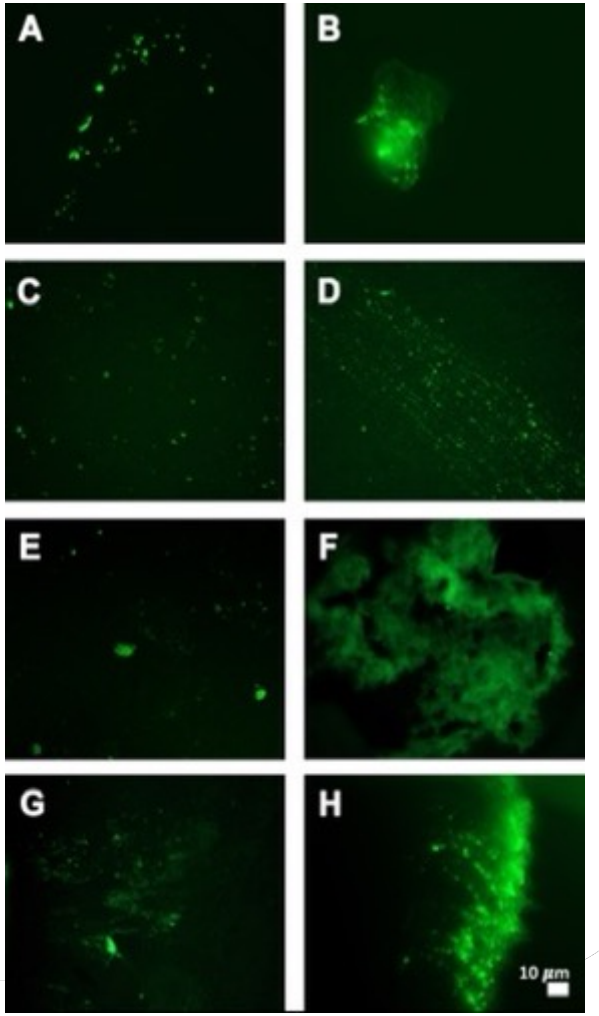
Healthy Plasma



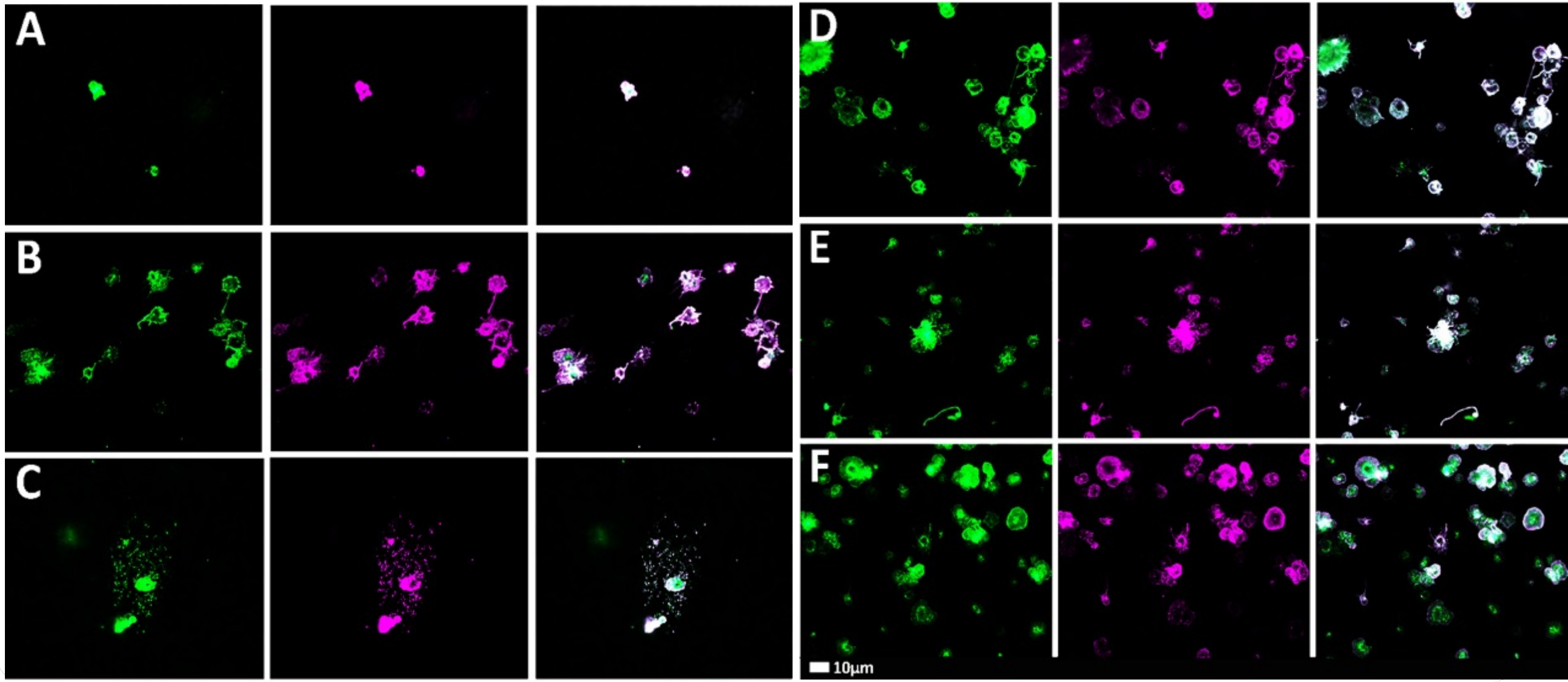
Type 2 Diabetes Plasma



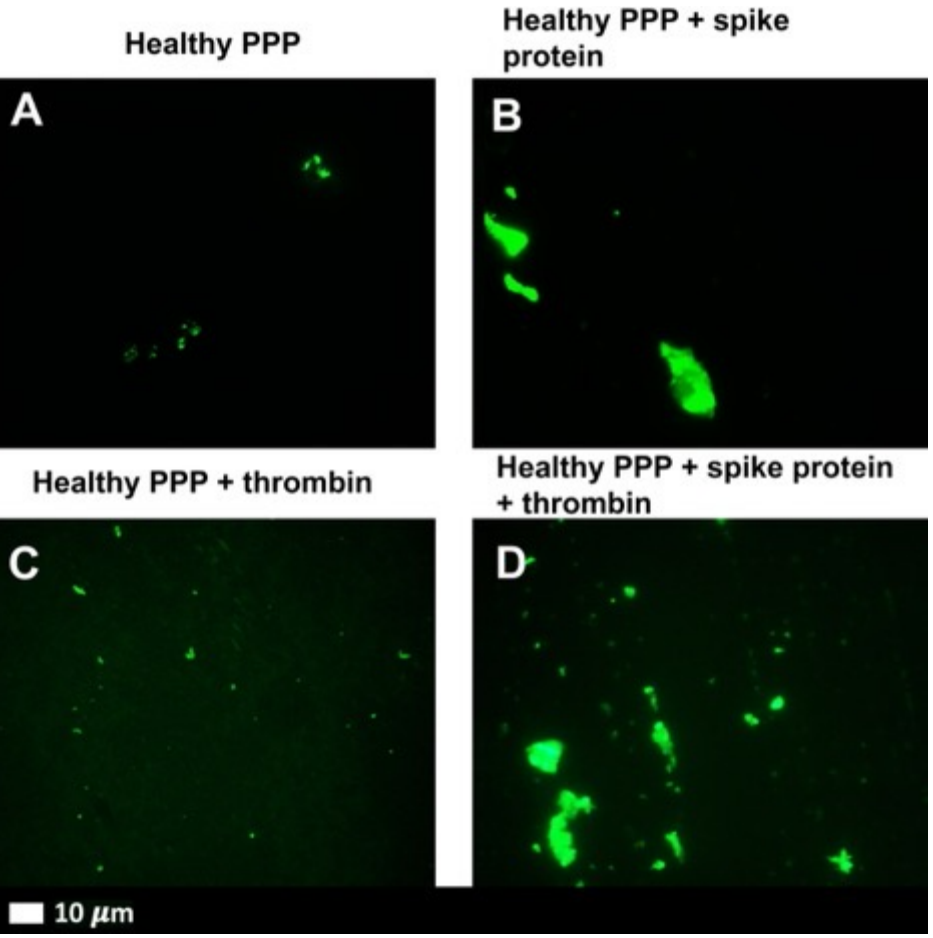
COVID-19 Plasma

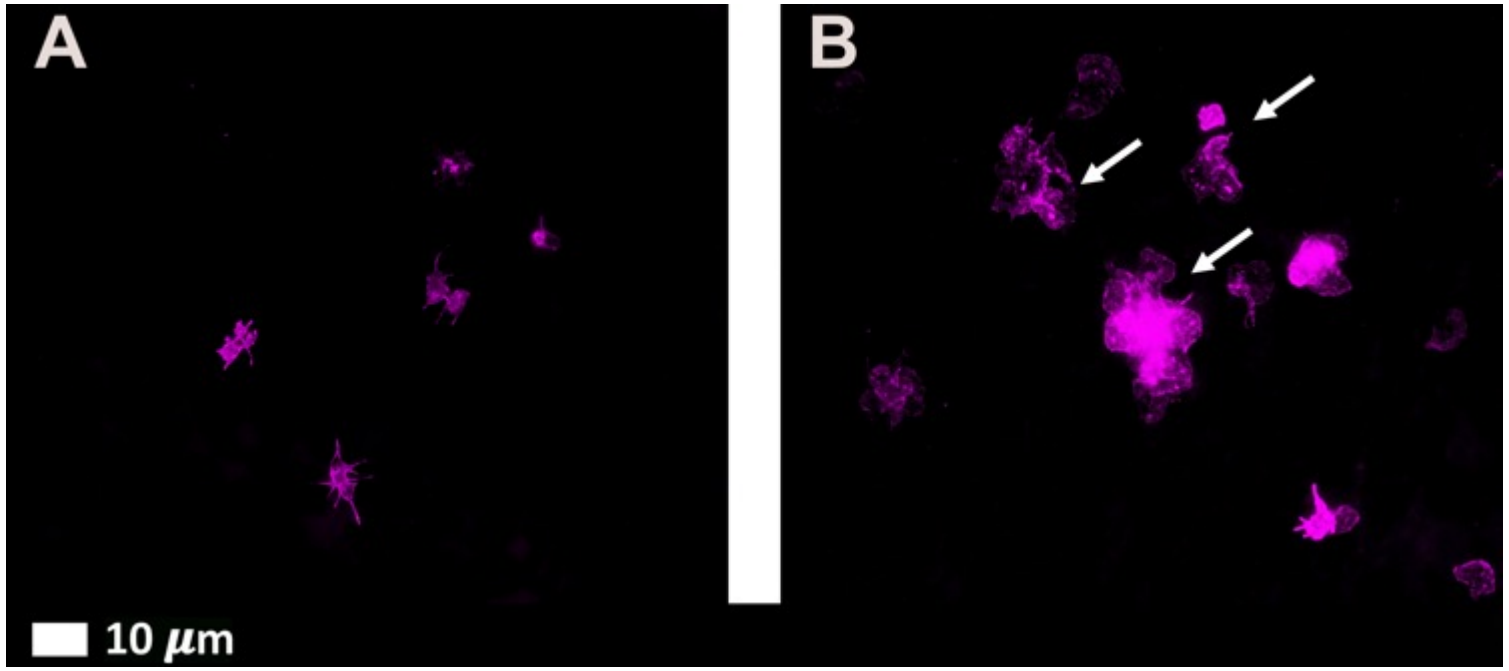


Platelets in controls (A) and Acute COVID-19 (B-F)



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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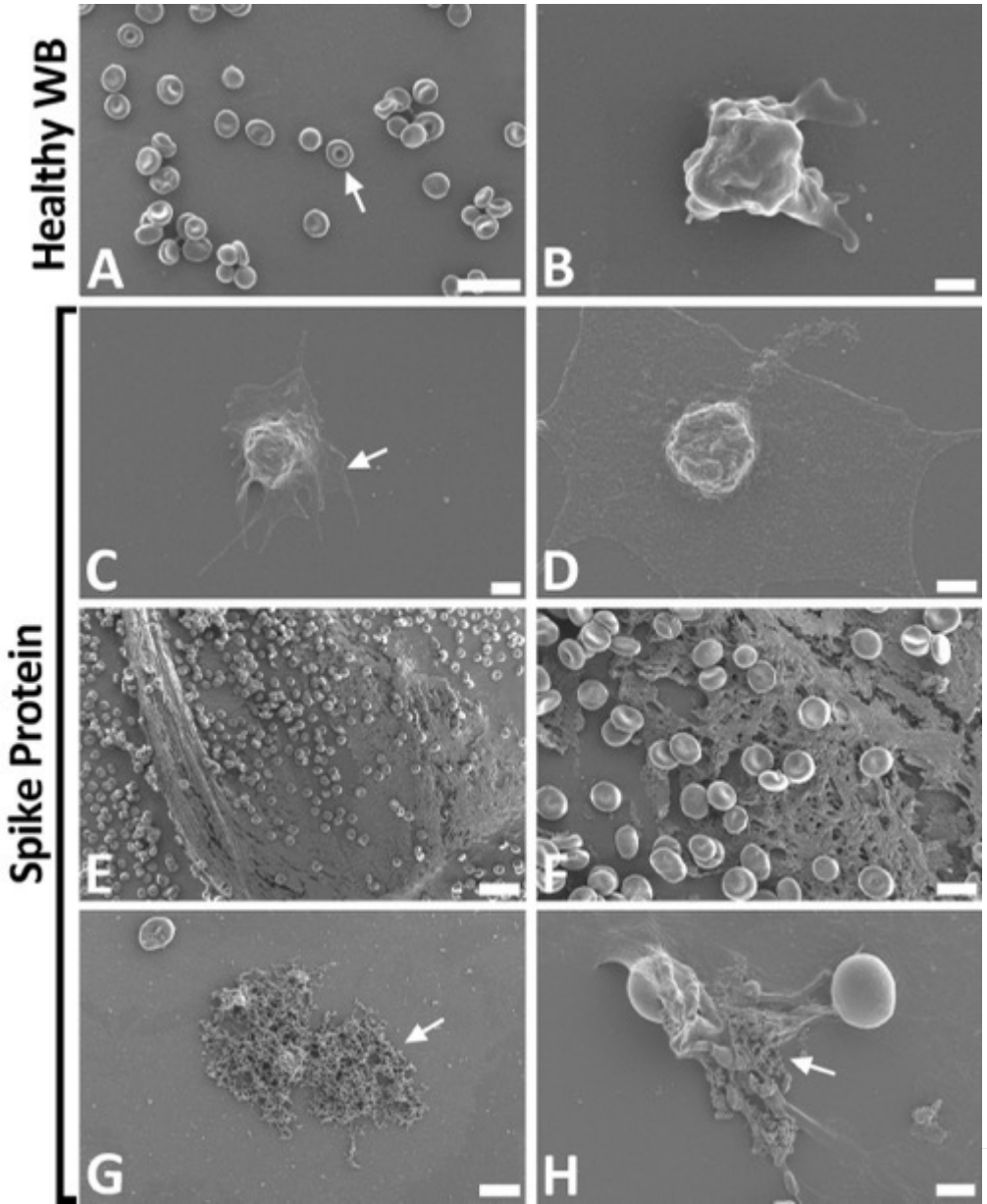
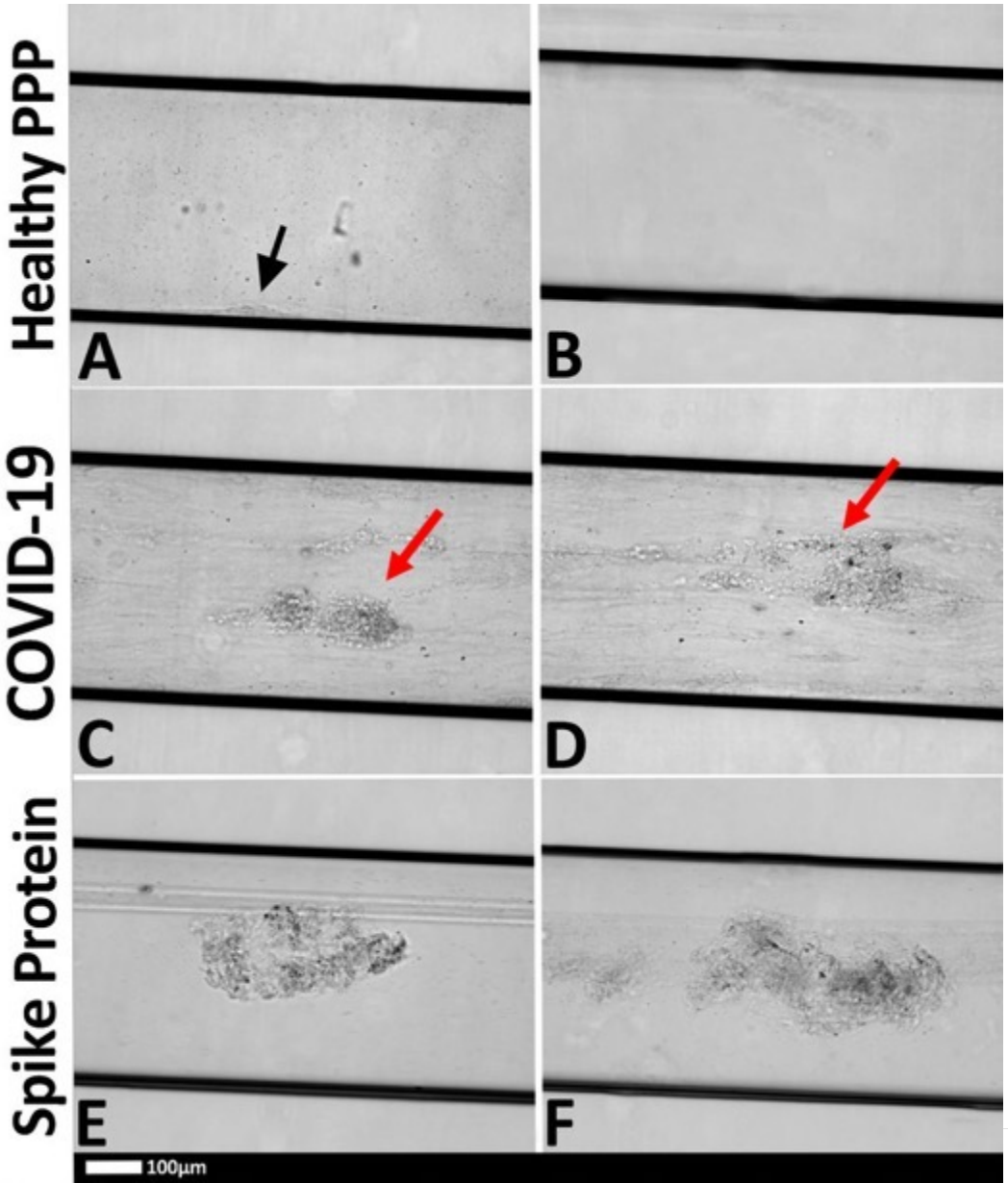
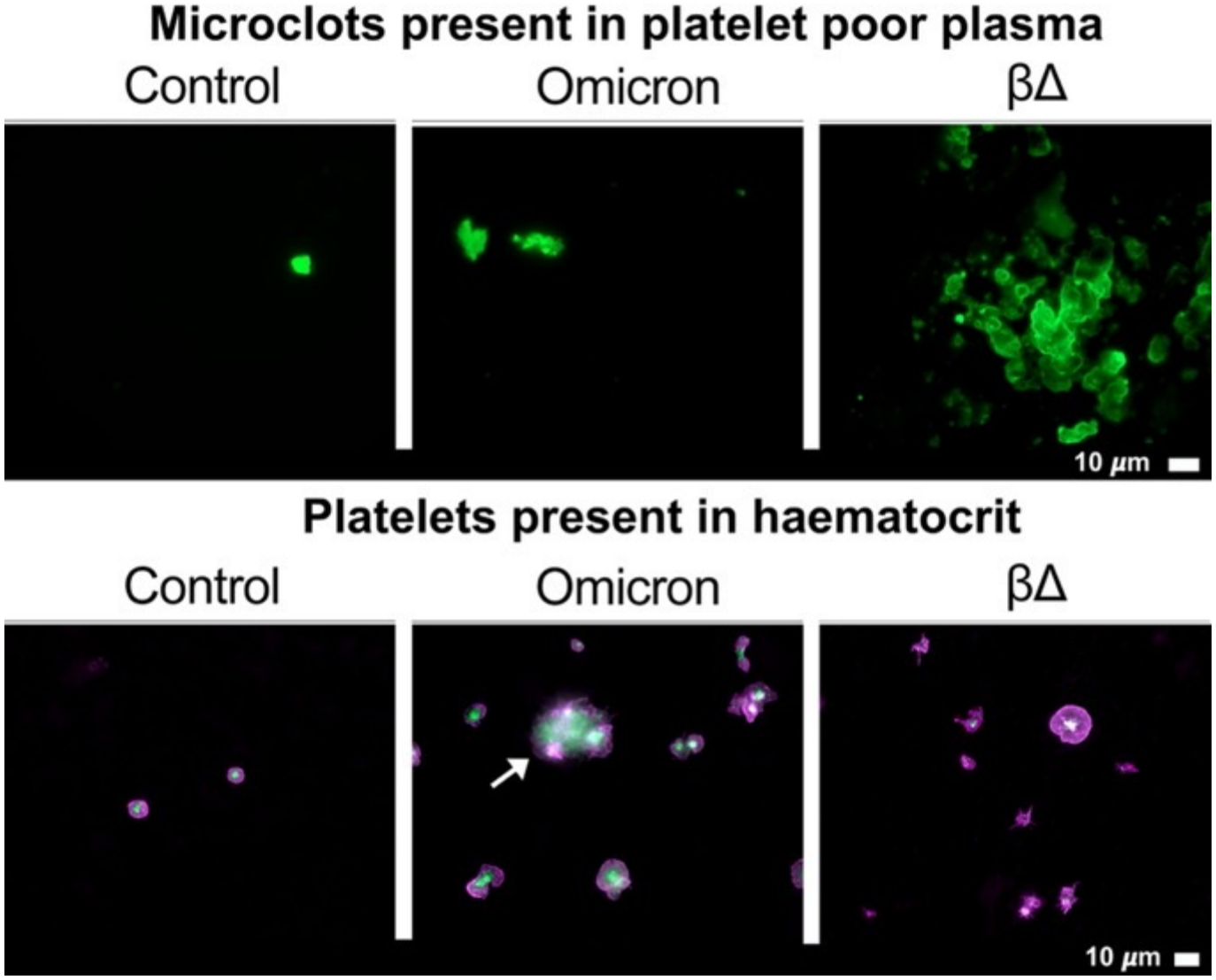




Healthy plasma versus older beta/delta vs Omicron



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.

Platelet ultrastructure

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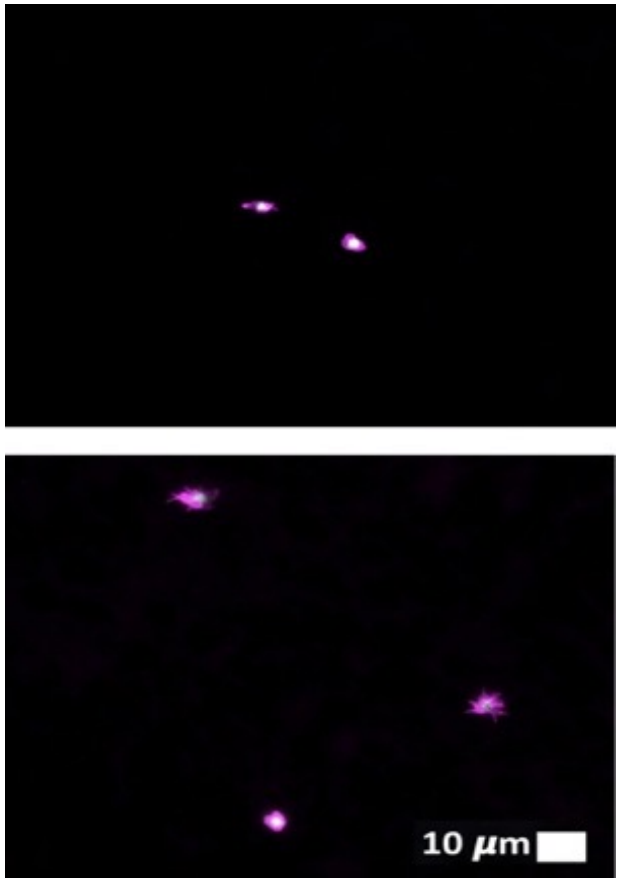
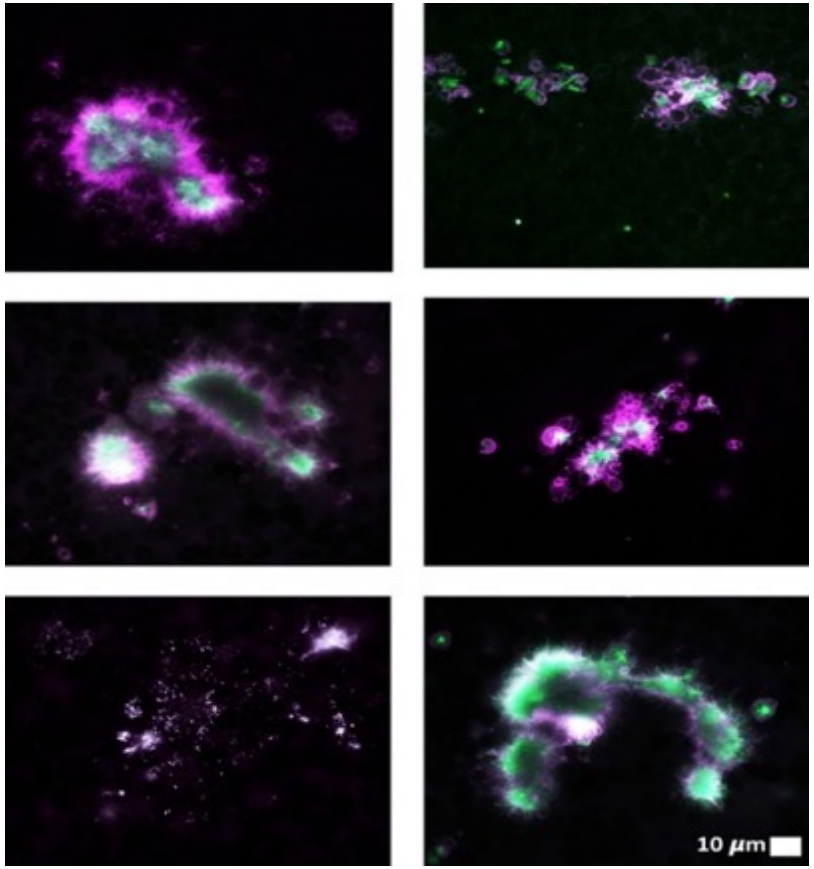
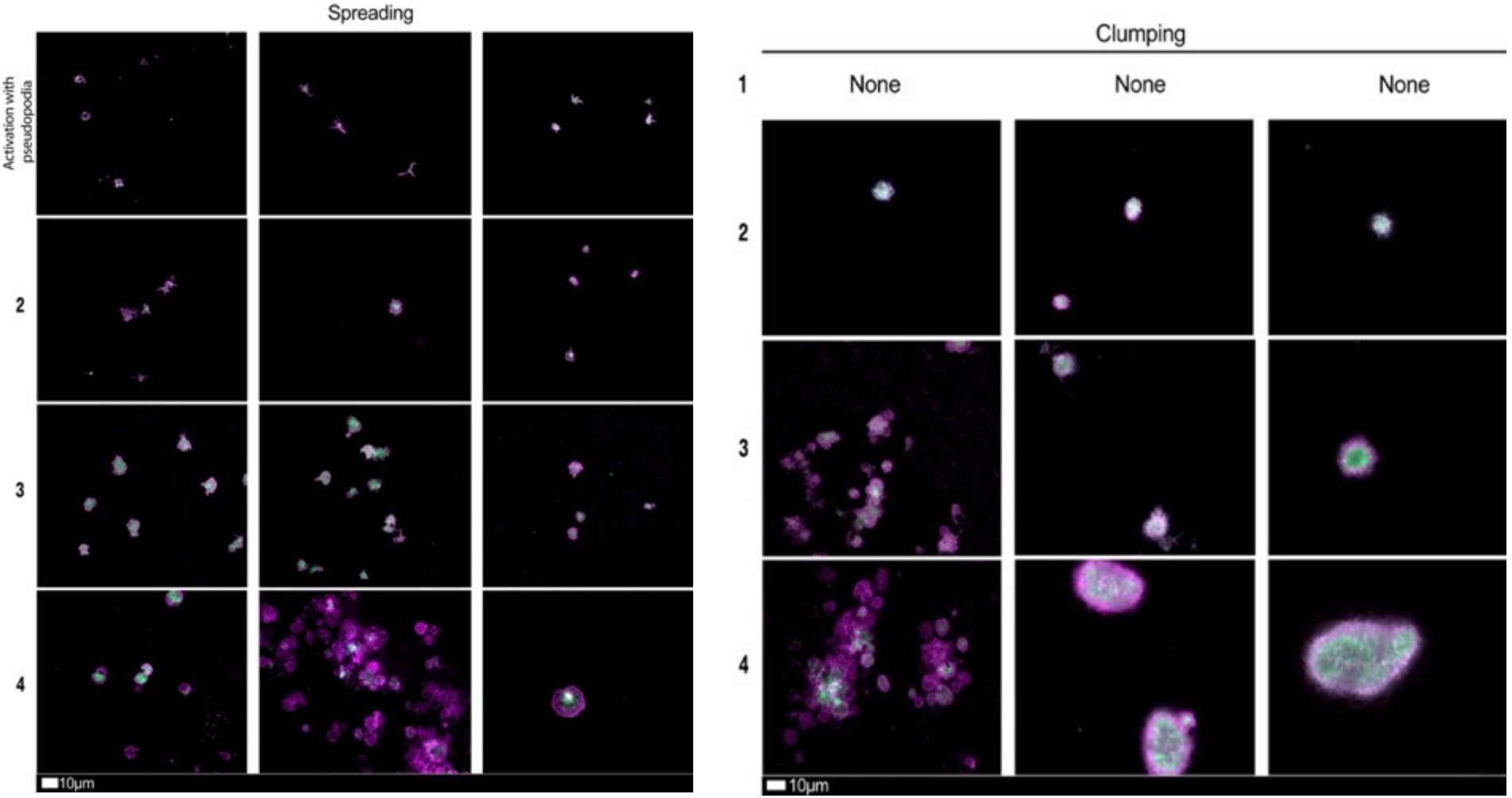
Platelets from Healthy Individuals



Platelets from Individuals with Long COVID



A Platelet Grading System

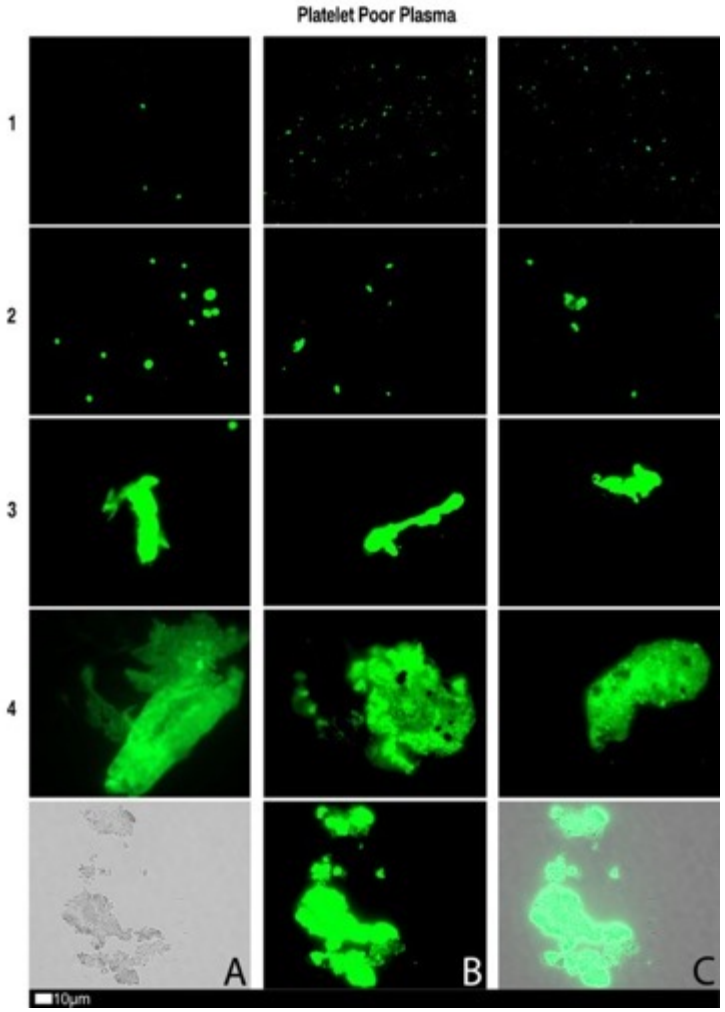


CD62P (PE-conjugated) (Pinkish signal) = P-selectin

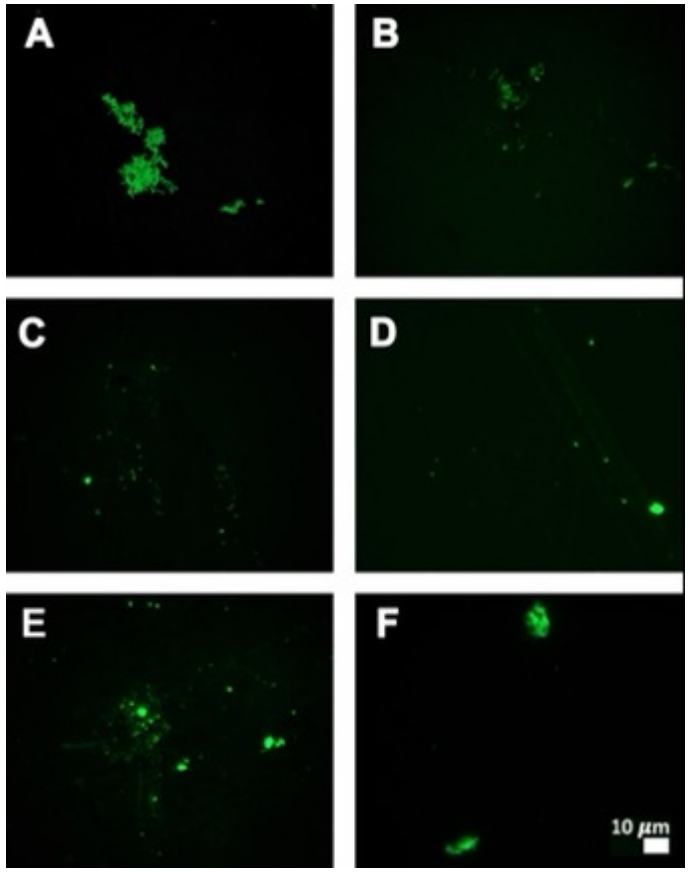
PAC-1 (green signal) = glycoprotein IIb/IIIa on the platelet membrane.

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



Type 2 Diabetes Plasma



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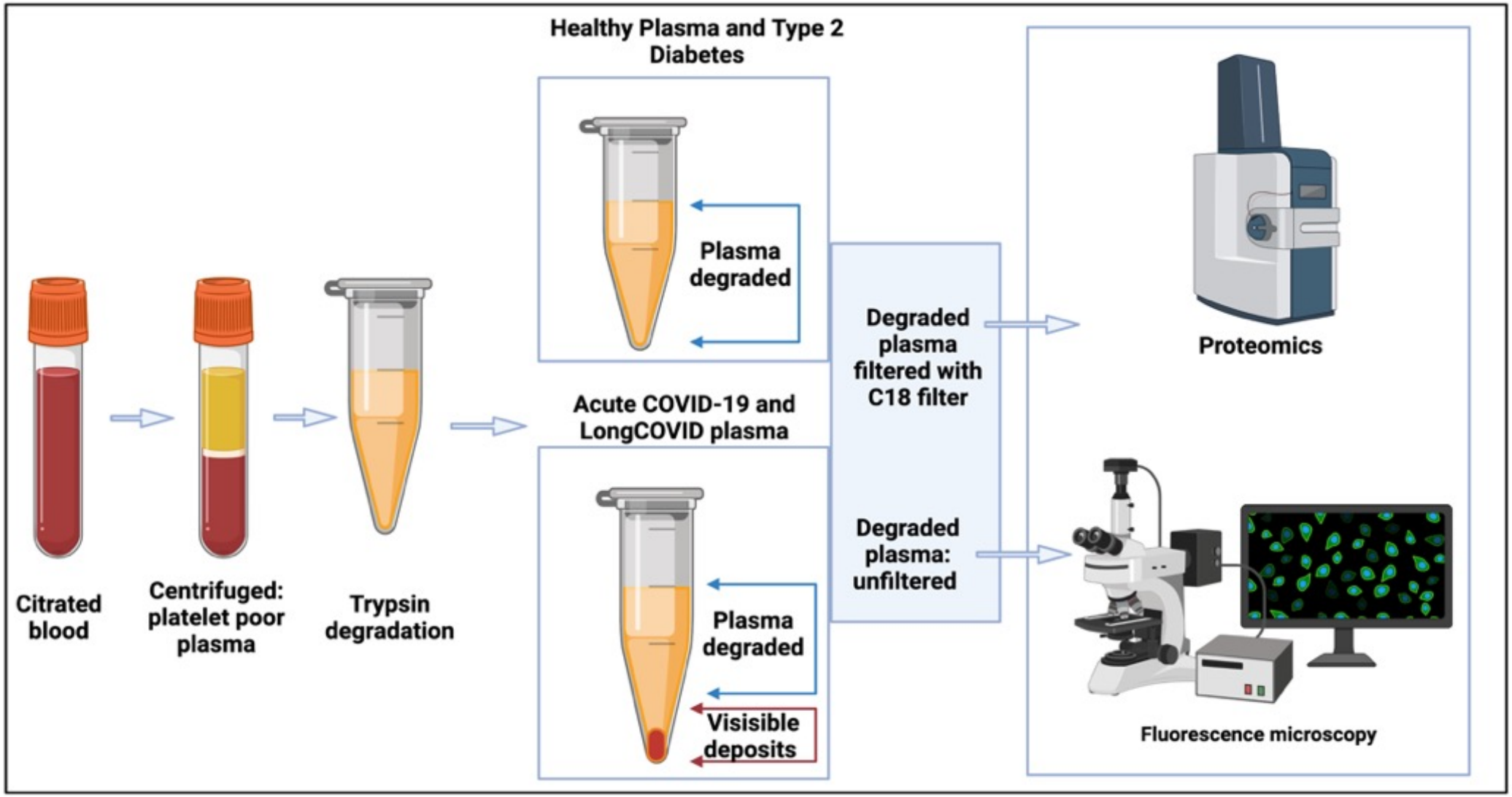
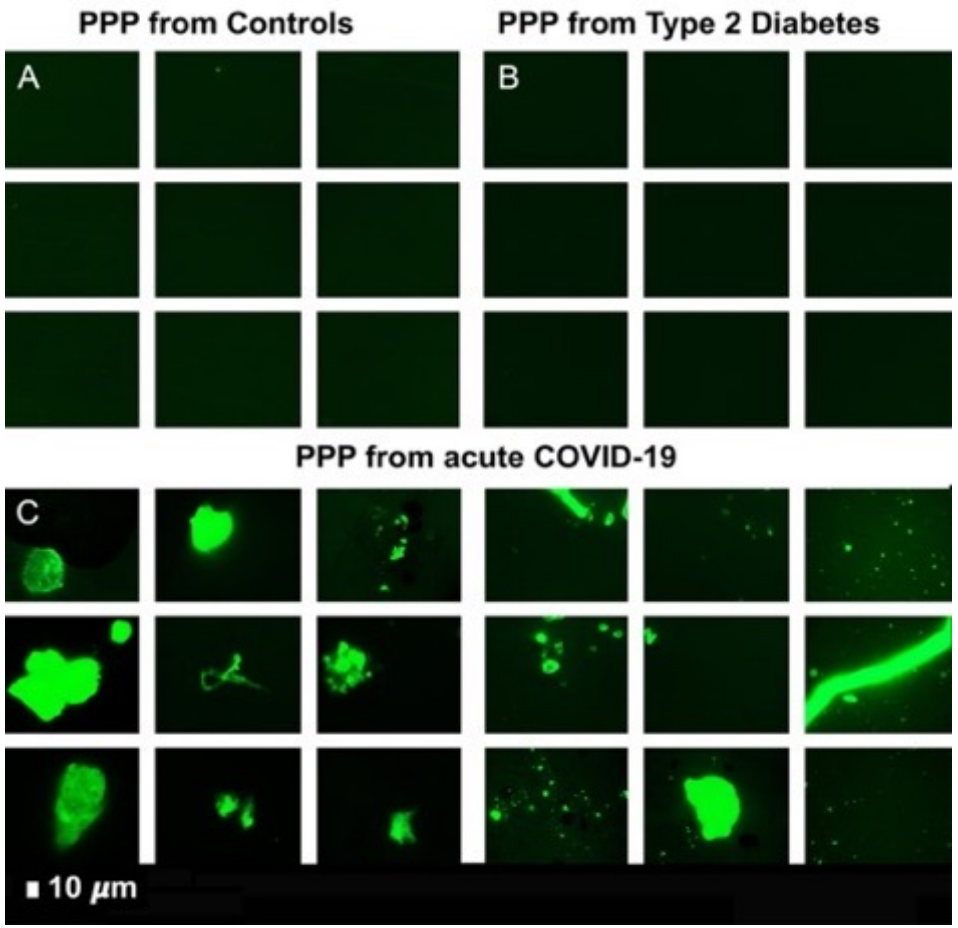
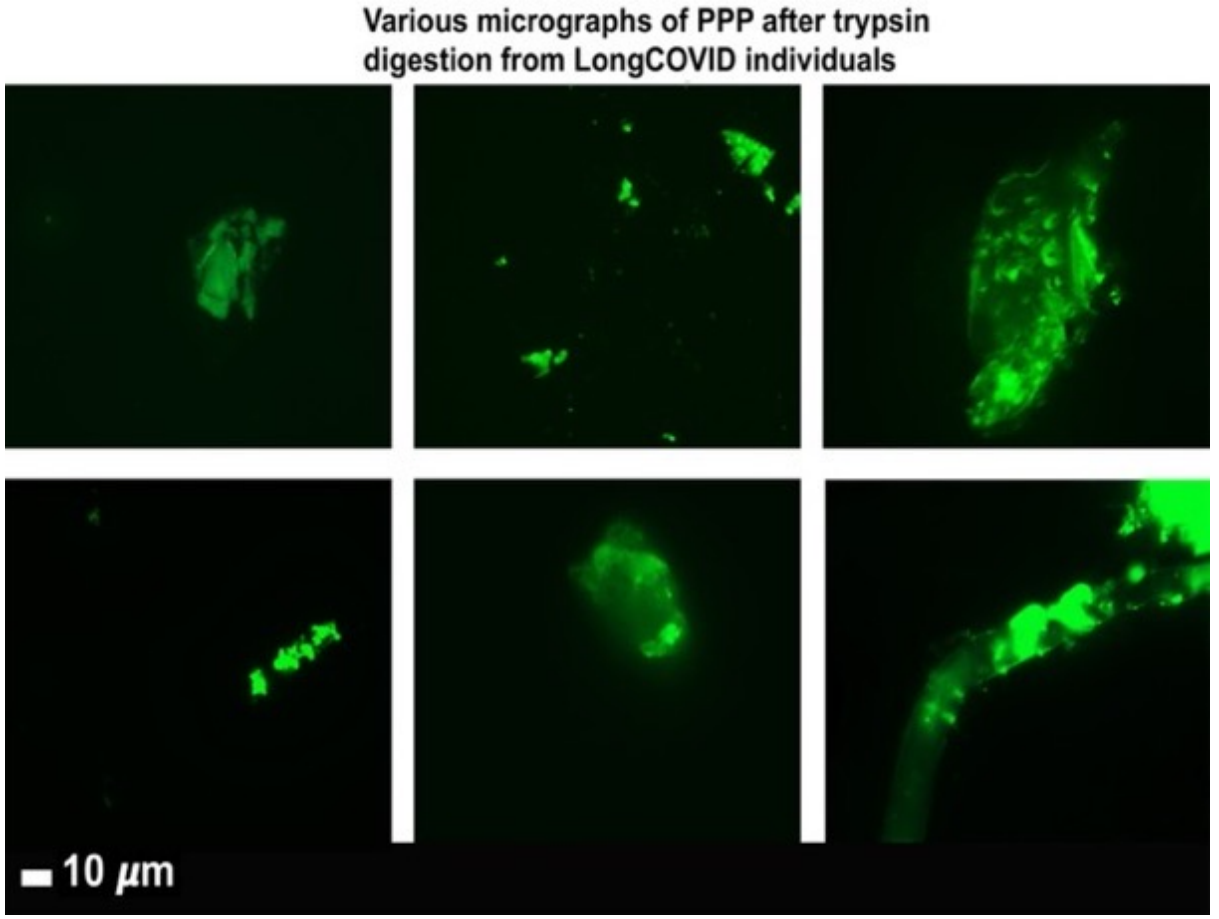
Proteomics of Plasma from Healthy, Diabetic, Acute COVID-19 and Long COVID



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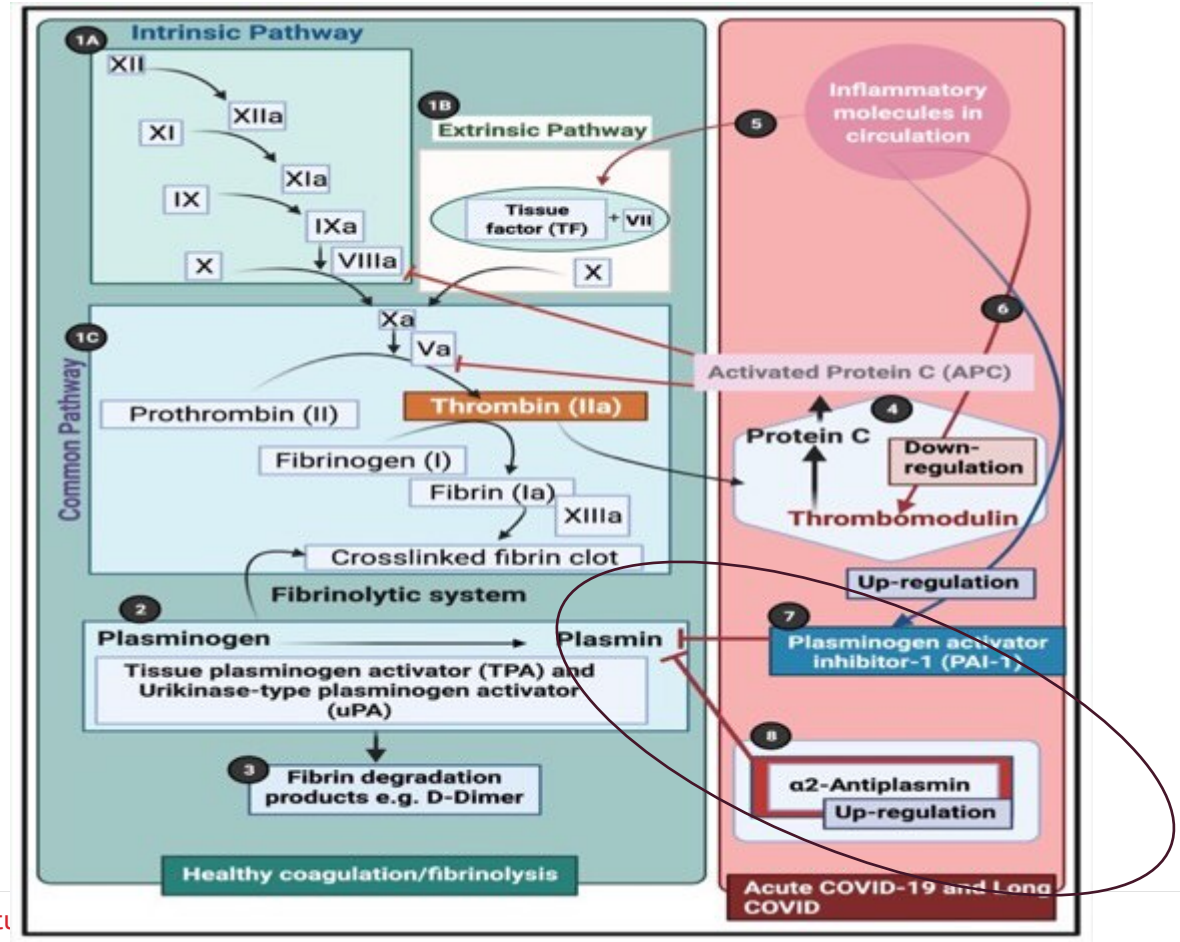
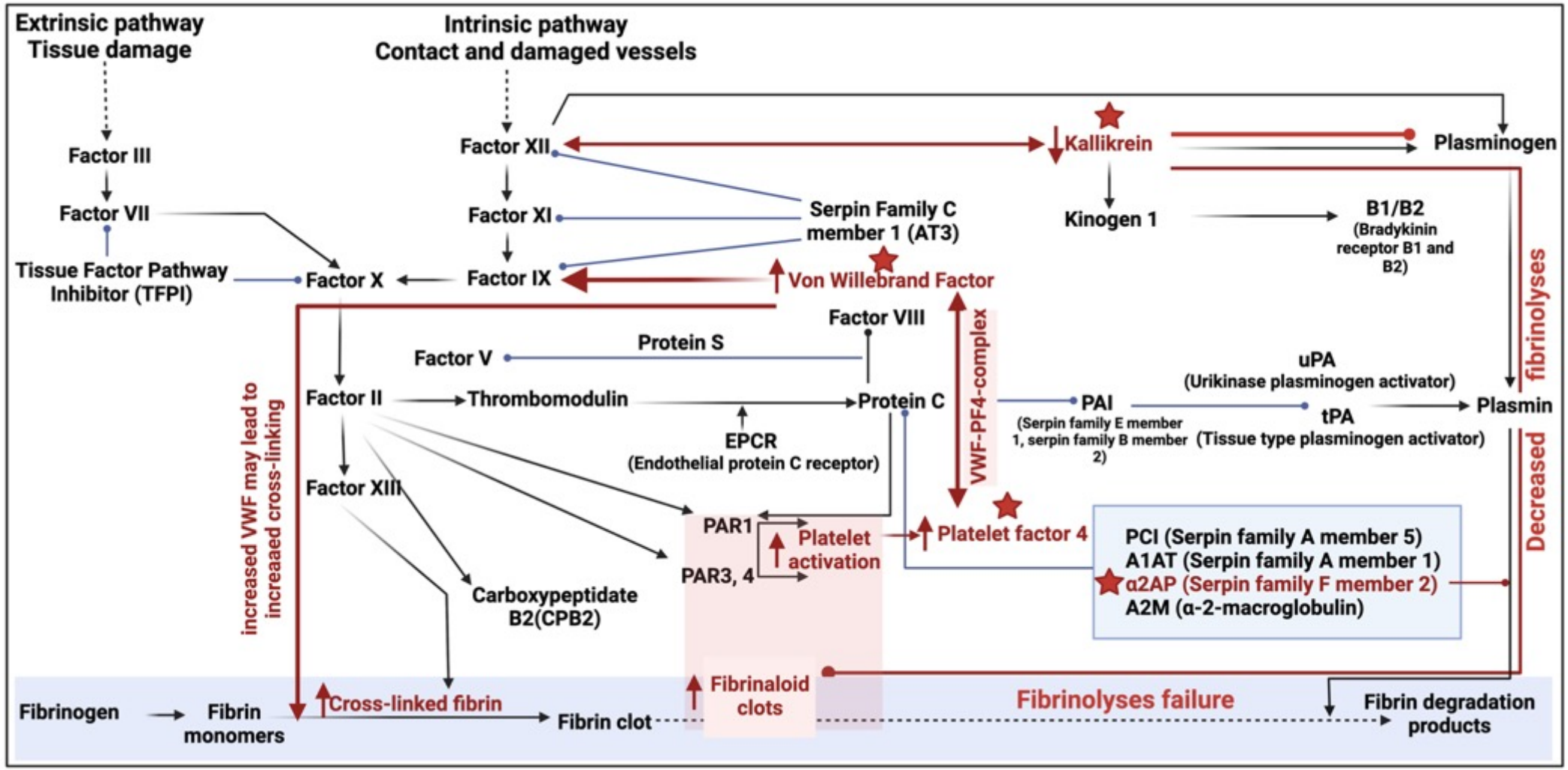
α2-antiplasmin (α2AP) inhibit plasmin and ultimately will prevent sufficient fibrinolysis to happen



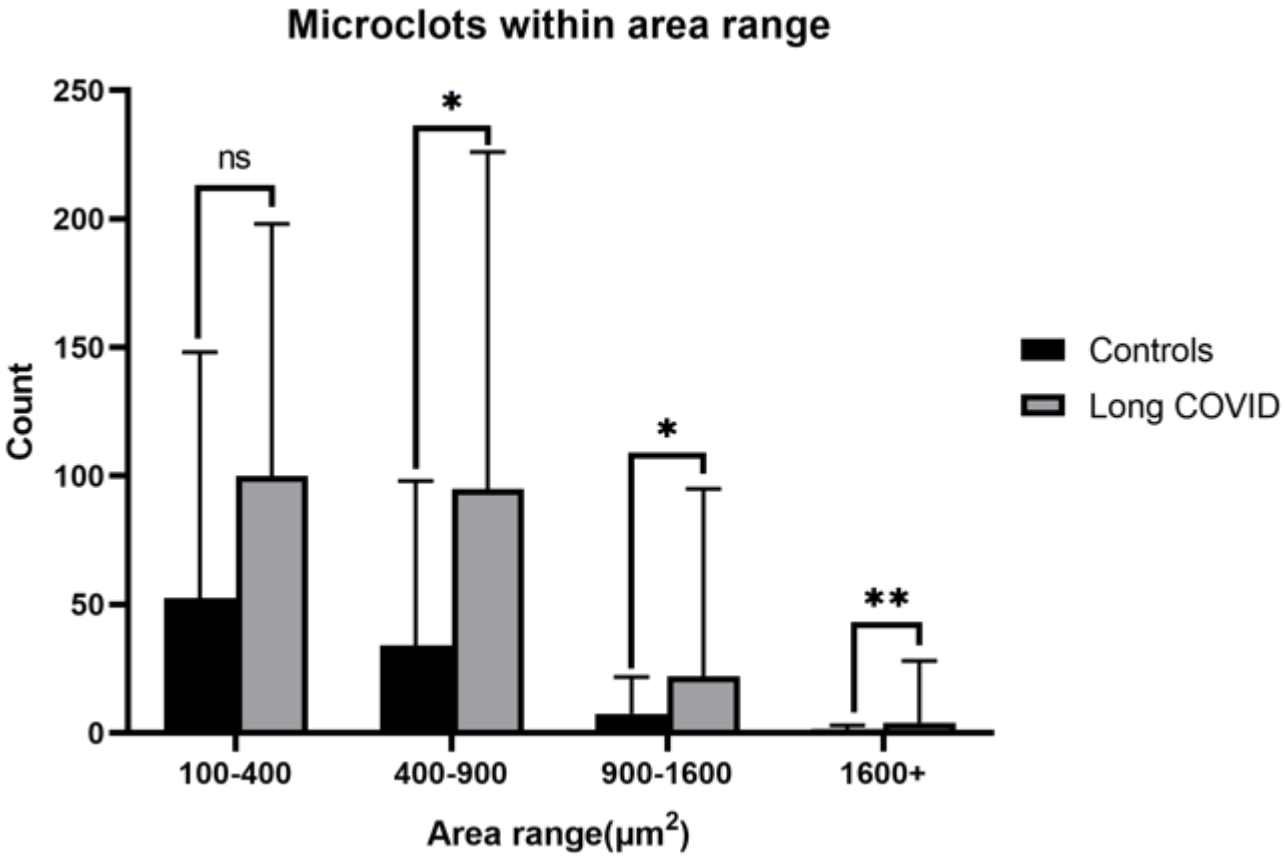
2022 Proteomics Analysis

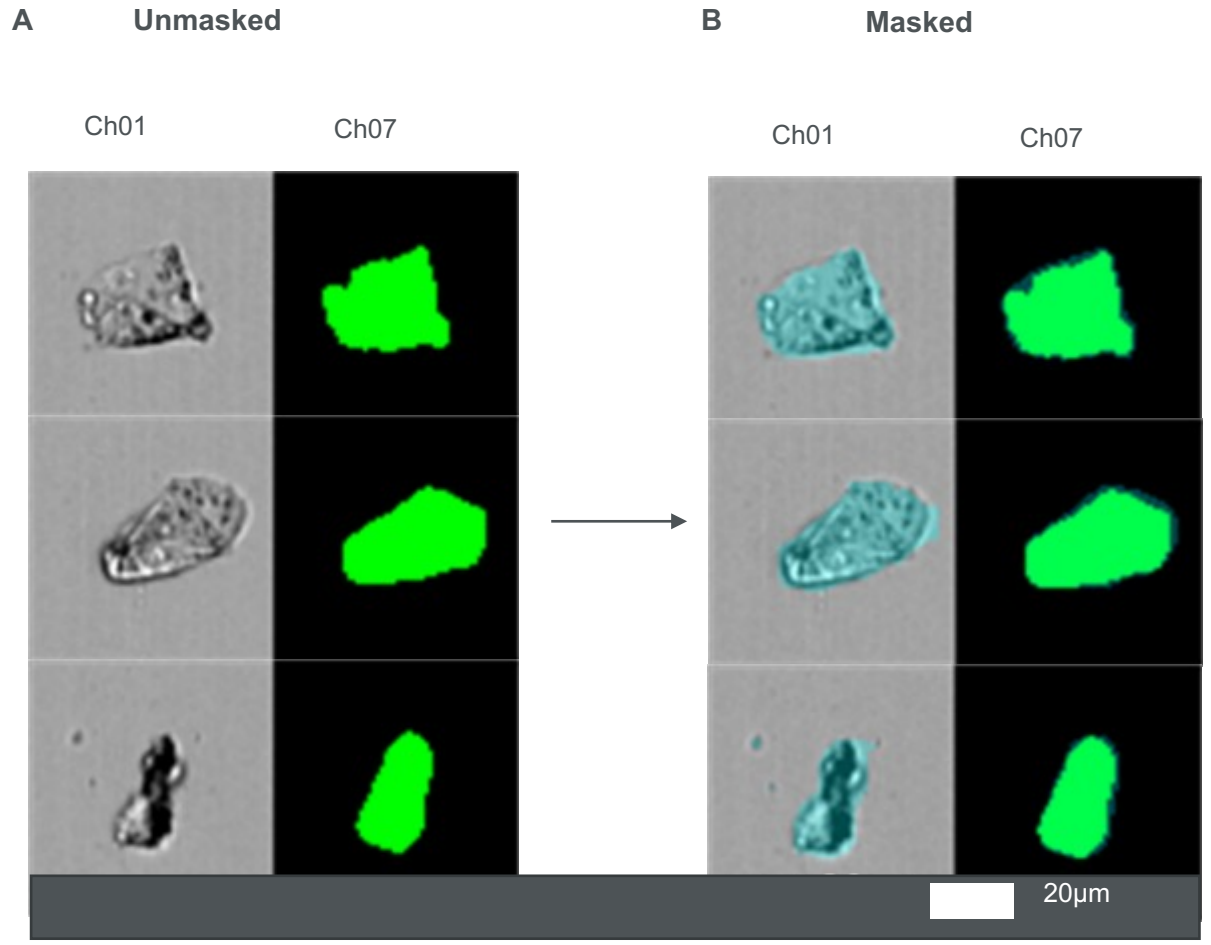


Kruger, A., Vlok, M., Turner, S., Venter, C., Laubscher, G.J., Kell, D.B., and Pretorius, E. (2022). Proteomics of fibrin amyloid microclots in long COVID/post-acute sequelae of COVID-19 (PASC) shows many entrapped pro-inflammatory molecules that may also contribute to a failed fibrinolytic system. Cardiovasc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

A Place for Imaging Flow Cytometry?

Kindly funded by KERNLS, Balvi and Polybio Research Foundation





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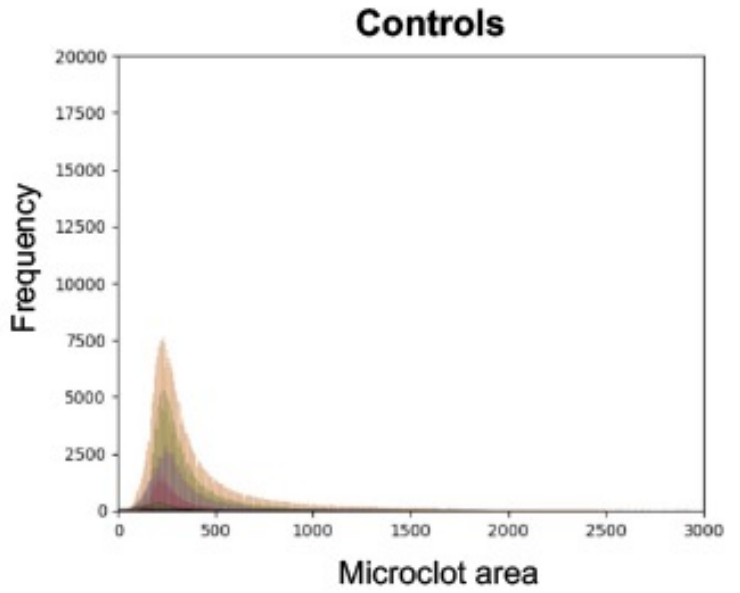
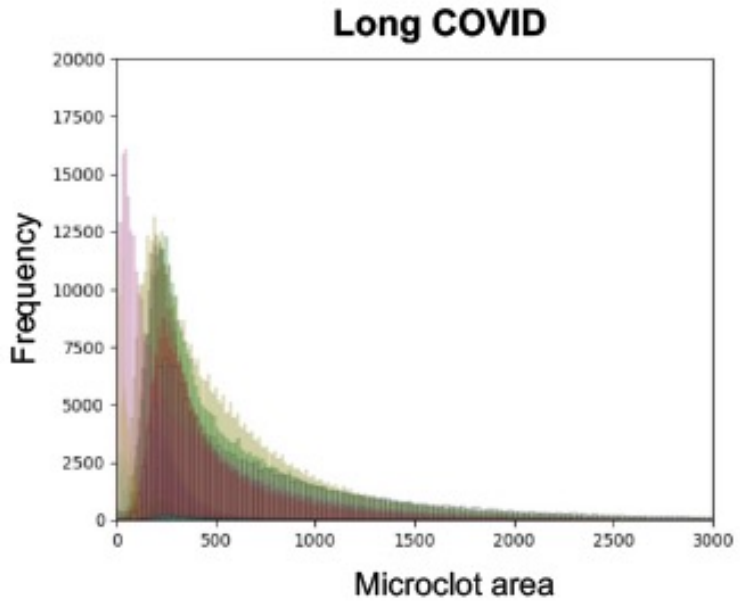
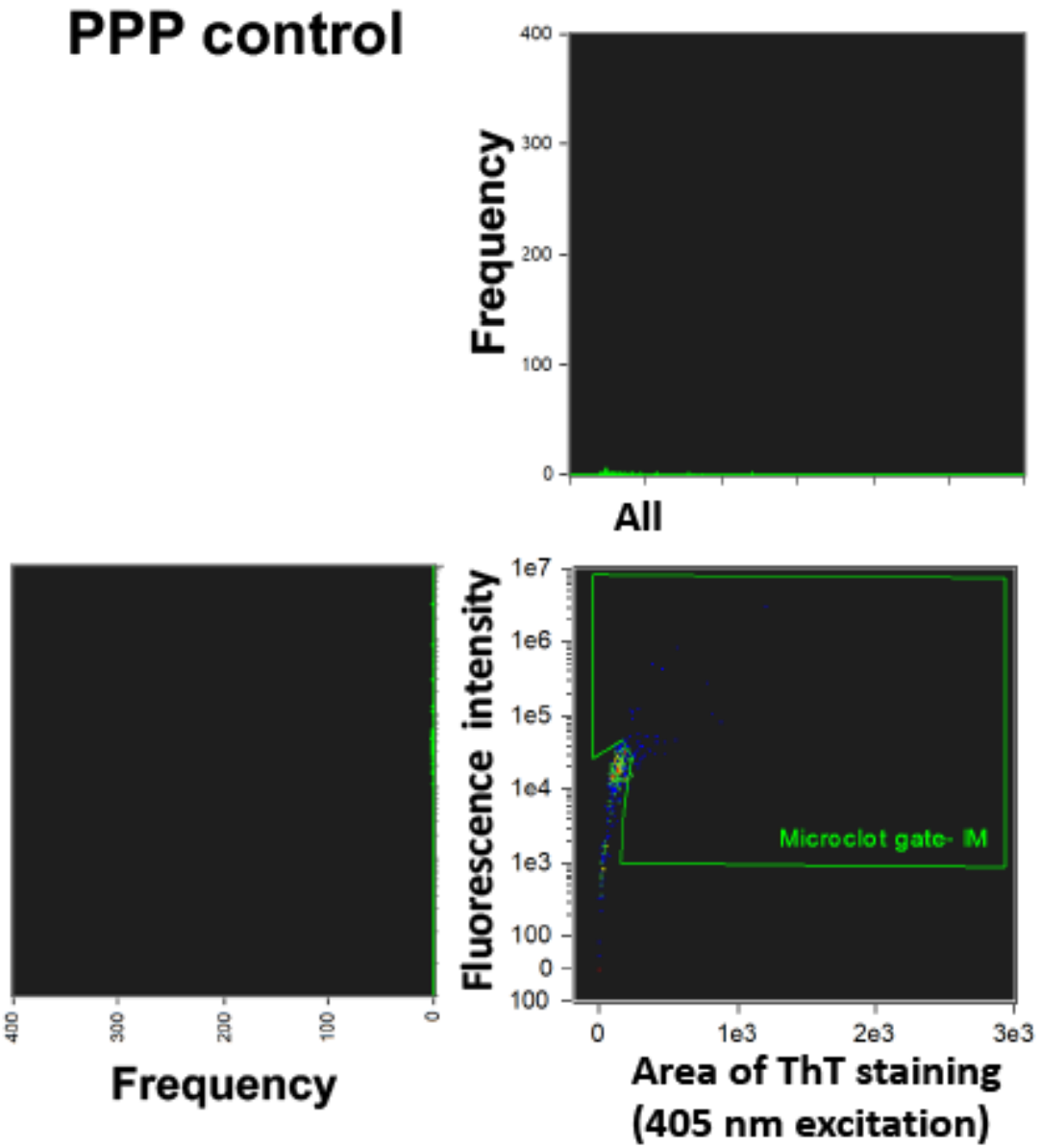
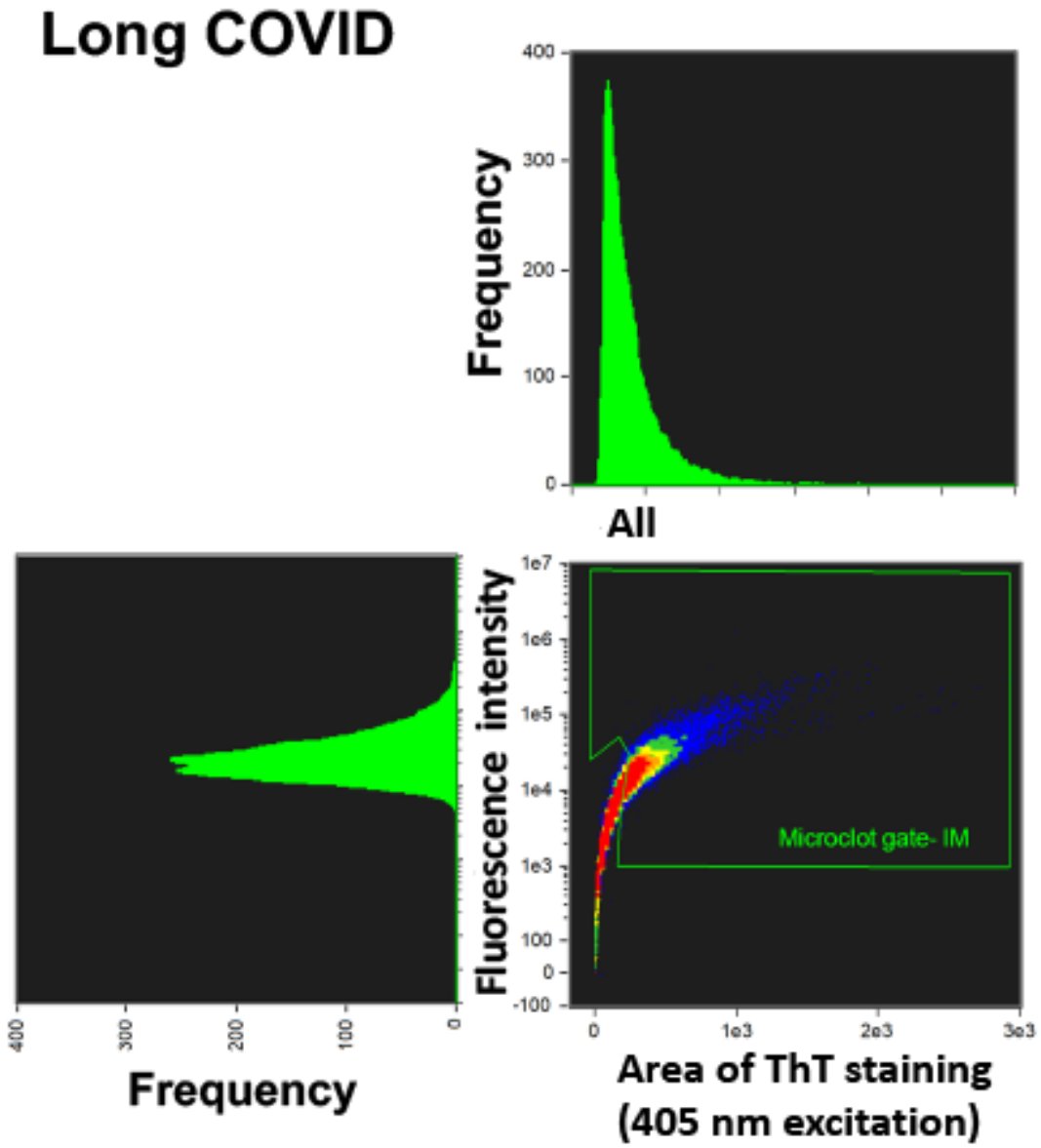




A Place for Imaging Flow Cytometry?





ME/CFS (Myalgic encephalomyelitis/chronic fatigue) syndrome

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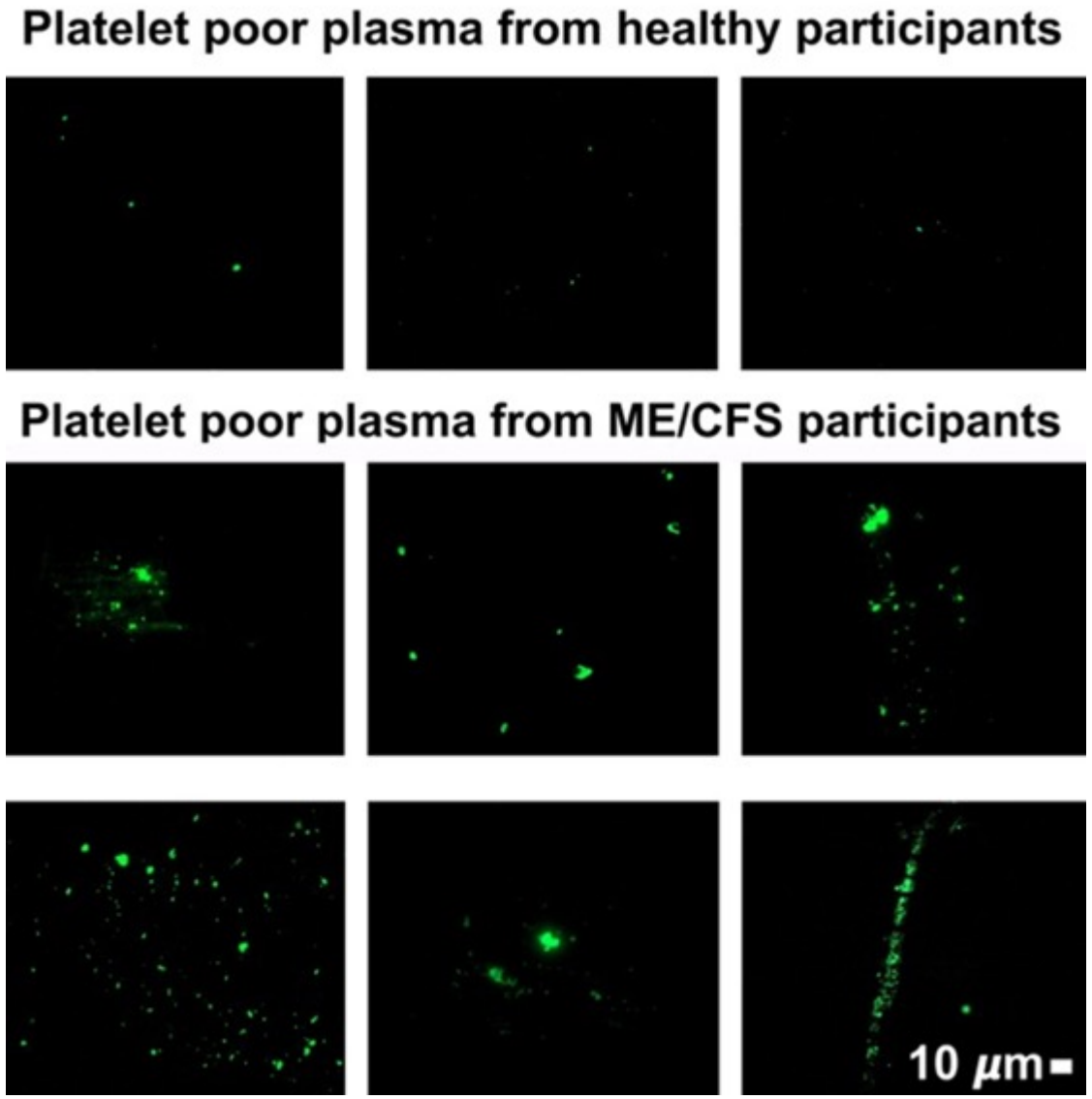

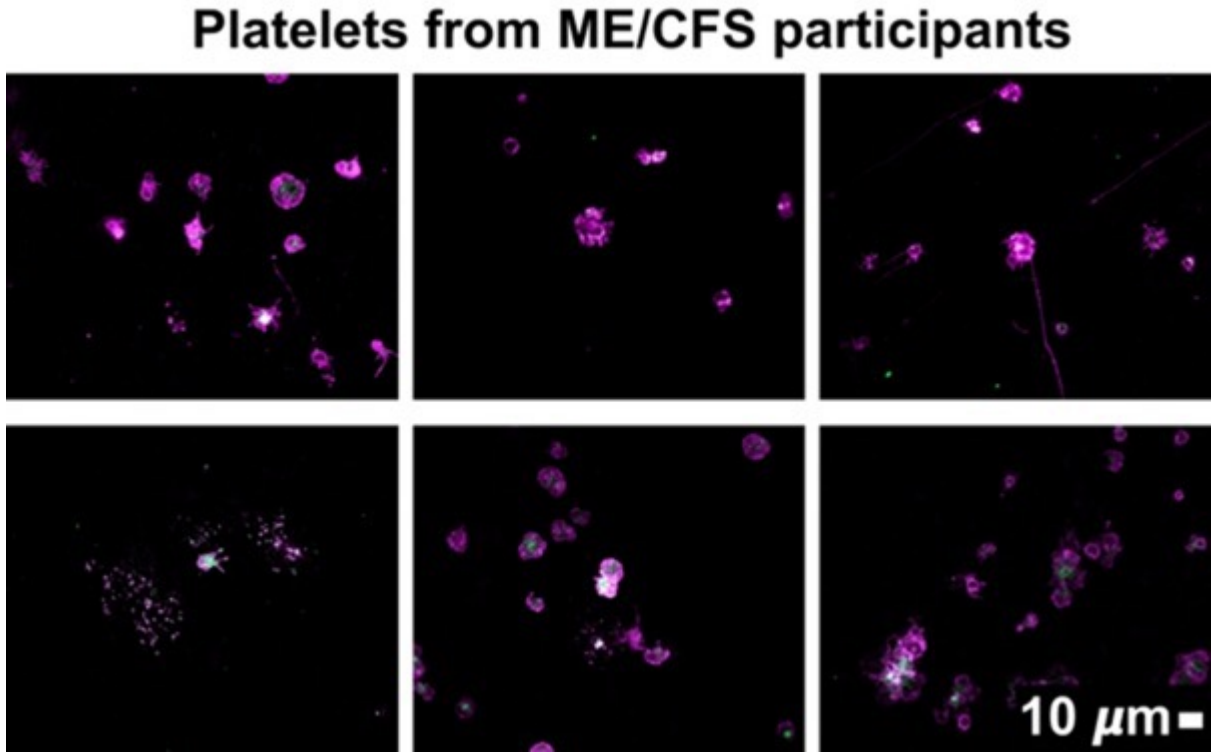

Long COVID sample stained with biofilm tracer

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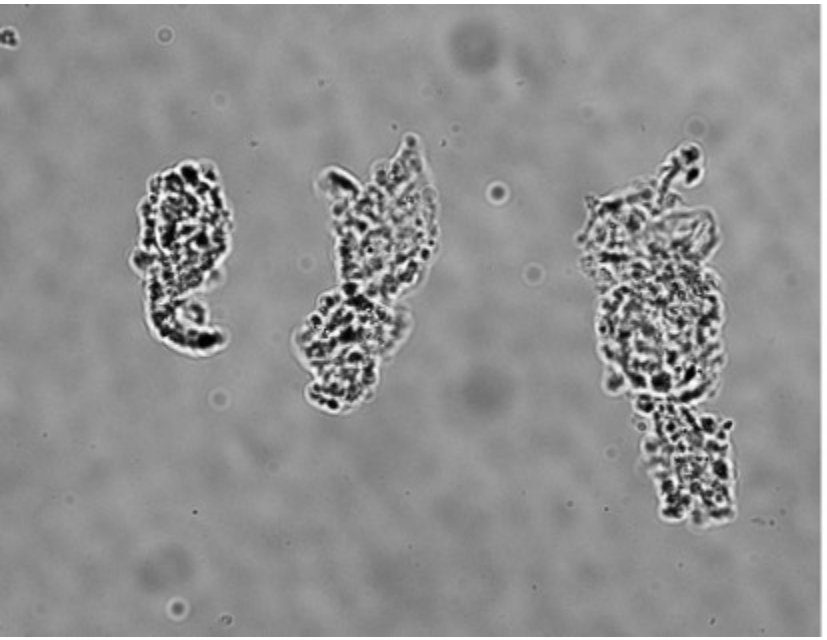
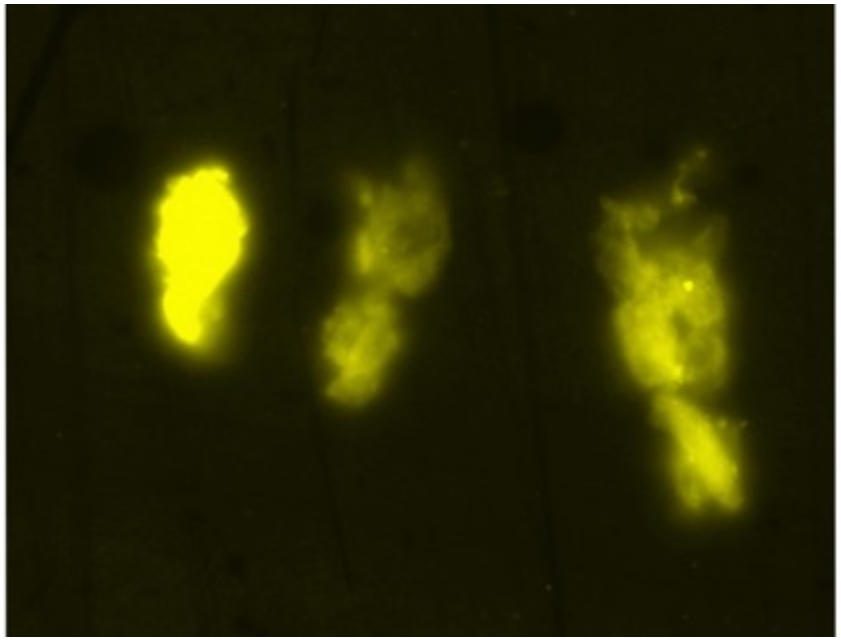
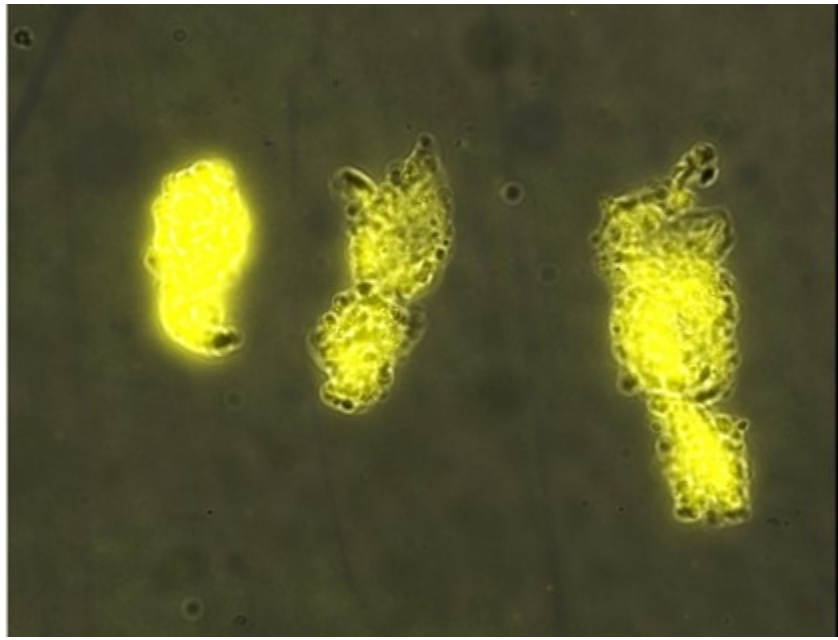

FilmTracer™ FM® 1-43 Green Biofilm Cell Stain

Long COVID sample stained with biofilm tracer and Amytracker

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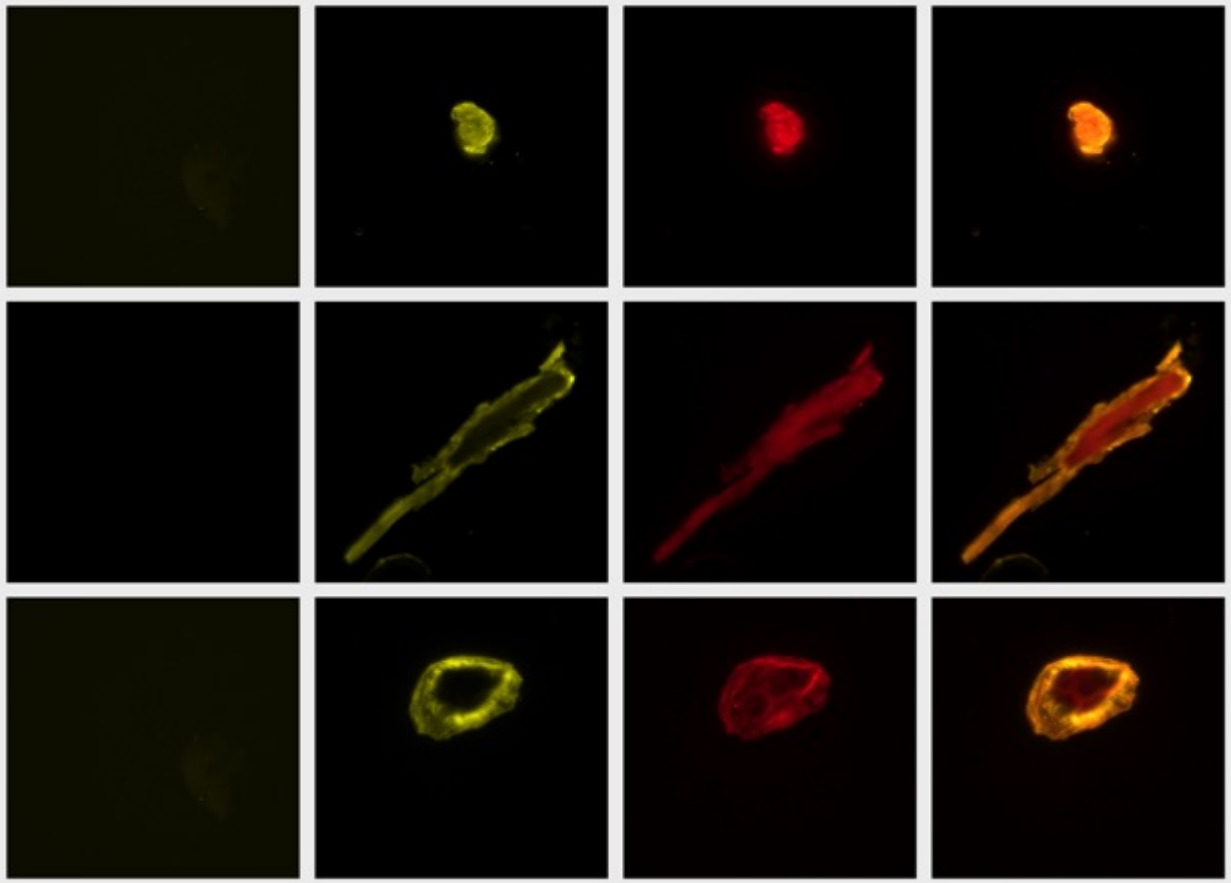

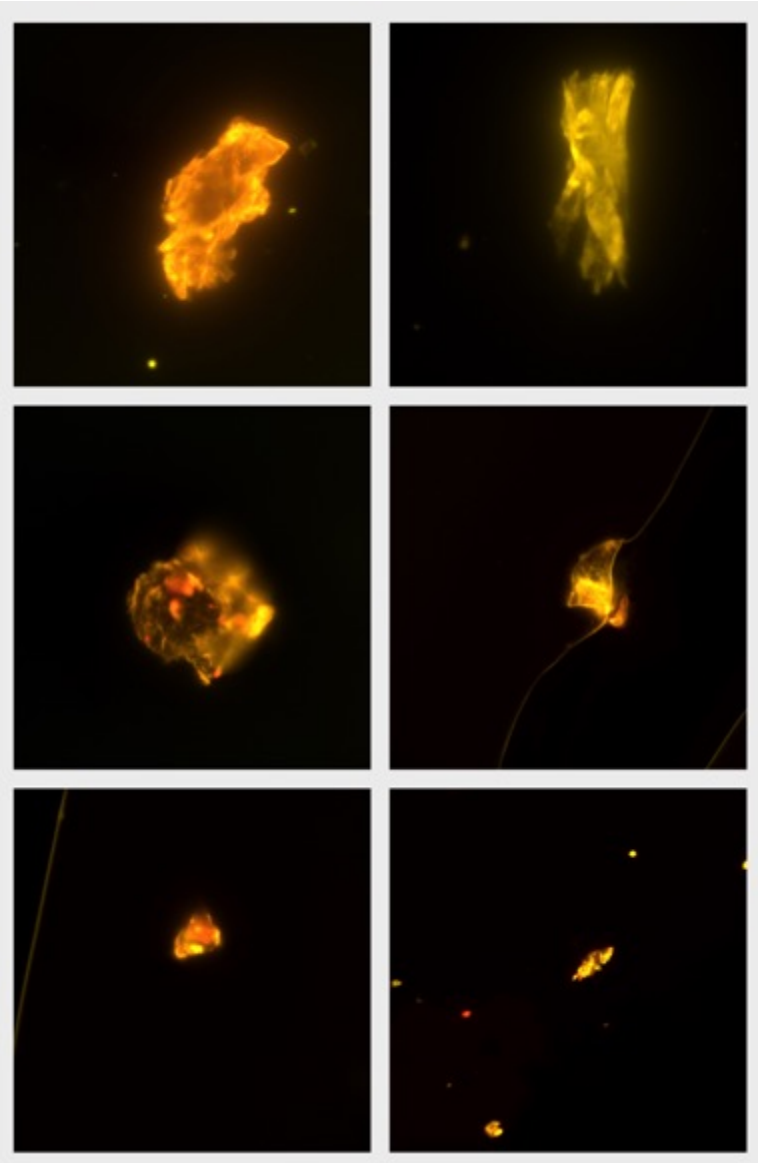

Endothelial debris in platelet poor plasma: ME/CFS and Long COVID?

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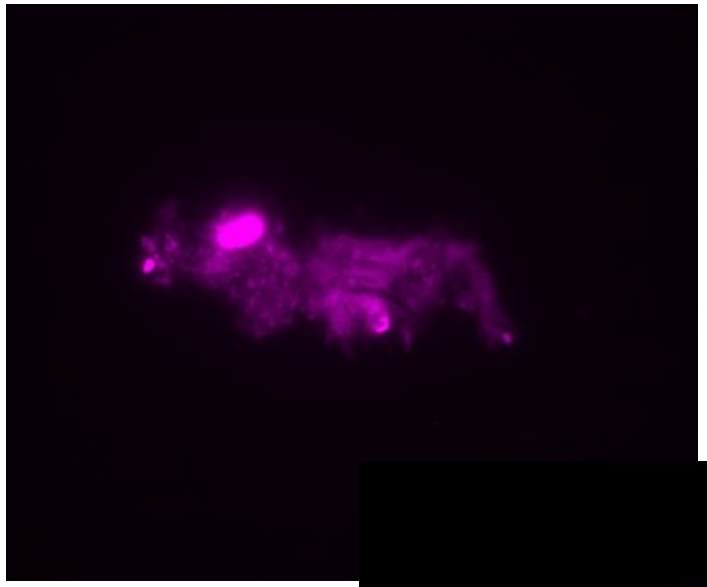
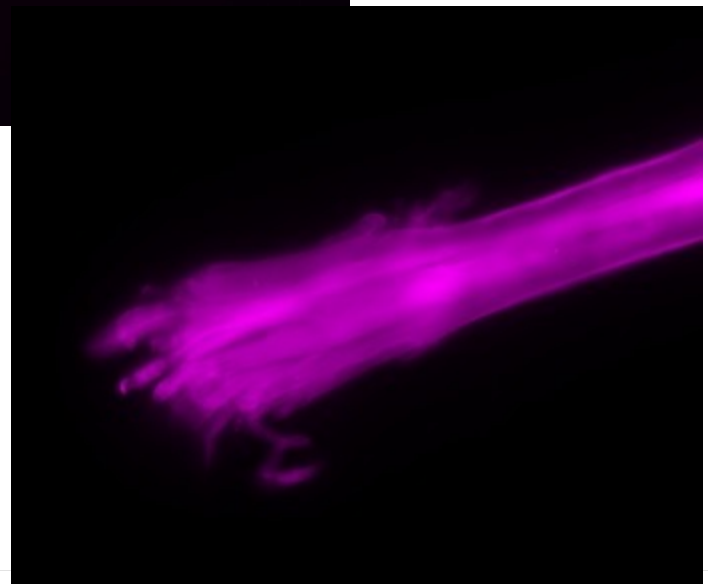
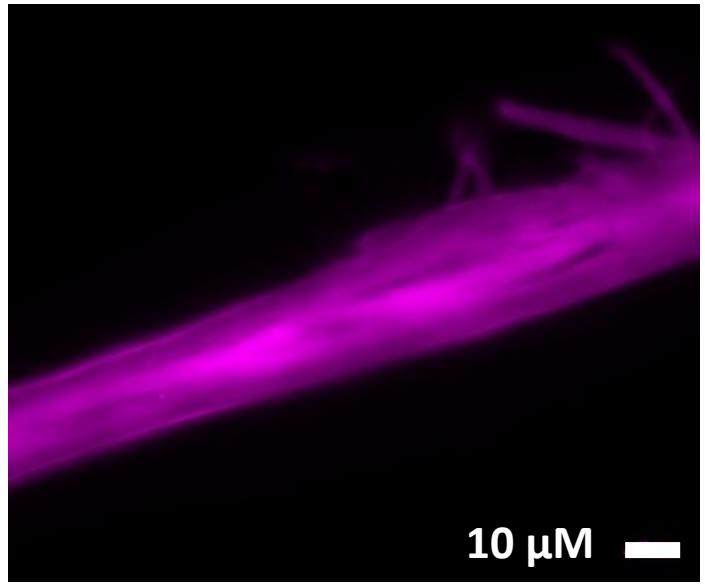
Control

Long COVID





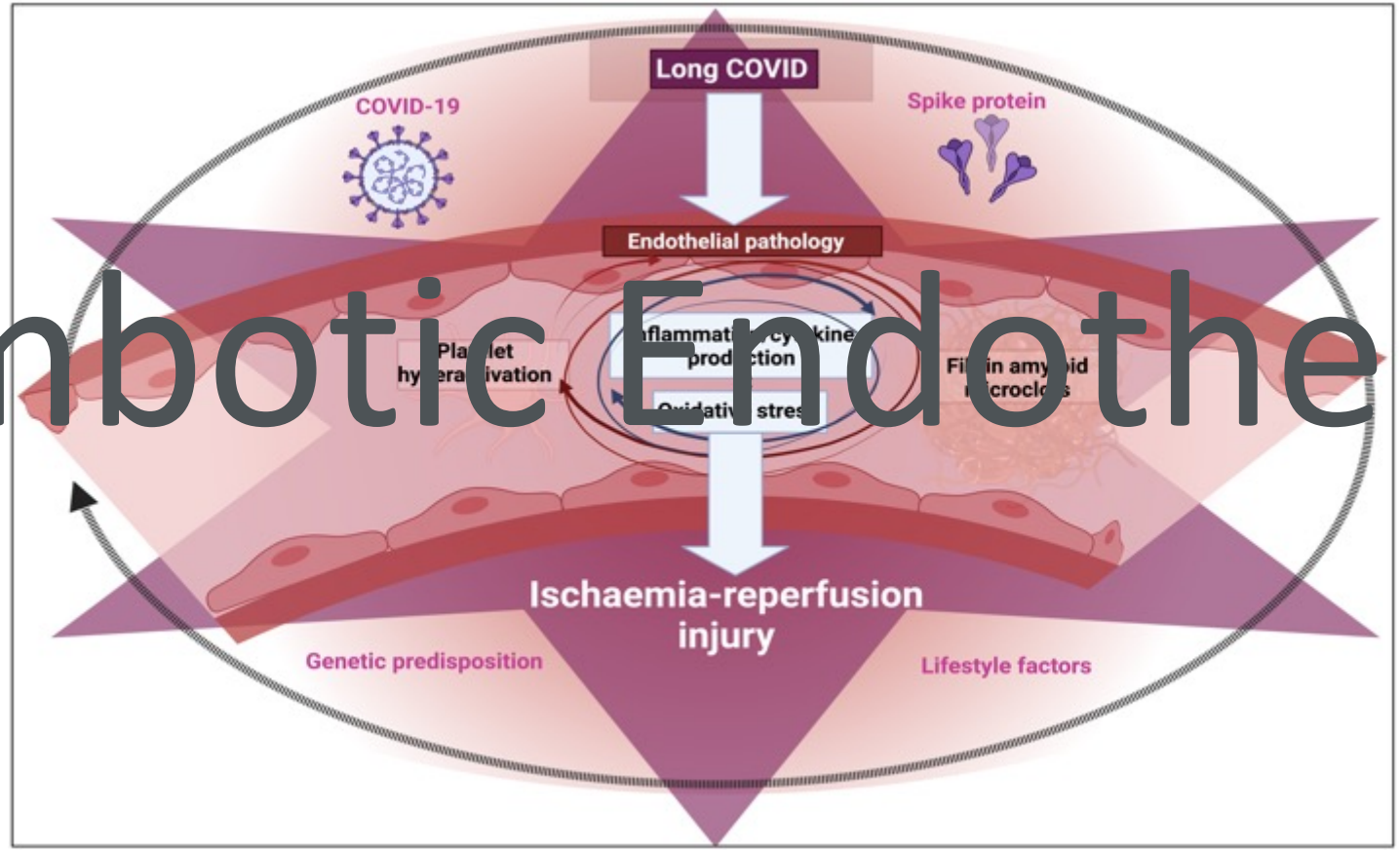
ME/CFS



E-selectin: CD62-E: BD Pharmingen™

Ischaemia-reperfusion (I-R) injury

Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT
forward together sonke siya phambili saam vorentoe

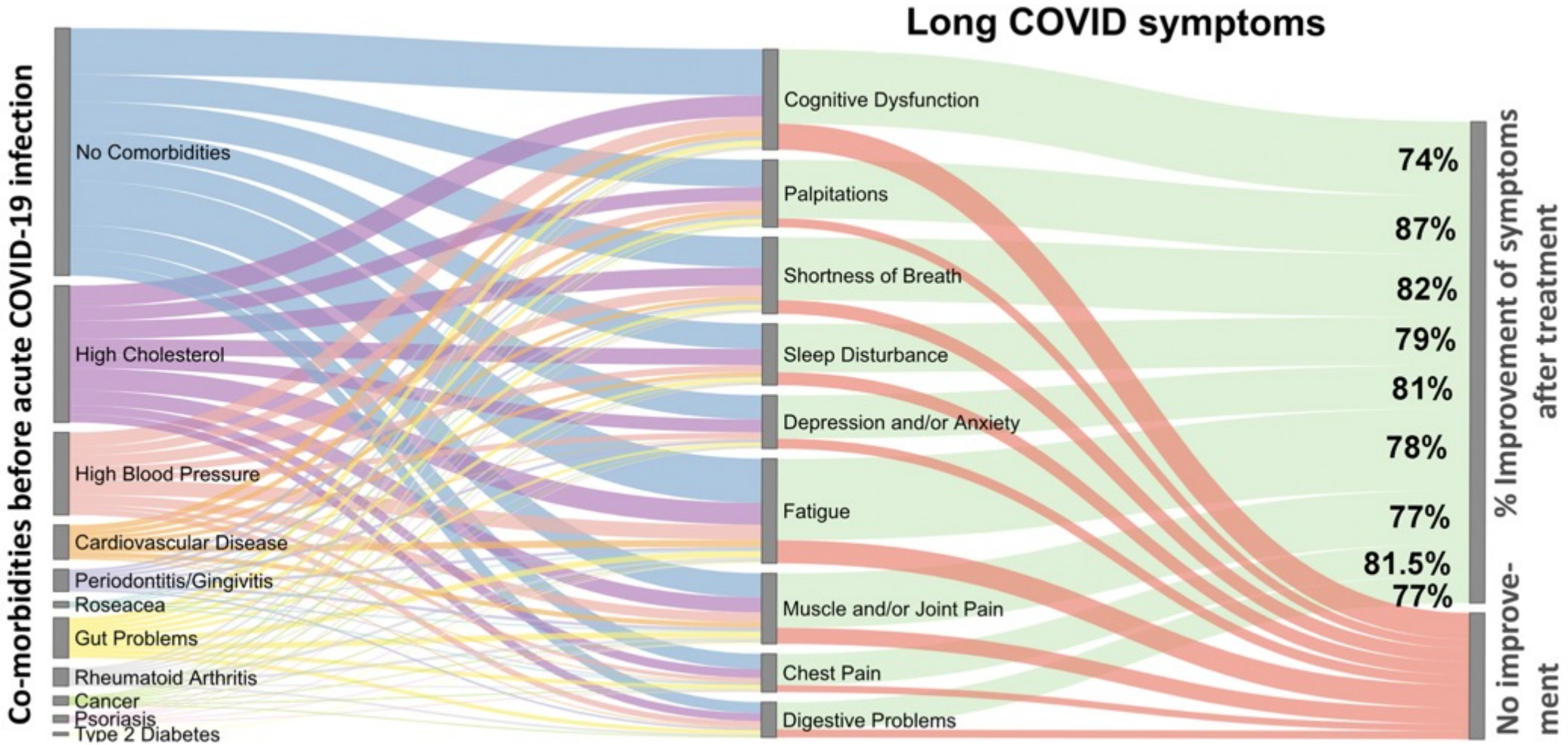


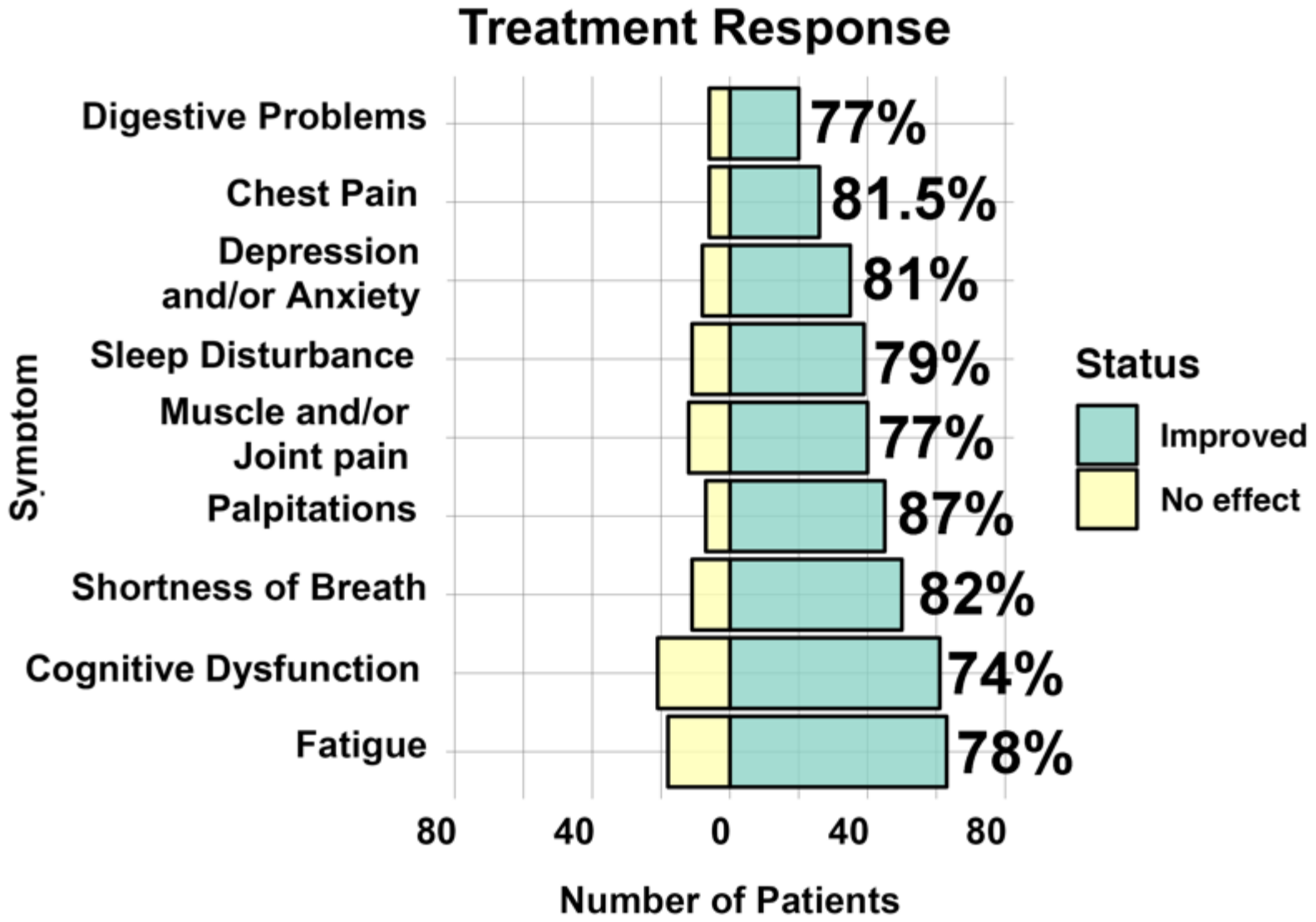
Thrombotic Endothelialitis

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.

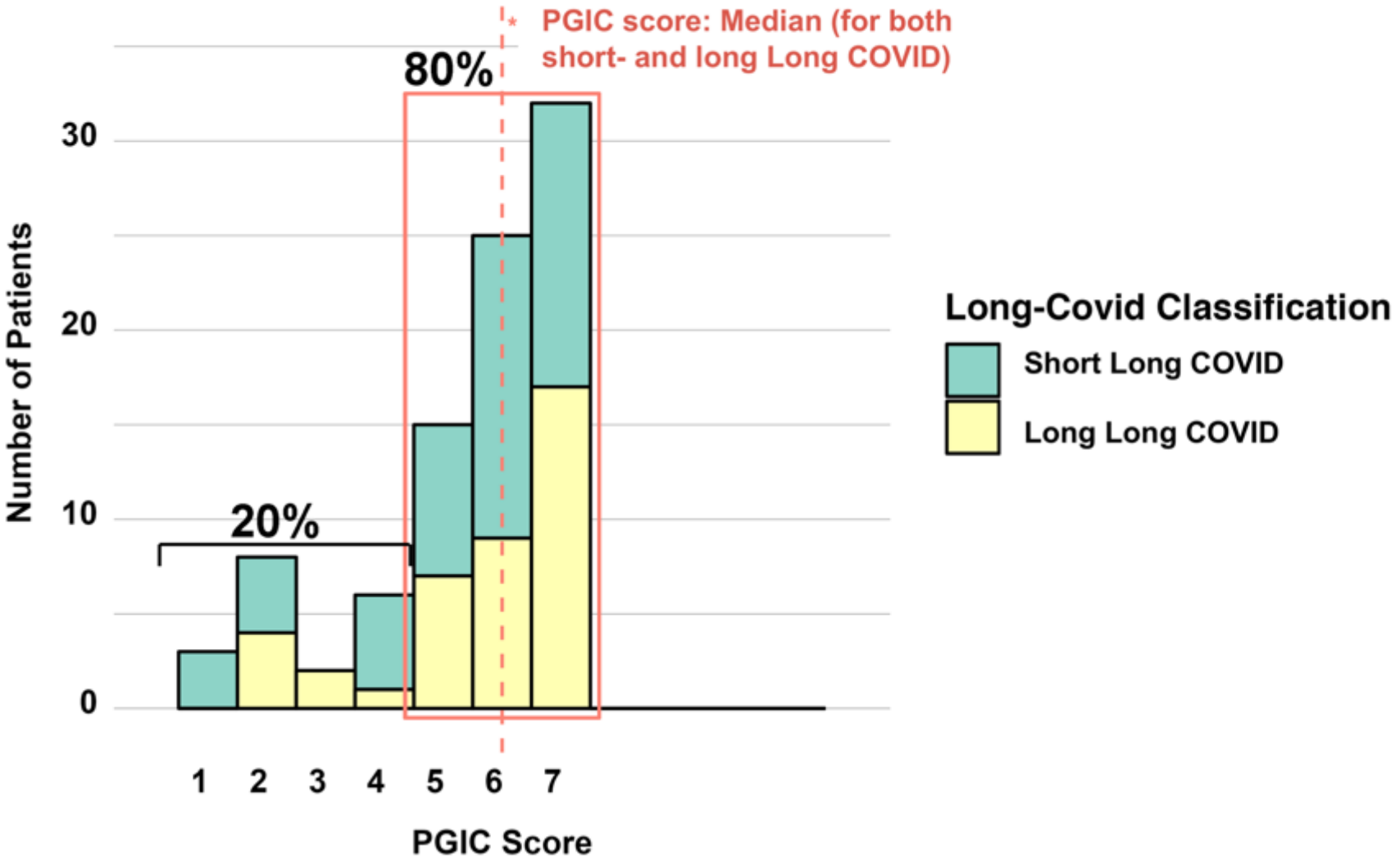
Turner, S., Khan, M.A., Putrino, D., Woodcock, A., Kell, D.B., and Pretorius, E. (2023). Long COVID: pathophysiological factors and abnormalities of coagulation. Trends Endocrinol Metab *34*, 321-344.

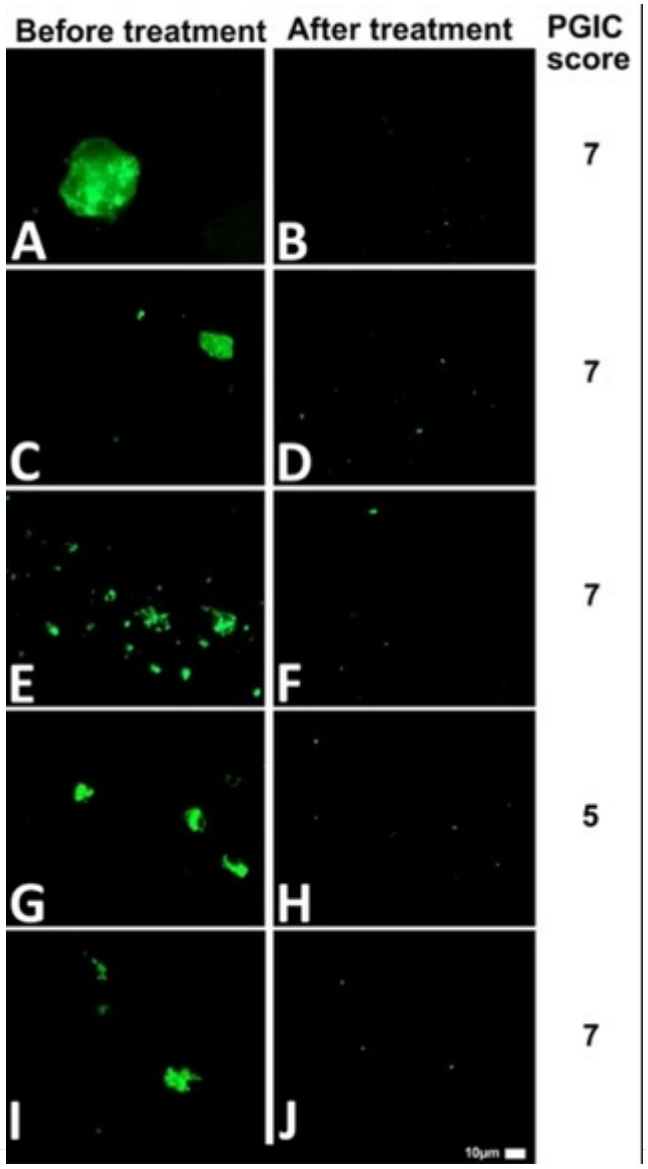
Data from a clinician-initiated treatment regimen




Treatment Response
Digestive Problems
Chest Pain
Depression and/or Anxiety
Sleep Disturbance
Muscle and/or Joint pain
Palpitations
Shortness of Breath
Cognitive Dysfunction
Fatigue
77%
81.5%
81%
79%
77%
87%
82%
74%
78%
Symptom
Status
Improved
No effect
80
40
0
40
80
Number of Patients

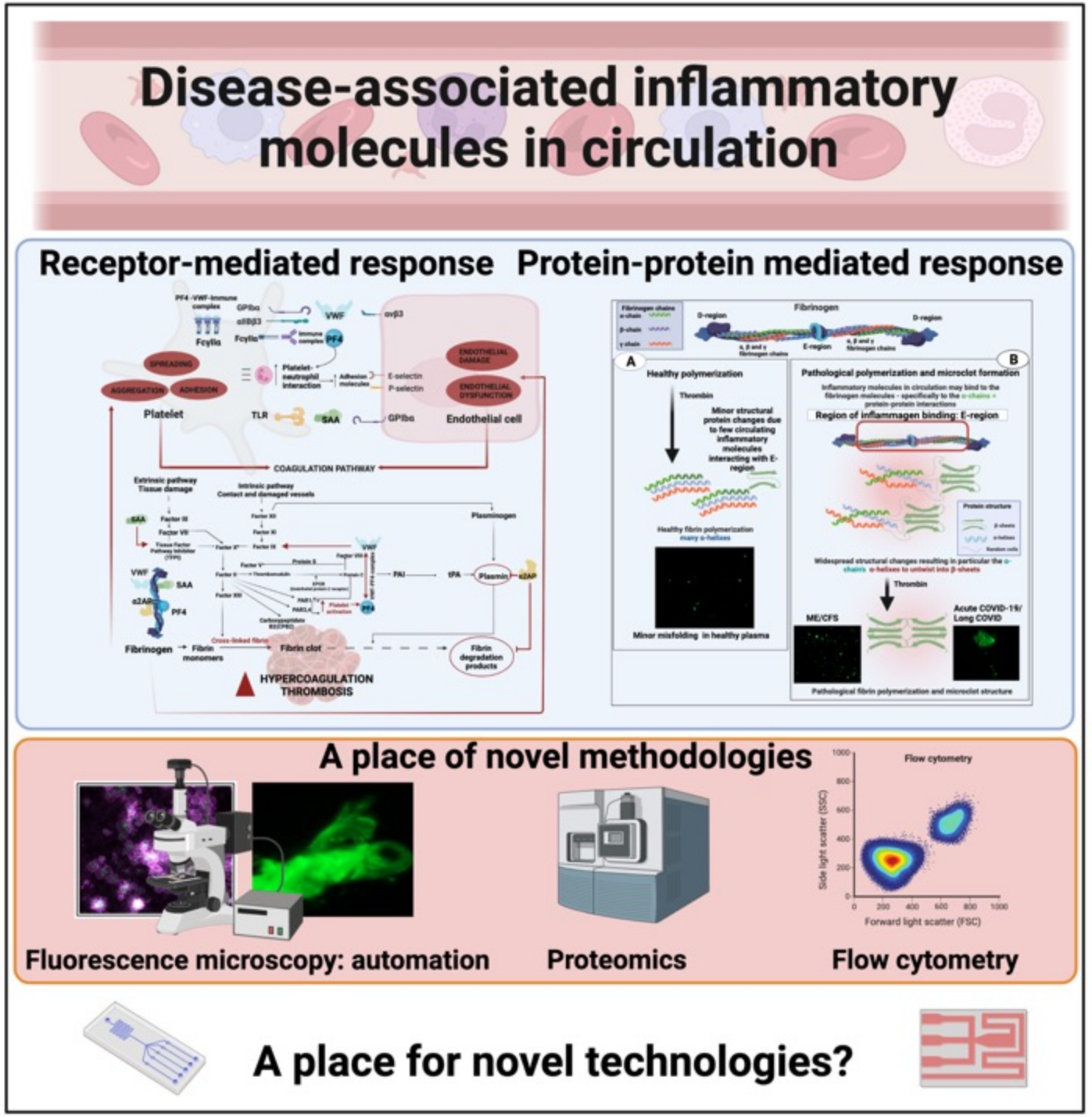
Histogram of PGIC Scores of Patients





In conclusion

- Relevance of receptor-inflammatory marker interaction in driving disease pathologies
- Relevance of direct protein-protein interactions in clotting pathologies
- The place of novel methods in diagnosing well-known and new diseases



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Thank you
Enkosi
Dankie