



Lecture 15: Endotheliopathy of Long COVID

Resia Pretorius



Stellenbosch

UNIVERSITY
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forward together
sonke siya phambili
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Collaborators and postgraduate students contributing to this presentation

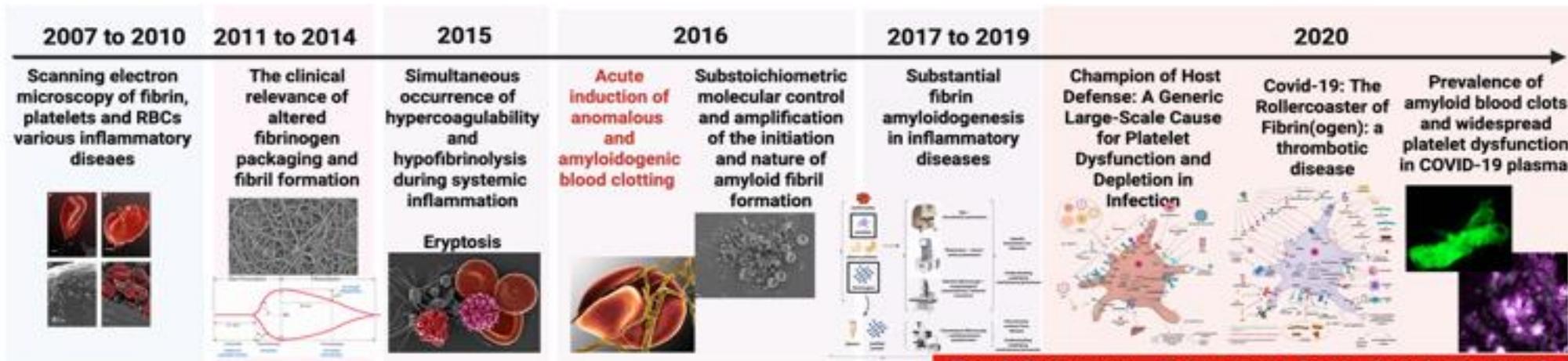
- Prof Douglas B Kell (University of Liverpool)
- Dr Massimo Nunes (Postdoc)
- Dr Jaco Laubscher, Dr Arneaux Kruger, Dr Uvi Naidoo, Dr Elouise Kroon
- Prof Alain Thierry and team (INSERM: France)
- Prof Melanie Walker (Department of Neurological Surgery (University of Washington))
- Dr Alakendu Sekhar (Department of Neurology, The Walton Centre, Aintree University Hospital)
- Dr Chantelle Venter (Blood lab manager: Stellenbosch University)
- Dr Mare Vlok (Biochemist)
- Dr Justine Grixti (Postdoc: University of Liverpool)
- Dr Simone Turner (Biocode Technologies)
- Tom Usher, Anel Thompson, Maxine Waters, Liz Copley (postgraduate students: Stellenbosch University)
- Prof Maritha Kotze, Prof Kanshu Rajaratnam (Stellenbosch University)
- Fritz van Schalkwyk and the University of the Free State team

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- Kanro Research Foundation
- KERNLS crowd funding initiative, together with patients and their family members, clinicians and researchers
- StandingUpToPOTS (USA)
- MRC and NRF (South Africa),

Disclosure

- I am a Founding Director of Biocode Technologies



Biocode Technologies is registered **Various patents filed for the early detection of Inflammation using Serum Amyloid A**

EP: WHO panel member: expanding understanding of the post COVID-19 condition; EP, DBK various keynotes, presentations

EP: WHO panel member: formulating a Post COVID-19 Condition case definition for children and young people; EP, DBK various keynotes, presentations

2021

2022

Proteomics: Persistent clotting pathology due to microclots in Long COVID entrapping huge amounts of inflammatory molecules

TEG®, Microclot and Platelet Mapping for Guiding Early Management of Severe COVID-19 Coagulopathy

SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis

Combined triple treatment of fibrin amyloid microclots and platelet pathology in Long COVID

The potential role of ischaemia-reperfusion injury in chronic, relapsing diseases such as rheumatoid arthritis, Long COVID, and ME/CFS

A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications

Follow-up Proteomics study of Long COVID microclots

Cardiovascular and haematological pathology in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

Relative hypercoagulopathy of the SARS-CoV-2 Beta and Delta variants when compared to the less severe Omicron variants

A patent is filed: the detection of amyloid microclots in blood samples as the first diagnostic method for Long COVID

First clinics in Europe and US signs licencing agreement to use microclot detection method in Long COVID patients

EP: Invitation to US National Academies of Sciences and Germany Academy of Science; Cohost of KEYSTONE conference on Long COVID EP, DBK various keynotes online presentations

Various international collaborator publications are published where amyloid nature of microclots are shown

2023

2024 to 2025

Long COVID: pathophysiological factors and abnormalities of coagulation

Development of an Imaging Flow Cytometer method to detect microclots in Platelet poor plasma

Proteomics of ME/CFS plasma reveals a dysregulated coagulation system

Viral Persistence Paper in Lancet Infectious Diseases

CPET study with Derby Team showing microclots break down and reform during exercise

Proteomics study with clinical team: Post COVID/ Vaccine Injury study

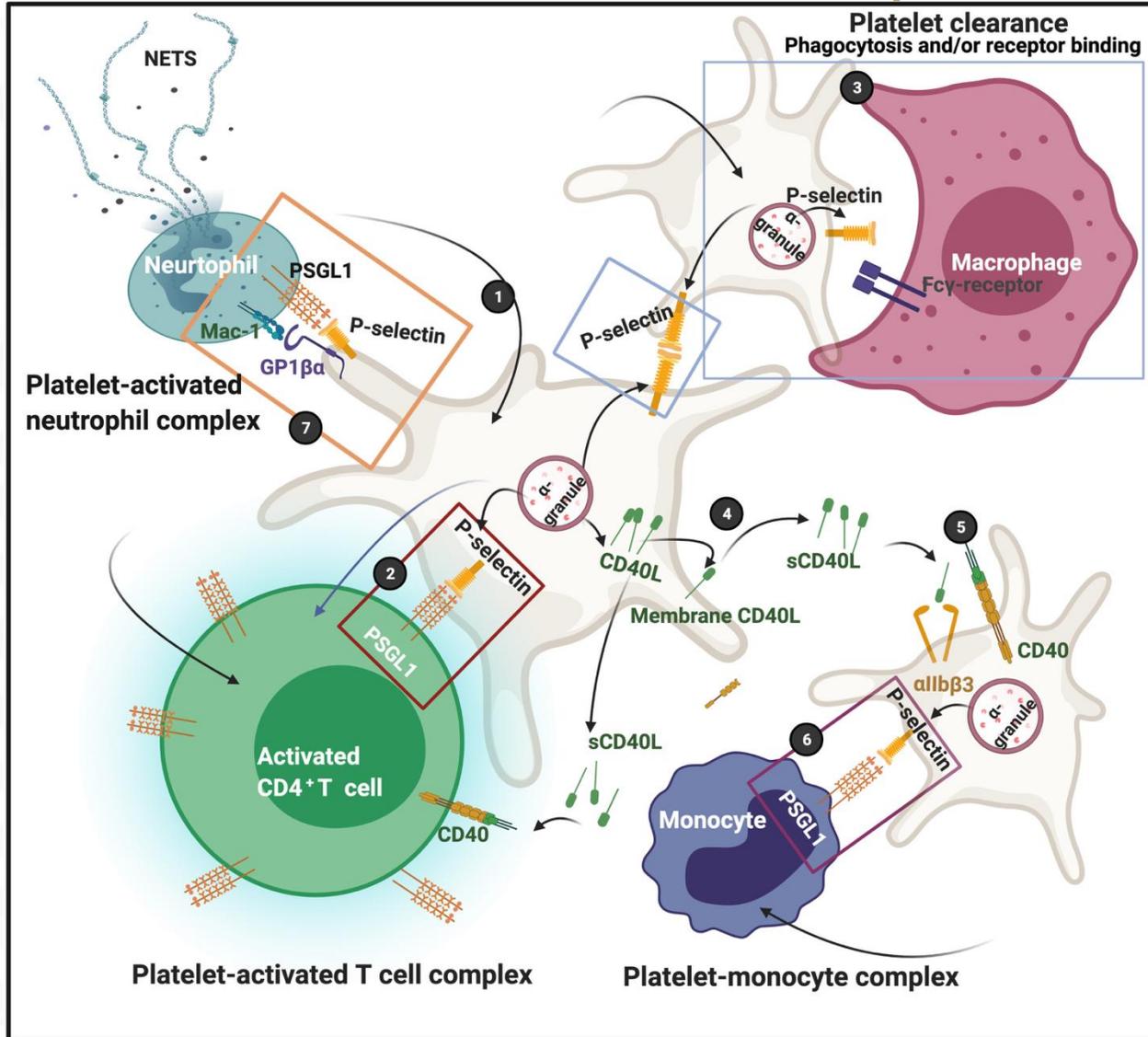
Extracted macroclots from stroke patients contain amyloid

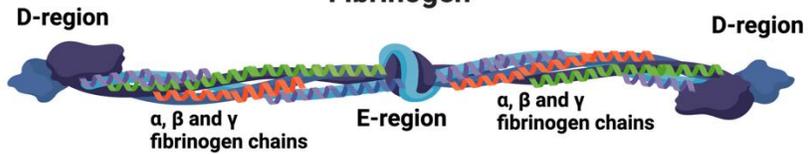
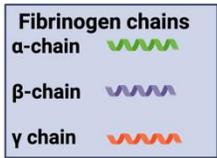
A Delphi paper with international collaborators

Various licencing agreements are signed to use microclot detection methods to identify microclots in Long COVID

Platelets Interactions

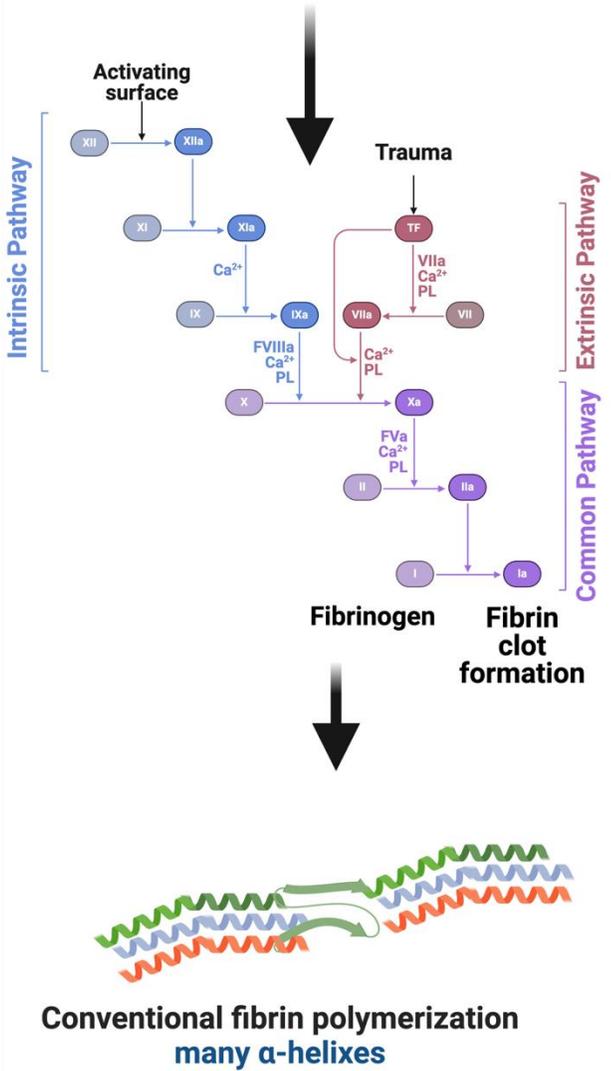
Platelet-Immune Cell Complexes





A

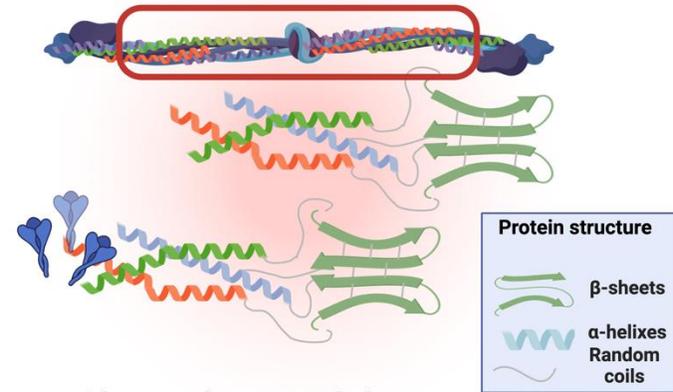
Conventional polymerization via the traditionally known clotting cascades



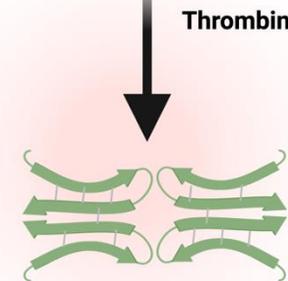
B

Pathological polymerization and microclot formation via direct binding of inflammatory molecules

Inflammatory molecules in circulation like the spike protein may bind to the fibrinogen molecules - specifically to the α -chains = protein-protein interactions

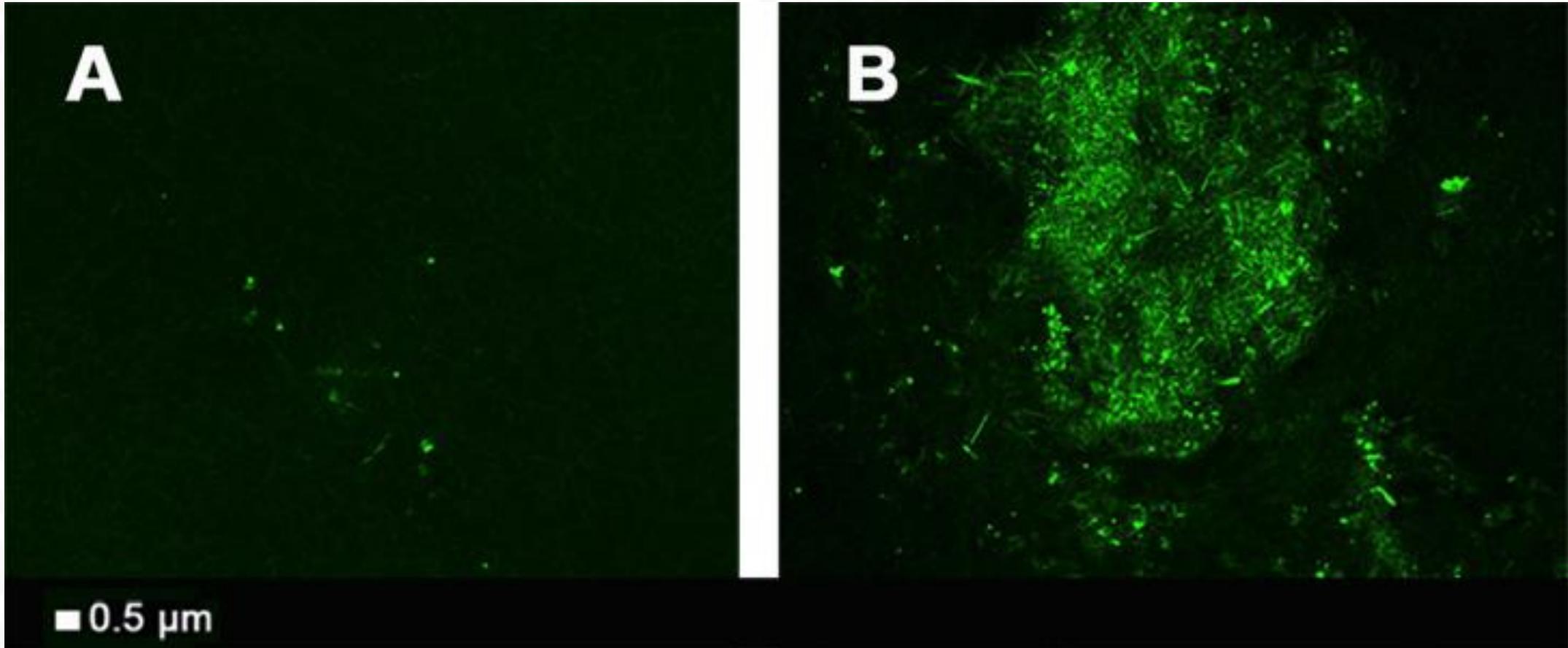


Widespread structural changes resulting in particular the α -chain's α -helixes to untwist into β -sheets



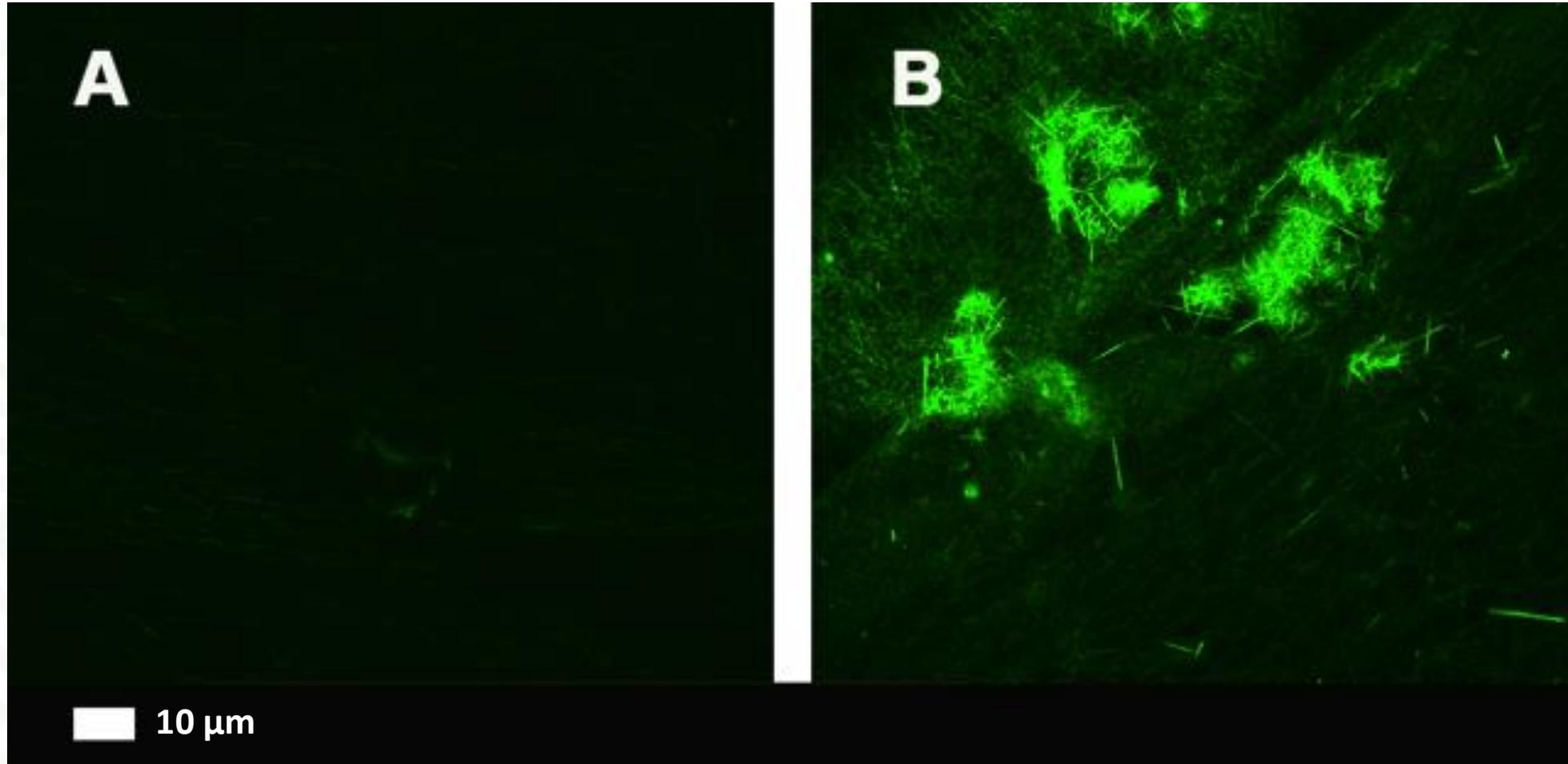
Pathological fibrin polymerization and microclot structure

Our earlier 2016 work: discovery that LPS can make fibrinogen amyloid



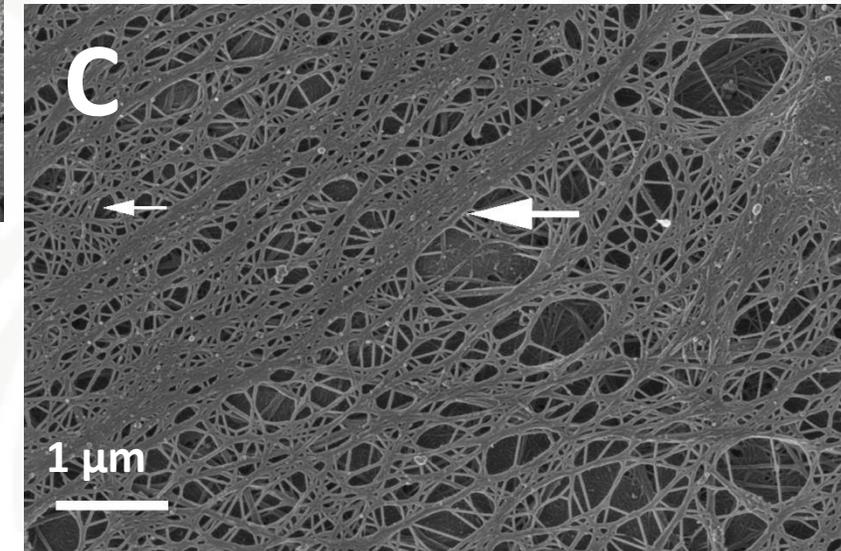
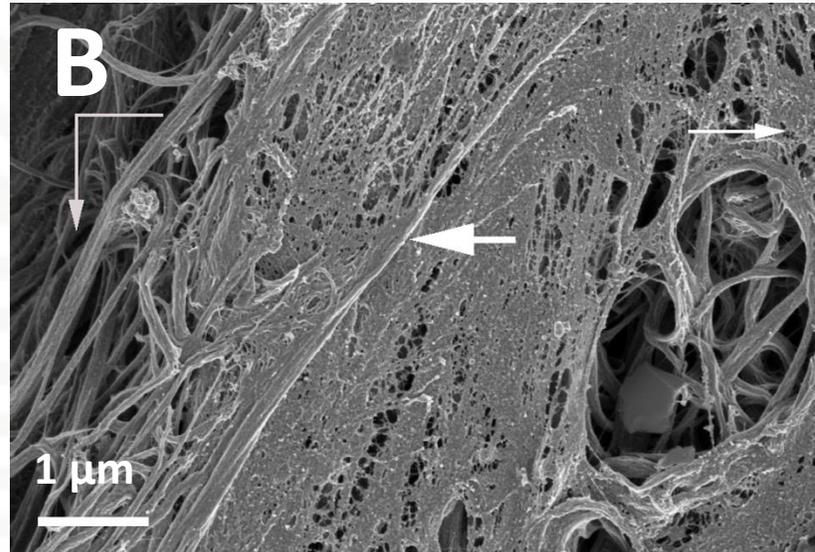
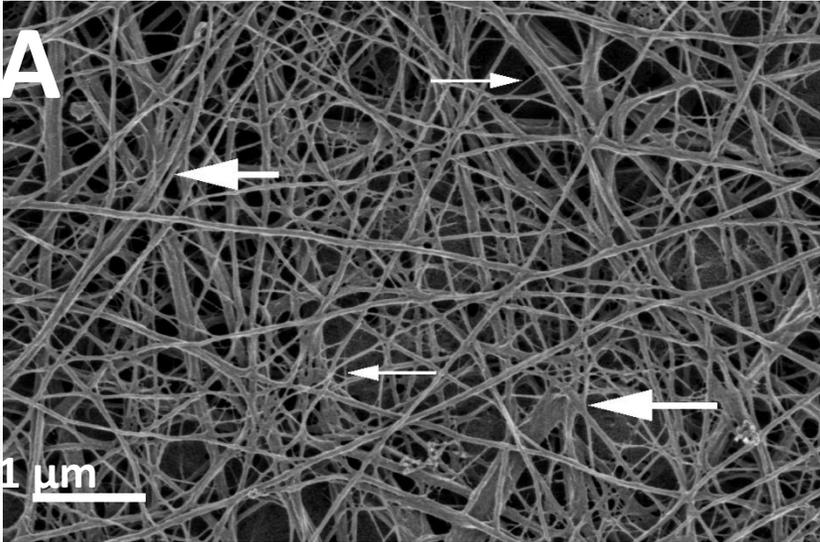
Stroke blood

Our early work



Fibrin nets in the presence of thrombin

Our early work



Haemochromatosis THE LANCET Clinical Picture

Thrombo-embolic-ischemic stroke

The effect of iron overload on red blood cell morphology



Etherson Pretorius, Natasha Vermeulen, Janette Bester, Jeanette L du Plooy, George S Genckle

A 76-year-old man with an extremely high serum ferritin concentration (33790 µg/L) was referred to the National Reference Laboratory for further laboratory testing to determine the cause. Genetic analysis showed that he was a carrier of a haemochromatosis-associated allele (H63D/heterozygous). Pathology confirmed renal insufficiency and liver damage, with an exceptionally high bilirubin concentration of 730 µmol/L and an unconjugated bilirubin concentration of 272 µmol/L. This light microscopy smear showed that most of the red blood cells have a changed morphology (figure). Scanning electron microscopy, by which high magnifications of the red blood cells can be viewed,

showed that the cells form pointed extensions with large membrane pores (figure). This abnormality is not seen in red blood cells from healthy individuals (figure). We suggest that the presence of the H63D haemochromatosis-associated allele is partly the cause of the exceptionally high serum ferritin concentrations, which brings about the abnormal red blood cell morphology.

Acknowledgments

Ethics approval was obtained from the Human Ethics Committee of the University of Pretoria. Dr Irma Ferreira PhD, Human Molecular Genetics Laboratory, AMPATH National Reference Laboratory, Concession, South Africa assisted with molecular genotyping of HFE mutations. We also thank the Unit of Microscopy and Microanalysis of the University of Pretoria for the use of the scanning electron microscope.

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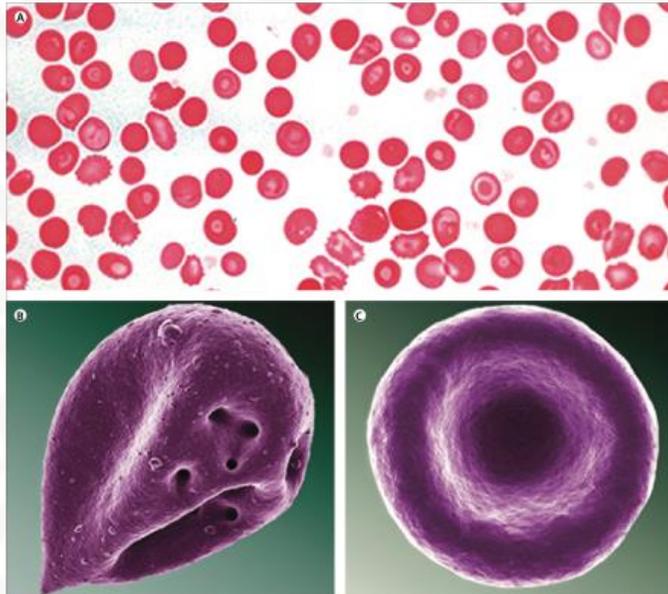
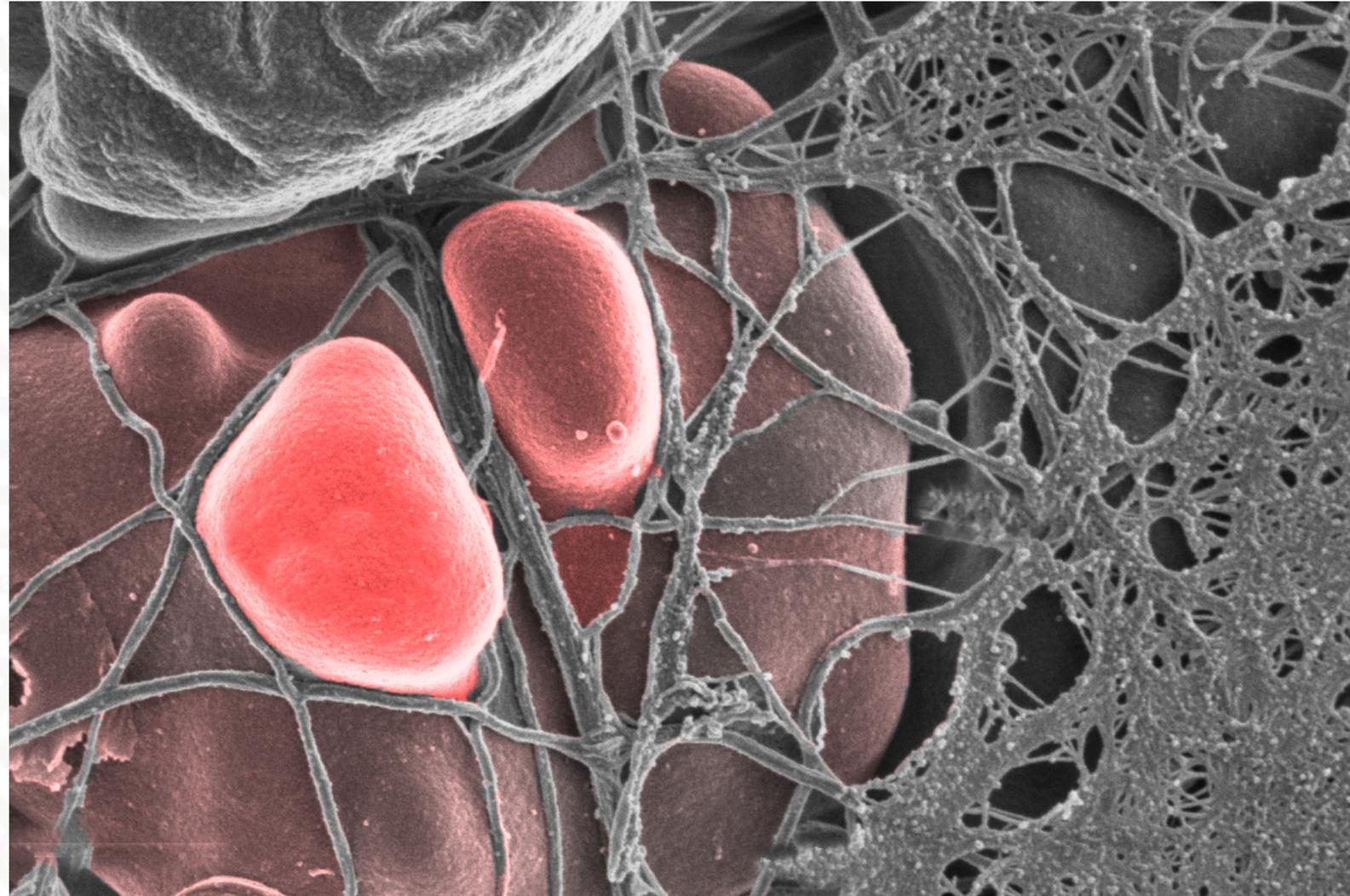
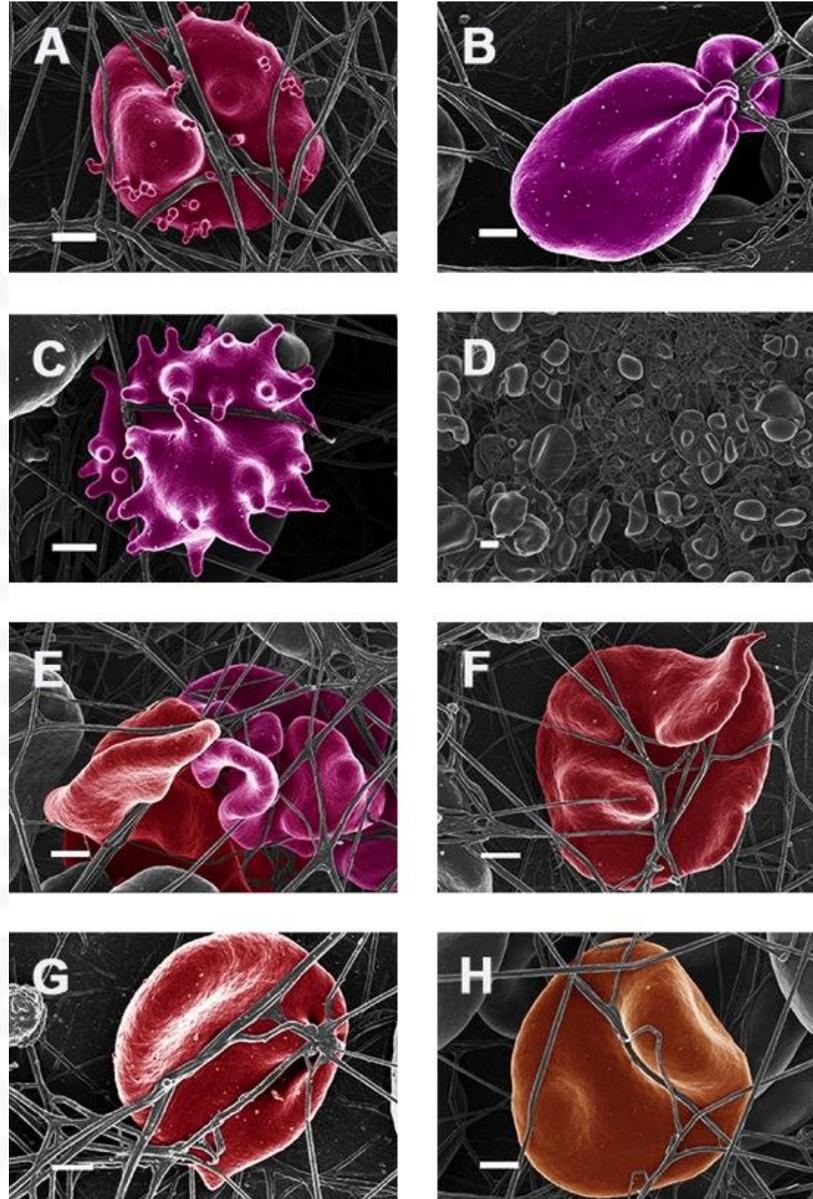


Figure: Abnormal red blood cell morphology
(A) 40× light microscopy smear of 76-year-old man with the H63D haemochromatosis-associated allele. (B) Scanning electron micrograph showing a red blood cell at high magnification. (C) A red blood cell from a healthy individual showing a typical discoid RBC.



Red blood cells and eryptosis in Parkinson's disease

Our early work



1 μ m

1 μ m

Hematological visualization of T-cell lymphoproliferative disorder

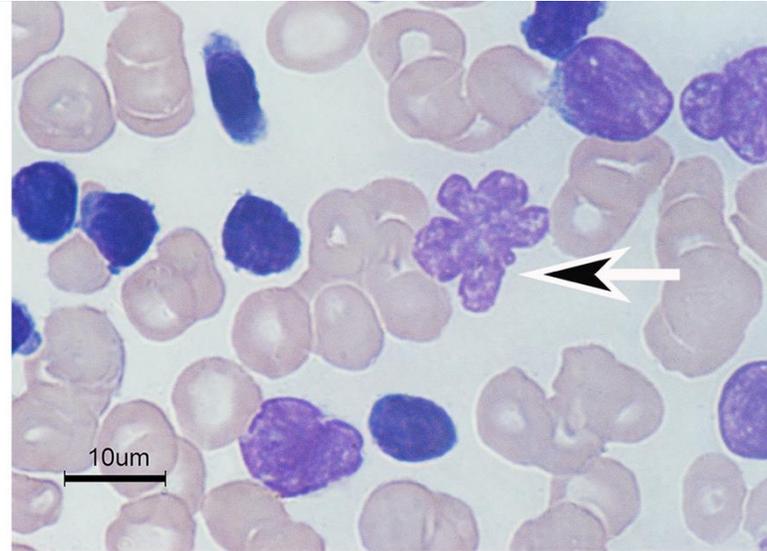
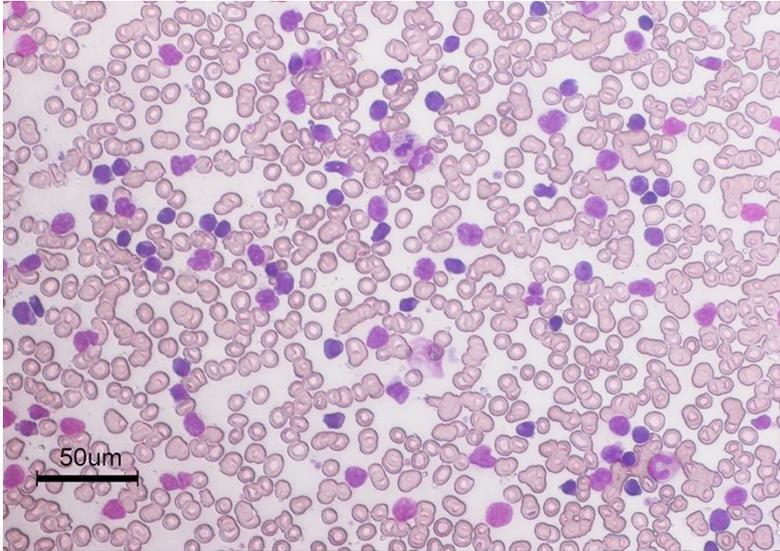
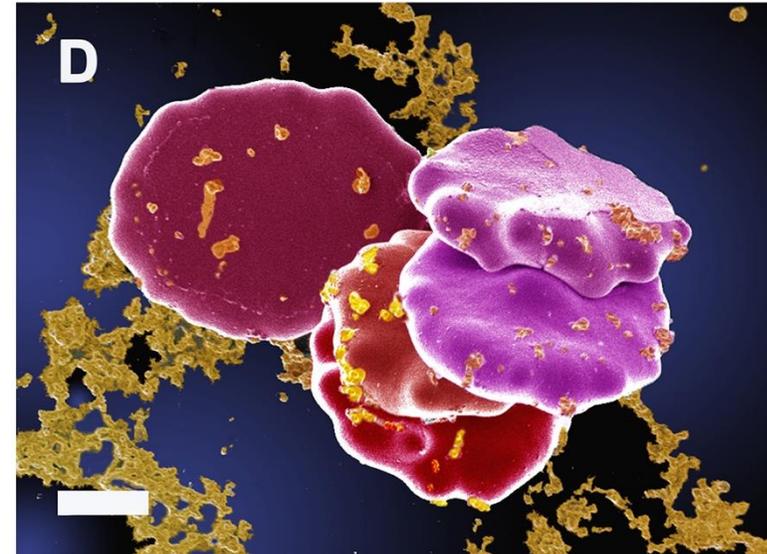
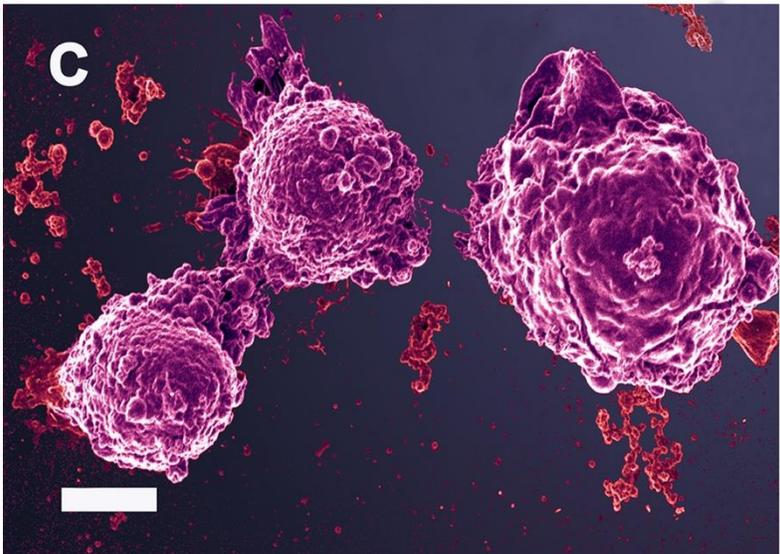


Plate 1: A and B) Light microscopy smear of whole blood, showing high concentration of white blood cells. **Arrow: atypical lymphoid cell with flower-cell morphology.** (A: 20x magnification and B: 100x magnification).

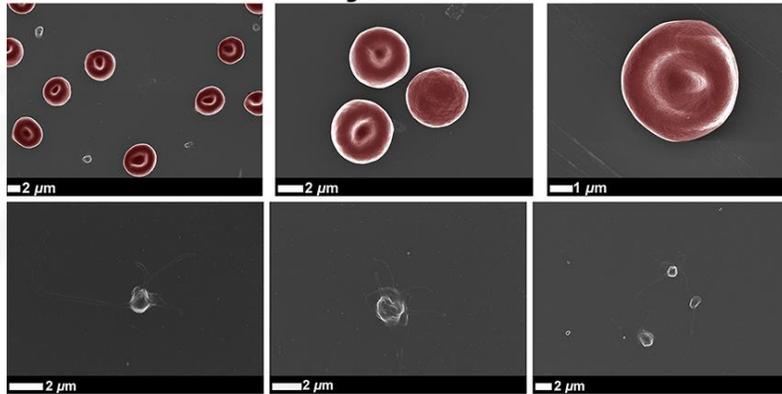
C) Scanning electron microscopy micrograph of a whole blood smear, showing 3 white blood cells and plasma protein debris.

D) Scanning electron microscopy micrograph showing Rouleaux formation of erythrocytes with plasma protein debris (Scale bar C and D: 2µm).

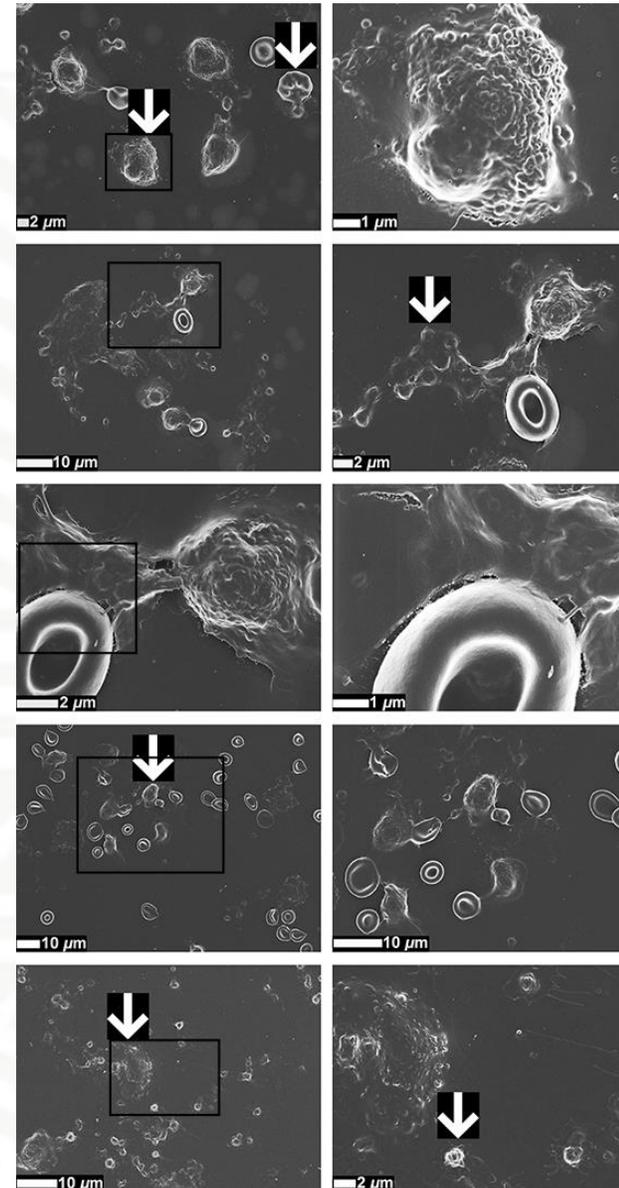
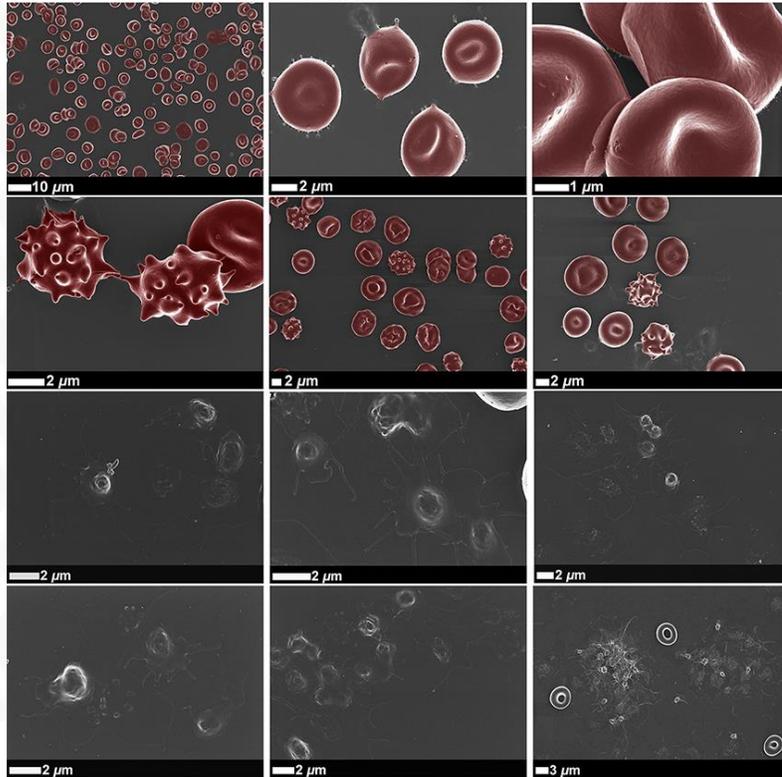


Colorectal cancer

Healthy individuals



Colorectal cancer



www.nature.com/scientificreports

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REPORTS

nature research

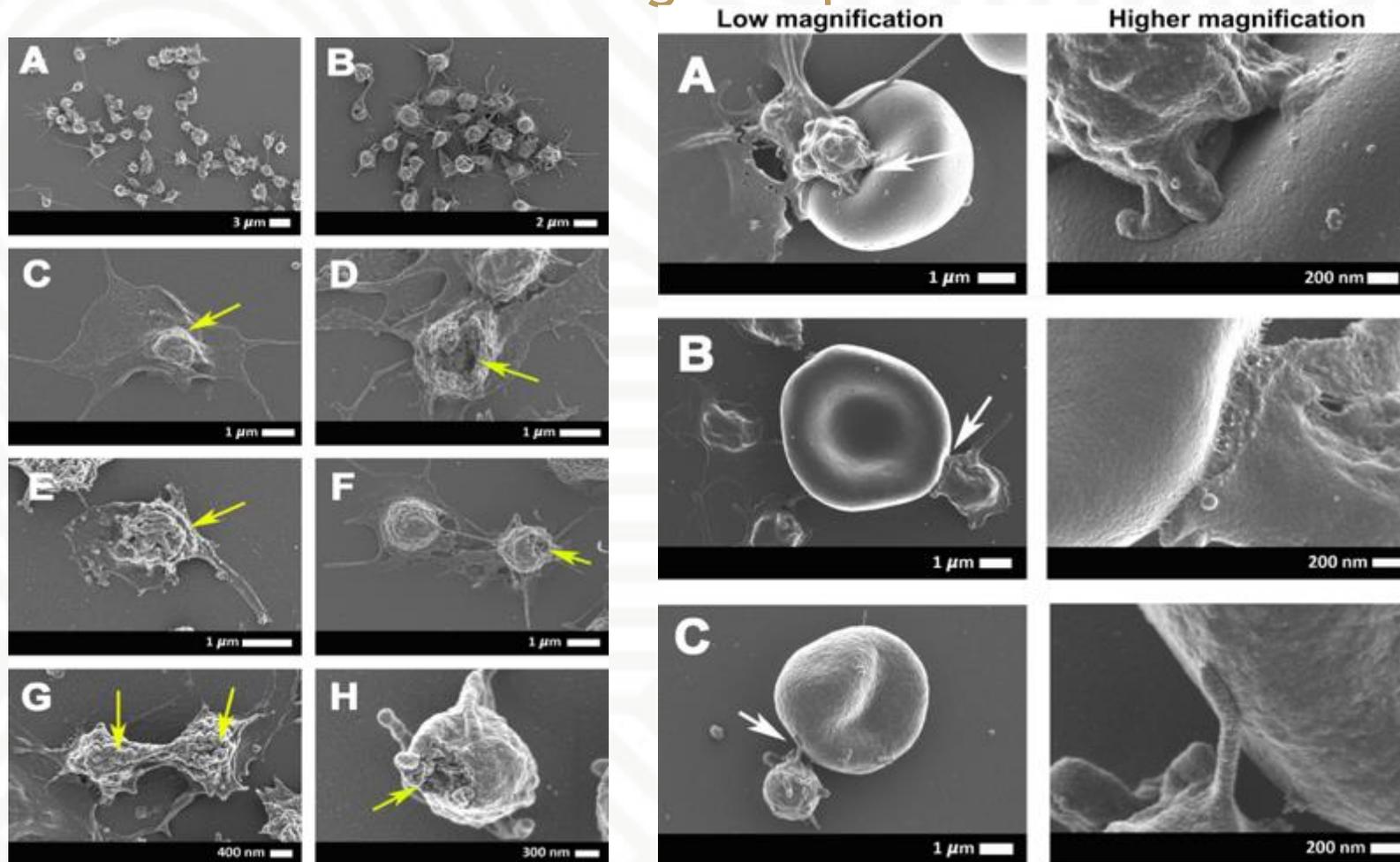
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OPEN Colorectal cancer is associated with increased circulating lipopolysaccharide, inflammation and hypercoagulability

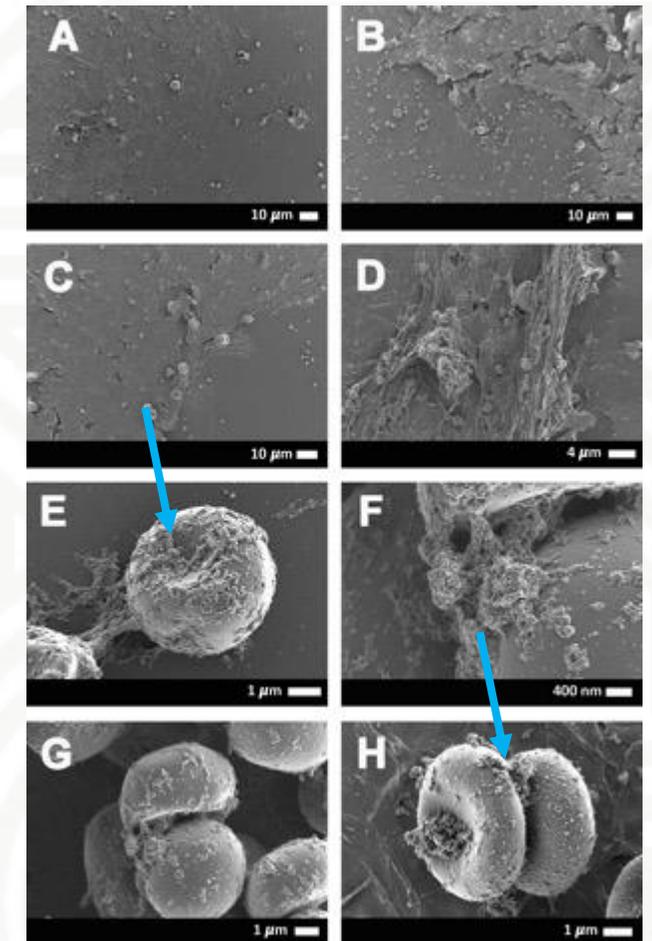
Greta M. de Waal¹, Willem J. S. de Villiers^{1,2}, Timothy Forgan³, Timothy Roberts^{1,4,5} & Ethersia Pretorius^{1,5}

Platelet and clotting pathologies in acute COVID-19

Structural changes in platelets

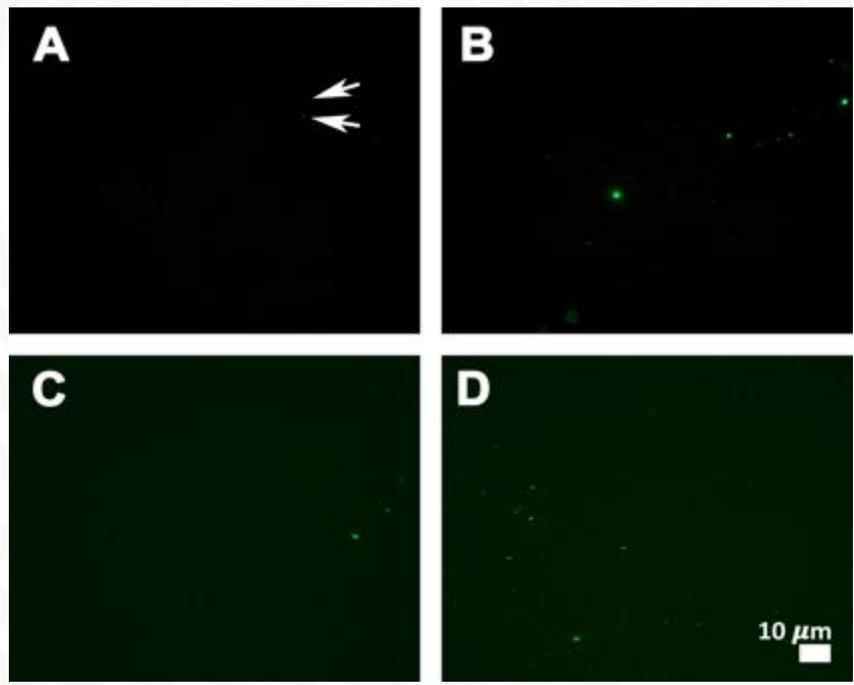


Microclots

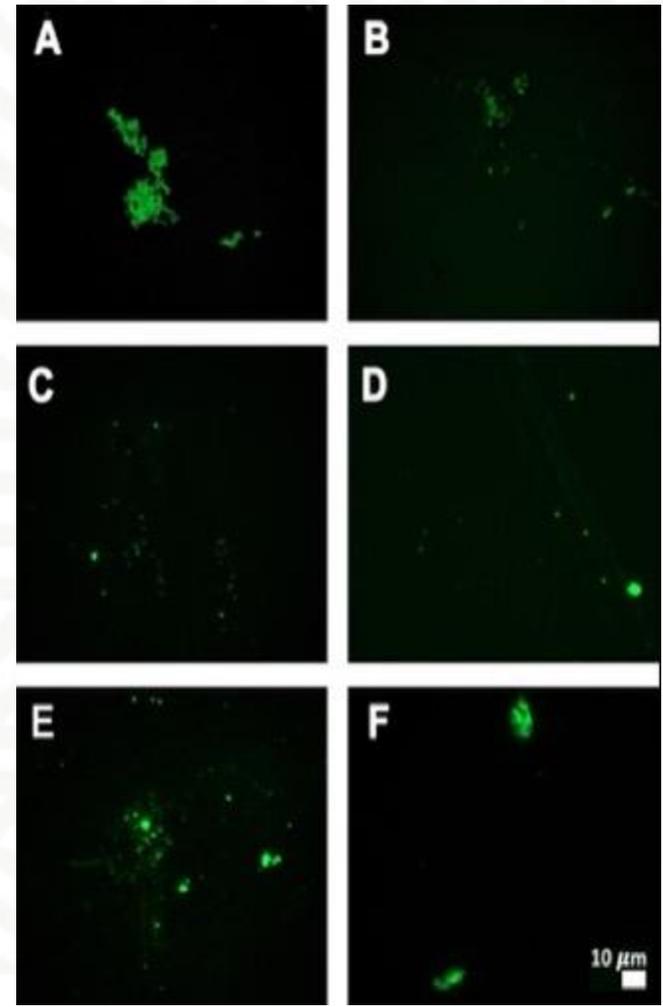


Structural changes in fibrin(ogen) in acute COVID-19 infection

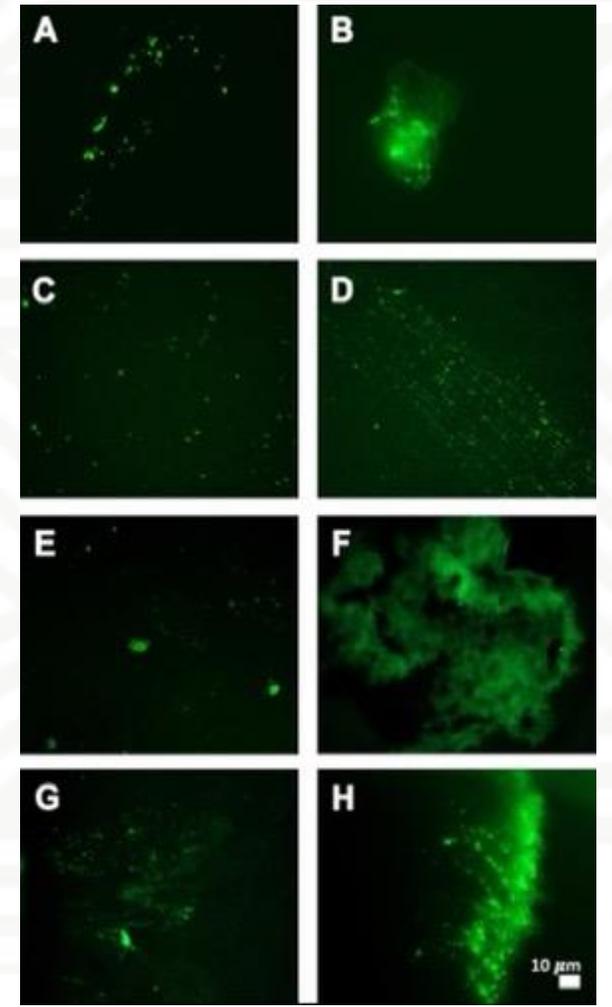
Healthy Plasma



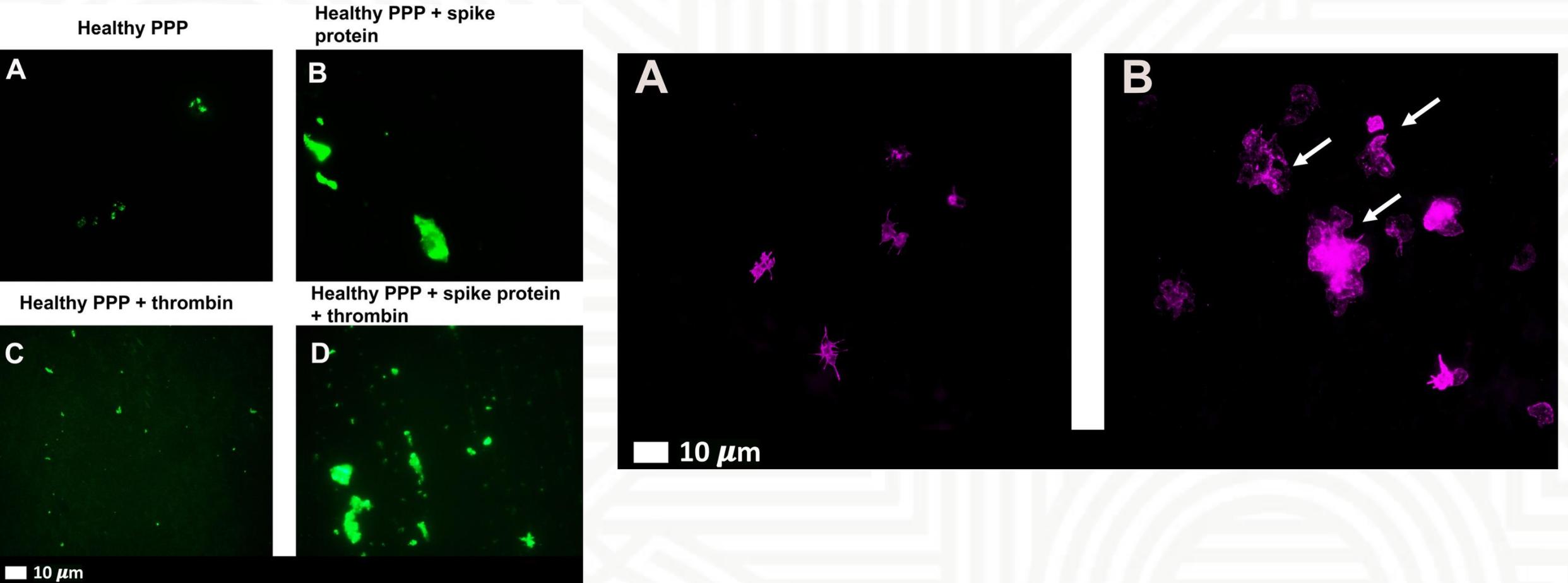
Type 2 Diabetes Plasma



COVID-19 Plasma

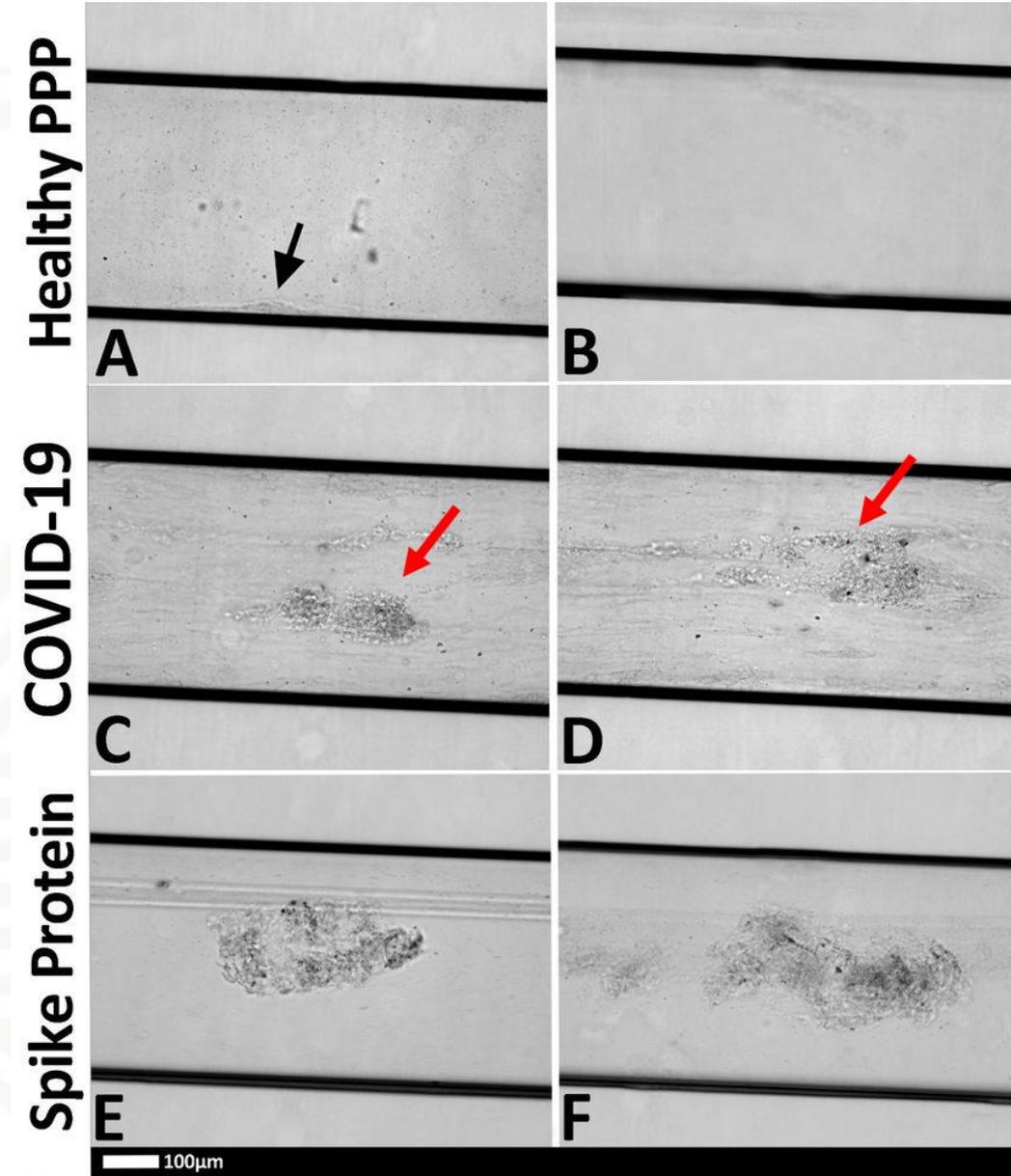
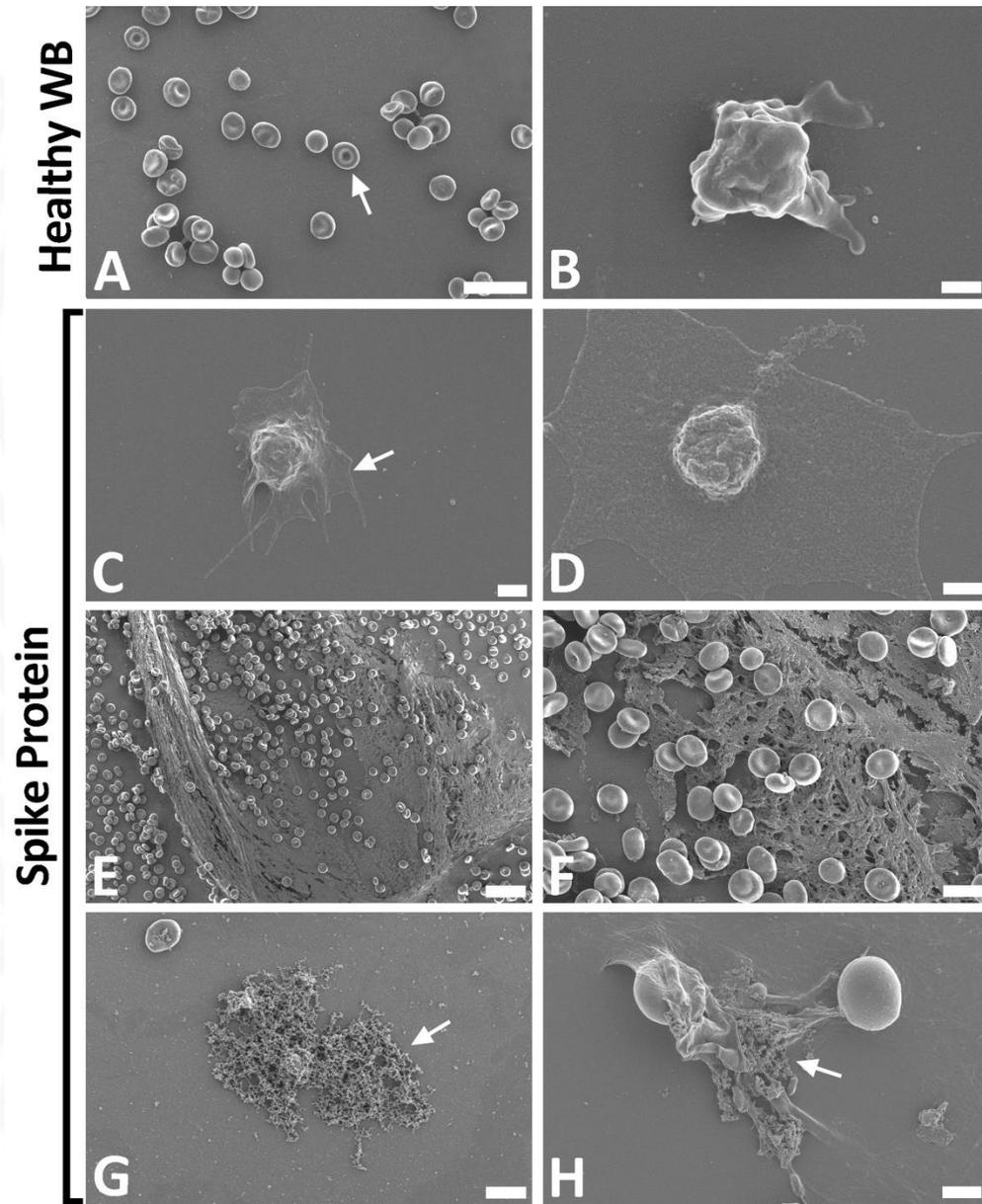


Spike protein S1 can induce fibrinolytic-resistant microclots and platelet hyperactivation



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 & microfluidics: Spike protein S1



Long COVID: first cases in SA, end of 2020

Long COVID

Addressing the global health crisis that isn't over yet

- 400 million people globally
- 10 to 30%
- Carrying an economic burden of \$1 trillion per year
- Excess mortality up to 3% higher than pre-pandemic levels even up to 2033

Understanding the Burden of Long COVID: Key Insights

Economic Impact

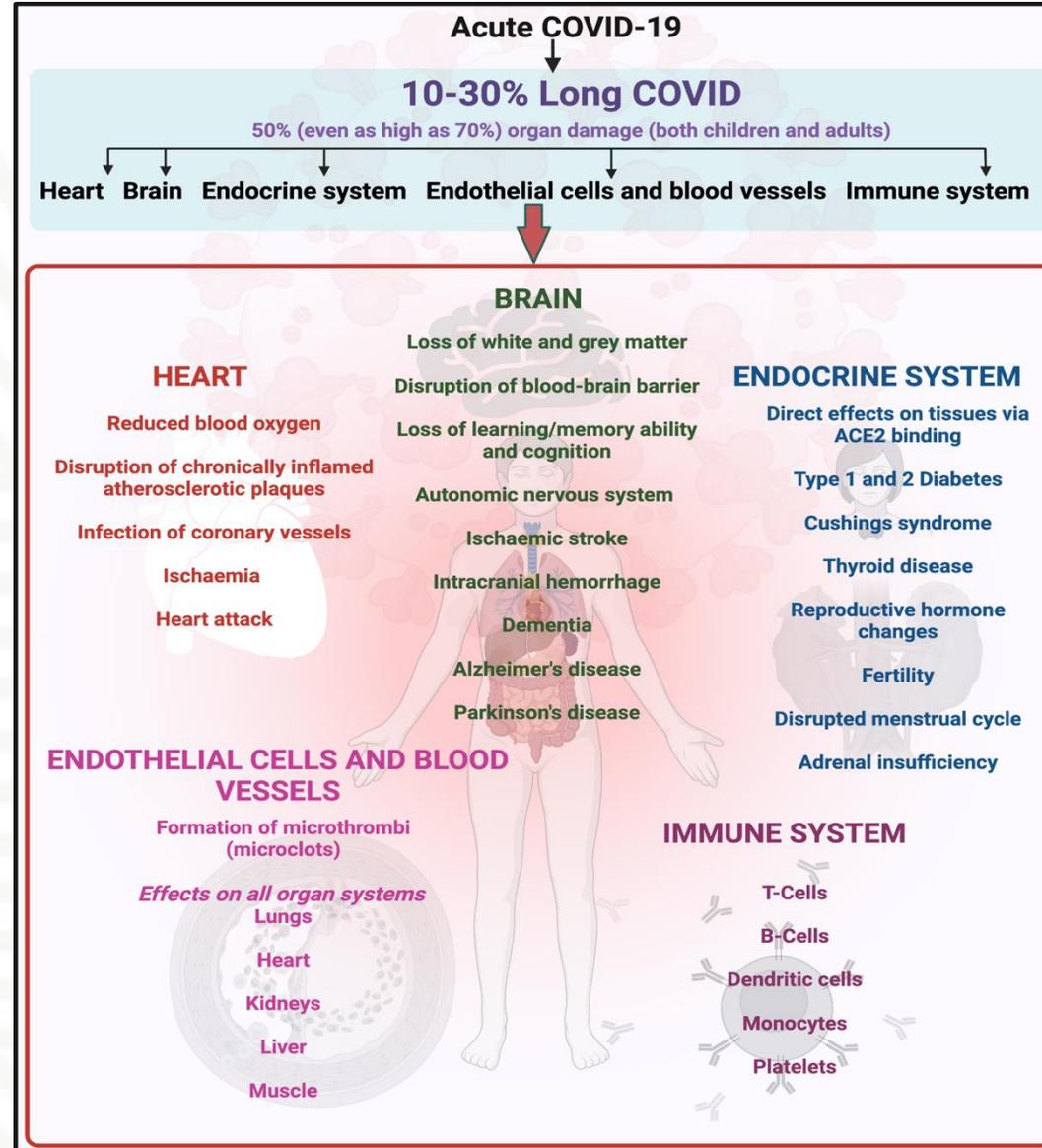
- Long COVID-related productivity losses are estimated to cost billions, with the UK alone seeing a GDP reduction of \$15 billion annually

Healthcare Strain

- Ongoing symptoms affect multiple organ systems, complicating patient care and recovery

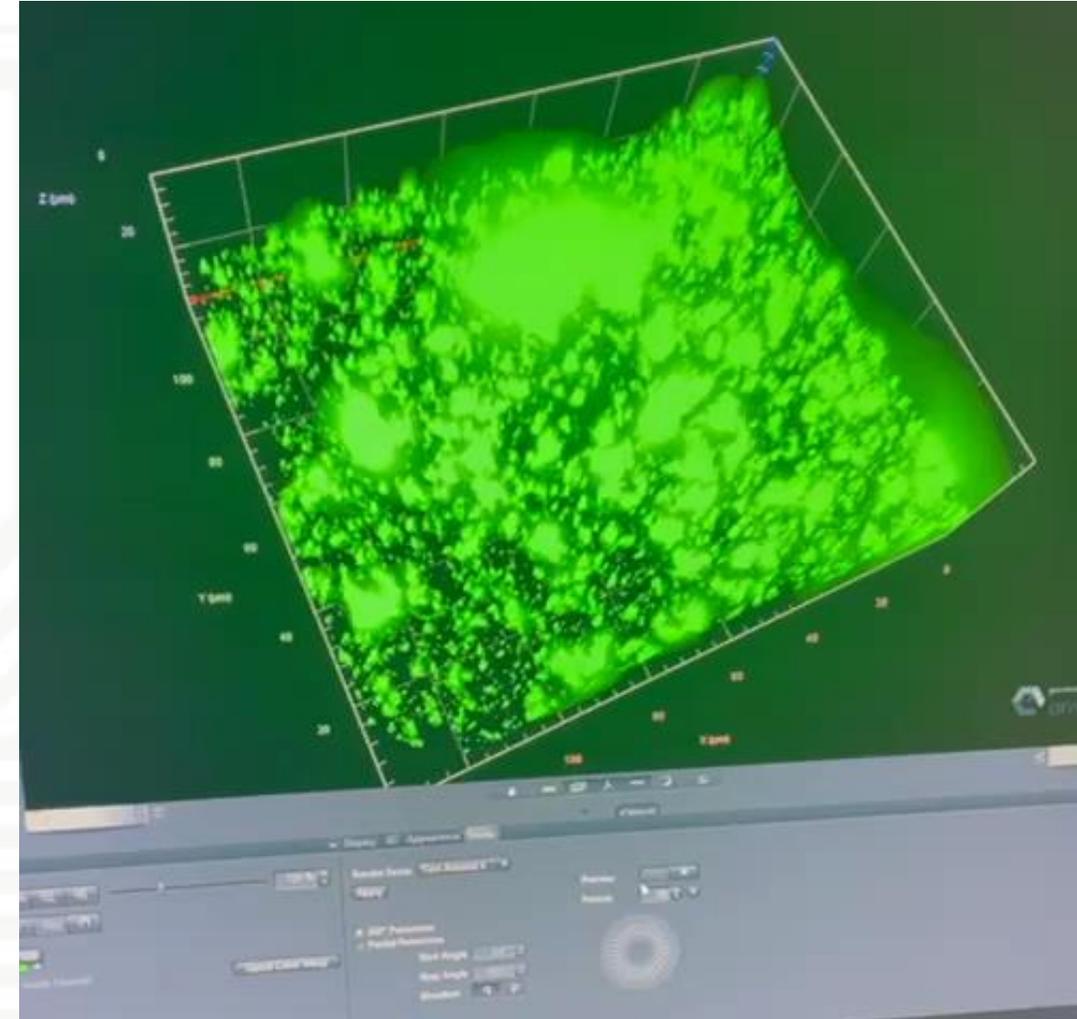
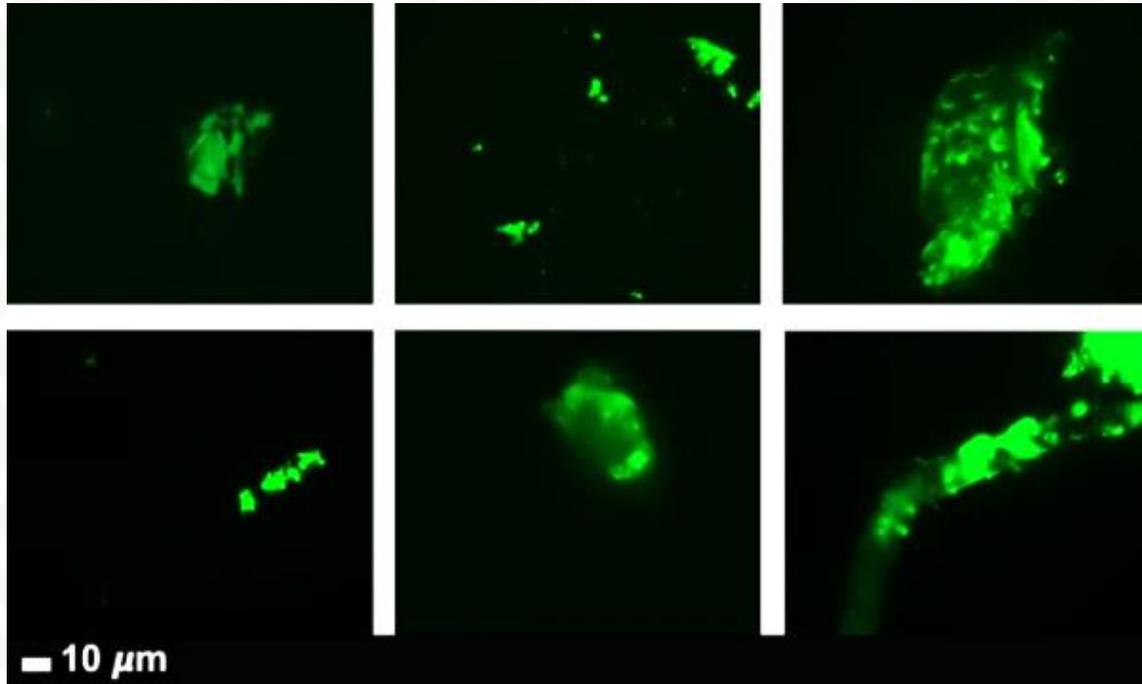
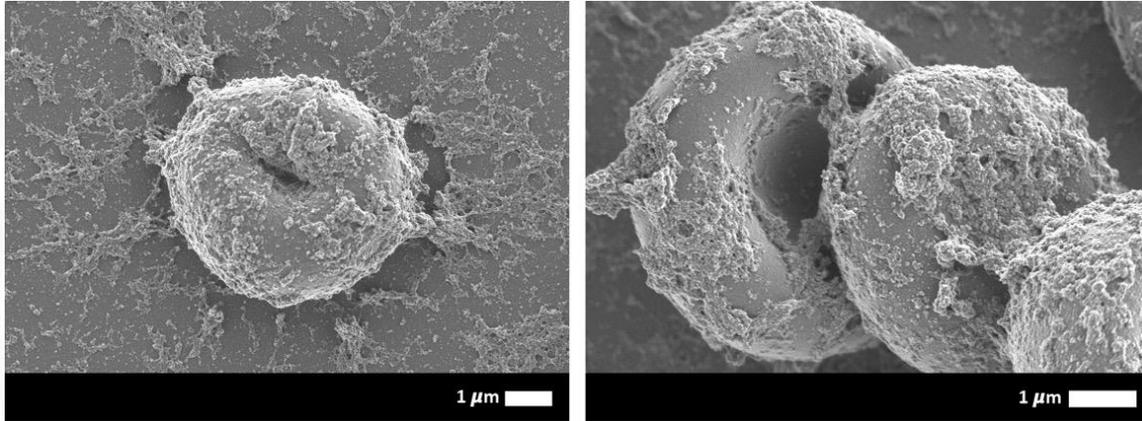
https://impact.economist.com/perspectives/sites/default/files/download/ei264_-_an_incomplete_picture_understanding_the_burden_of_long_covid_v8.pdf

Long COVID resulting in widespread organ damage

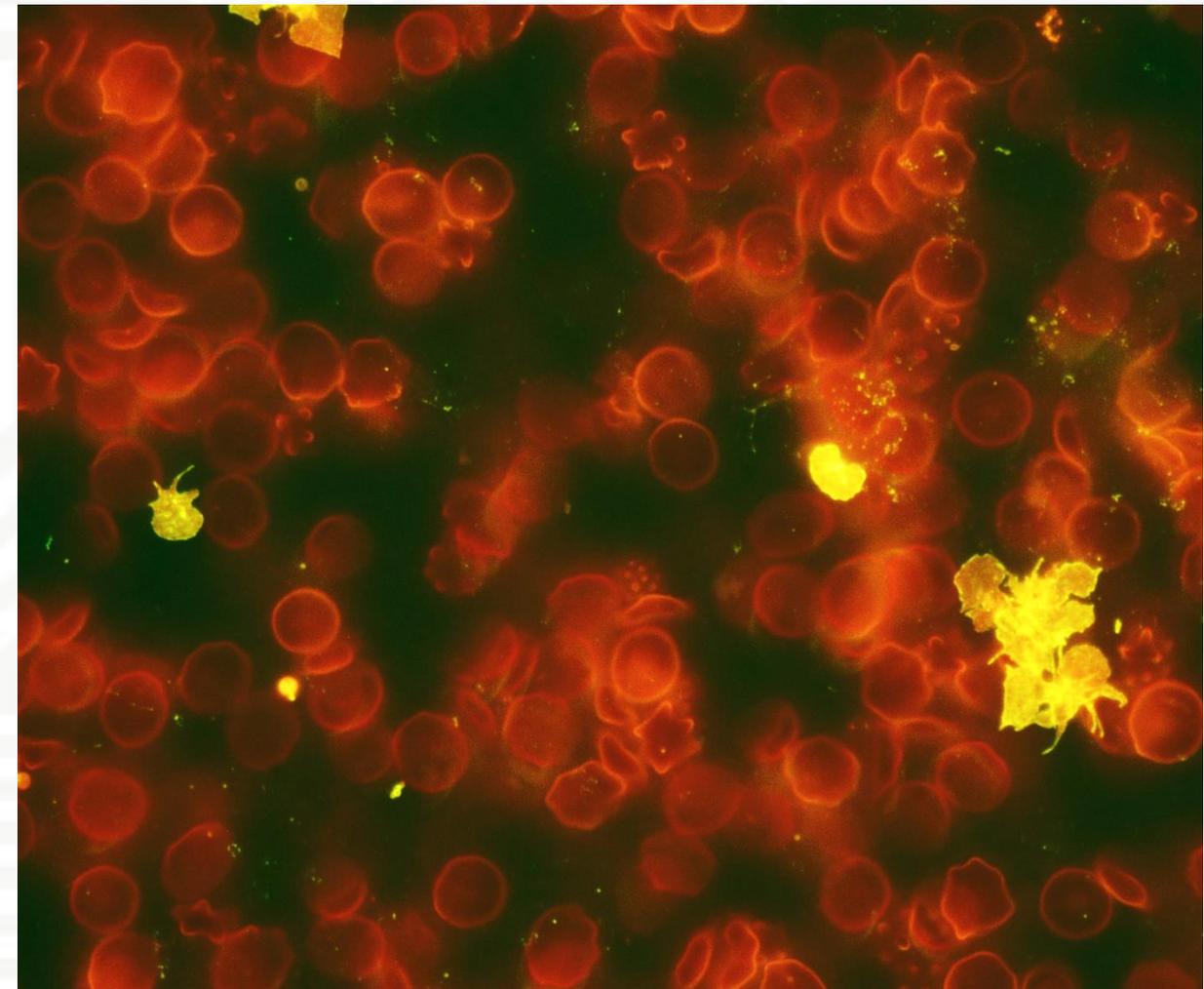
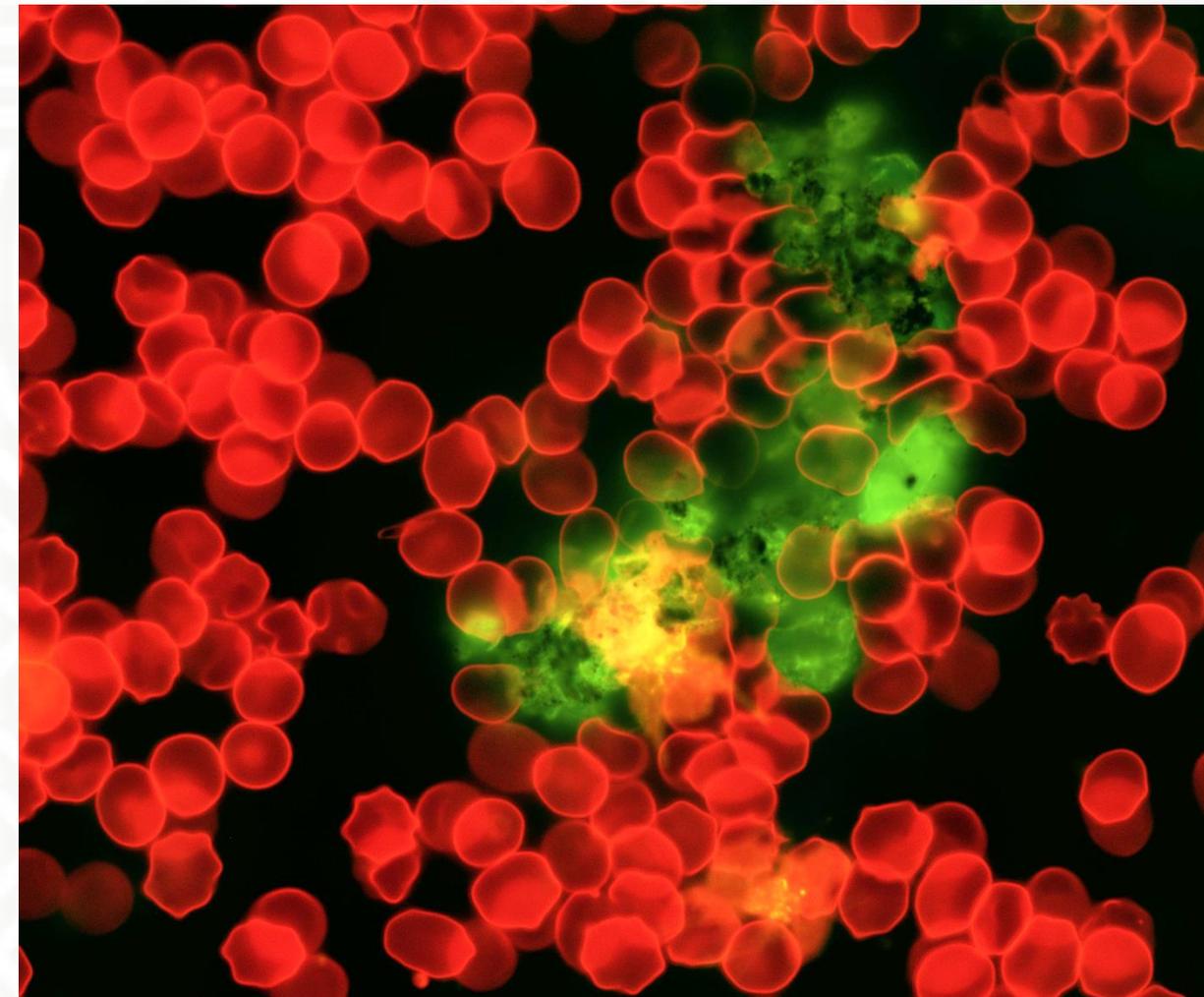


EWING, A. G., SALAMON, S., PRETORIUS, E., JOFFE, D., FOX, G., BILODEAU, S. & BAR-YAM, Y. 2025. Review of organ damage from COVID and Long COVID: a disease with a spectrum of pathology. *Med Rev (2021)*, 5, 66-75.

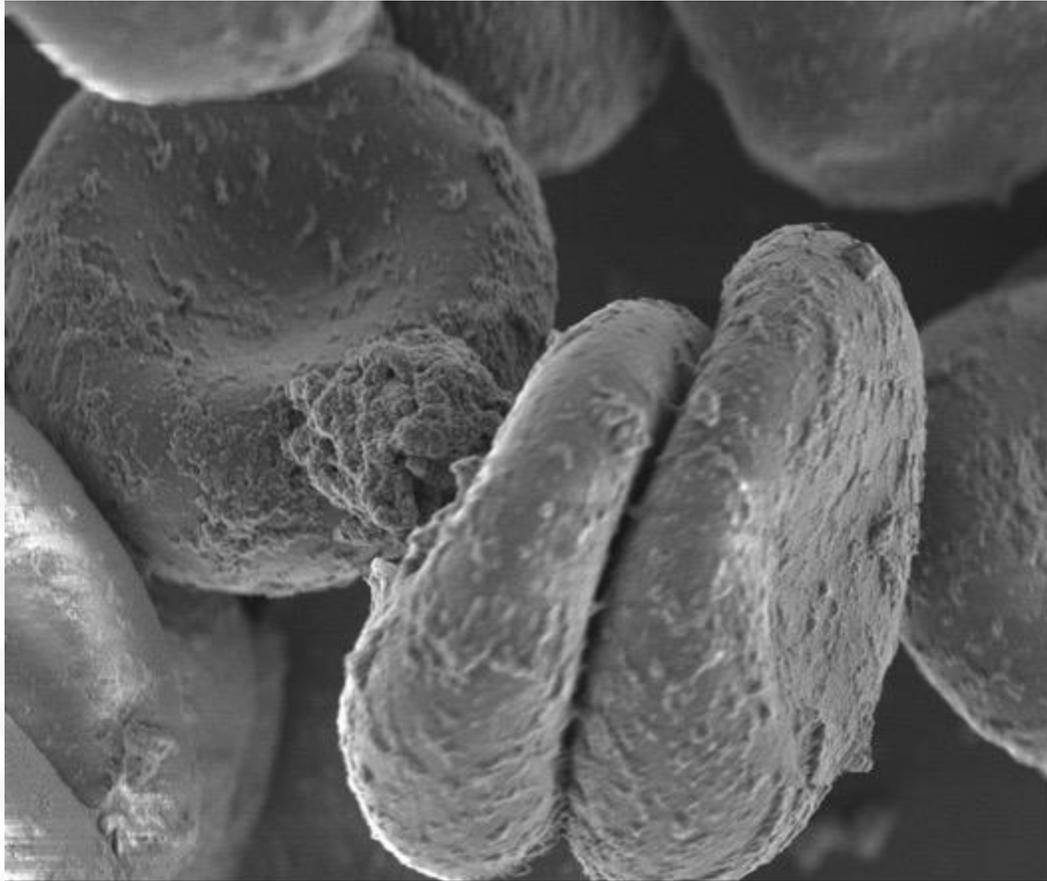
Fluorescence microscopy and scanning electron microscopy in Long COVID



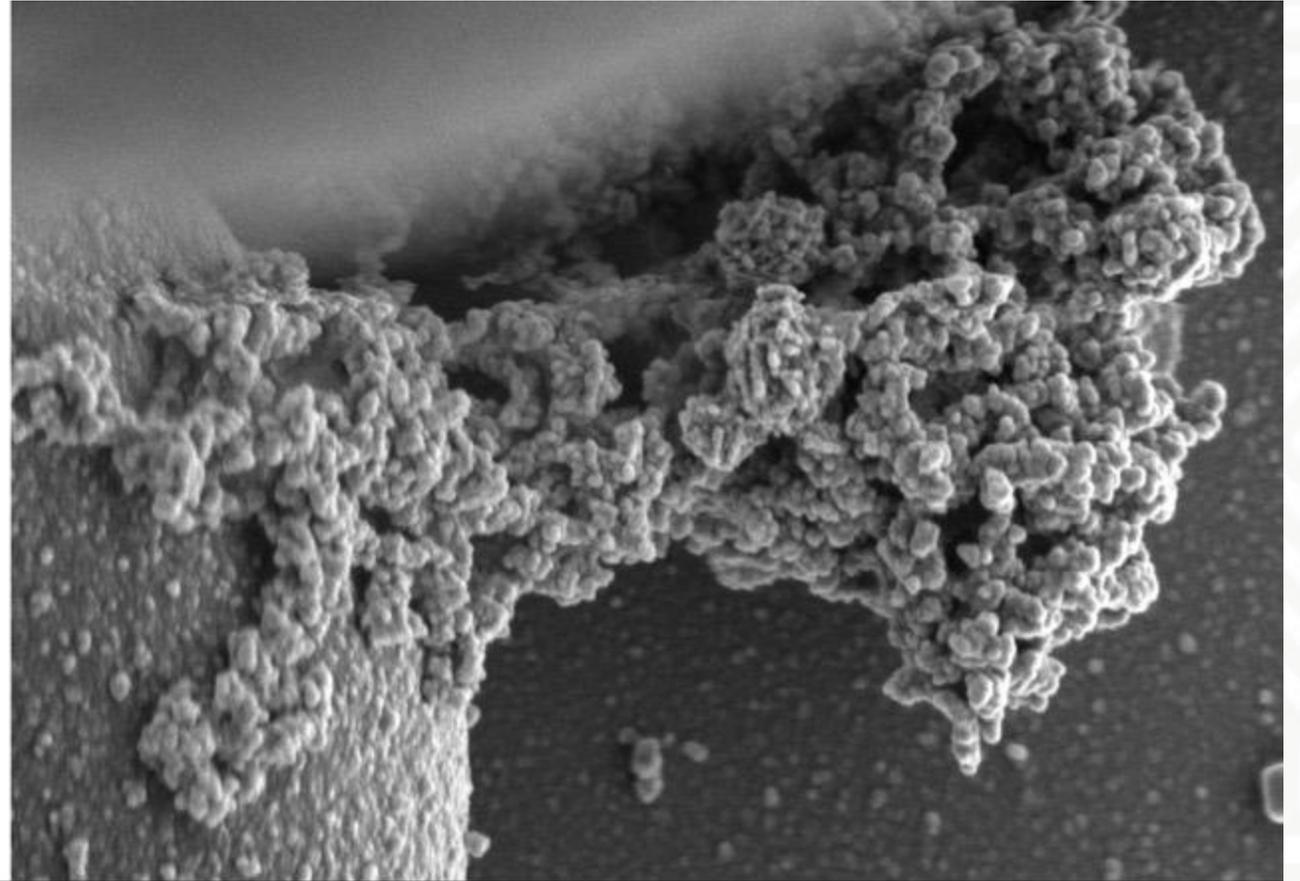
Fluorescence microscopy in Long COVID



Scanning electron microscopy in Long COVID

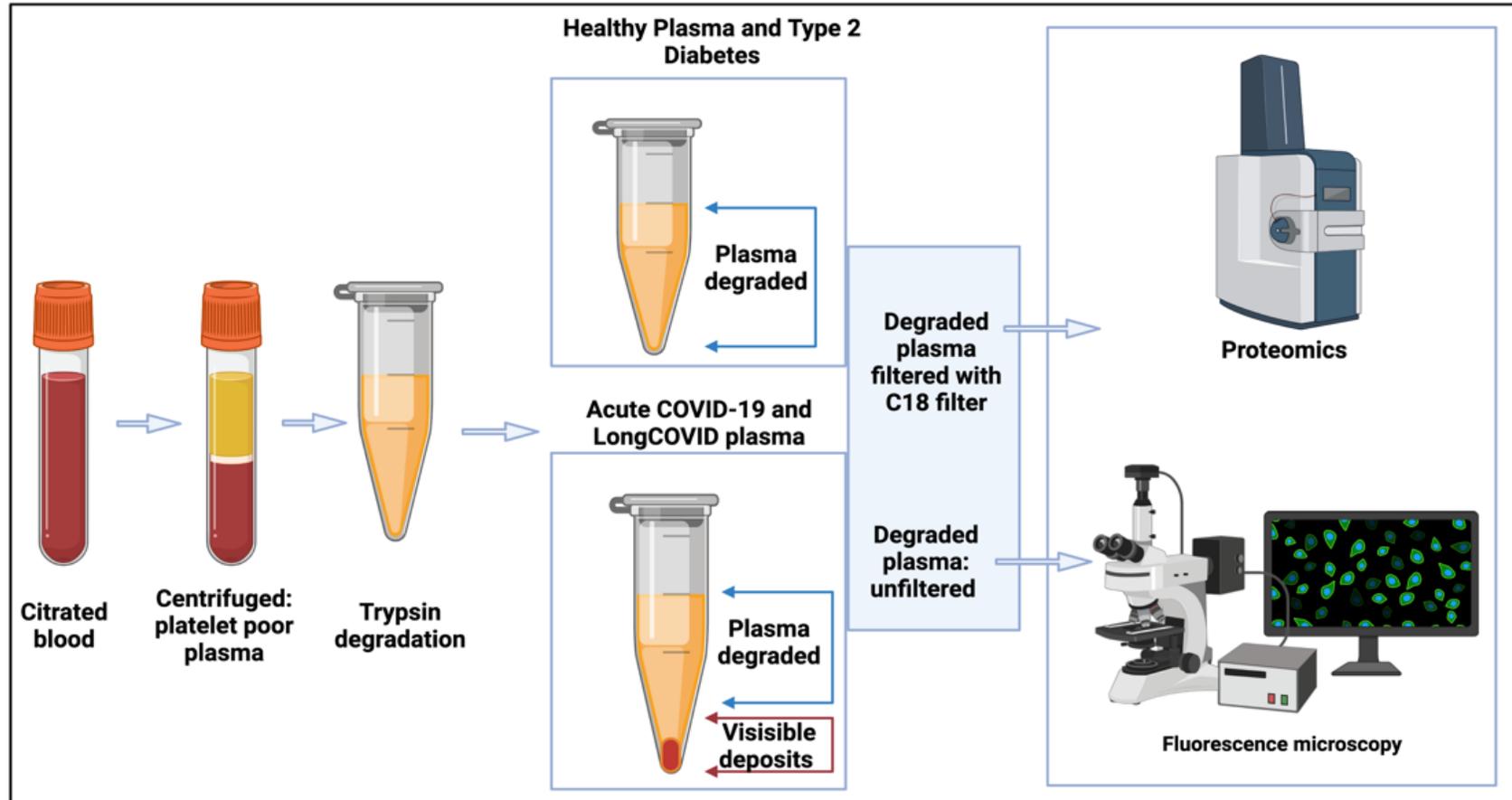


1 μm



300 nm

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



Pretorius, E., Vlok, M., Venter, C., Bezuidenhout, J.A., Laubscher, G.J., Steenkamp, J., and Kell, D.B. (2021). Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovasc Diabetol* 20, 172.

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.

2021 Proteomics Analysis: are they indeed “clots”?

Pretorius, E., Vlok, M., Venter, C., Bezuidenhout, J.A., Laubscher, G.J., Steenkamp, J., and Kell, D.B. (2021). Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovasc Diabetol* 20, 172.

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
α 2 antiplasmin (α 2AP)	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

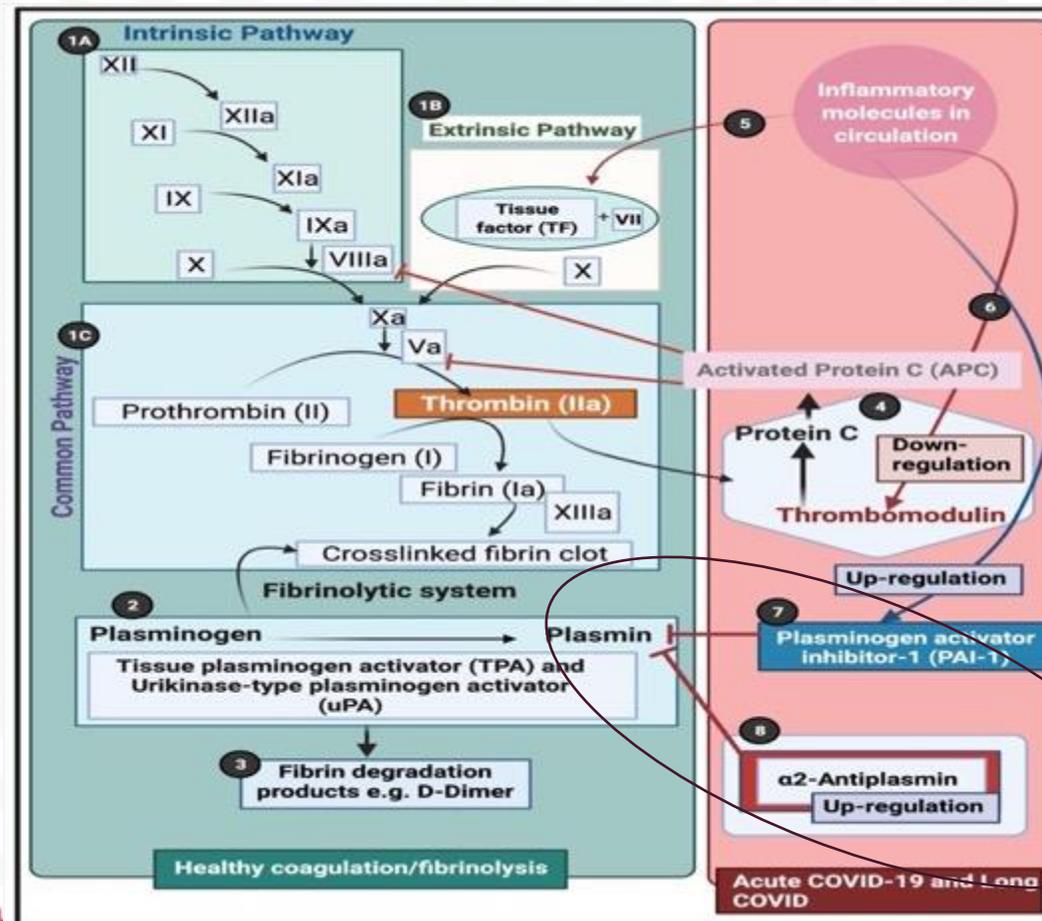
Digested pellet deposits from Long COVID/PASC microclots samples vs digested pellet deposits (microclots) from acute COVID-19 samples

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.

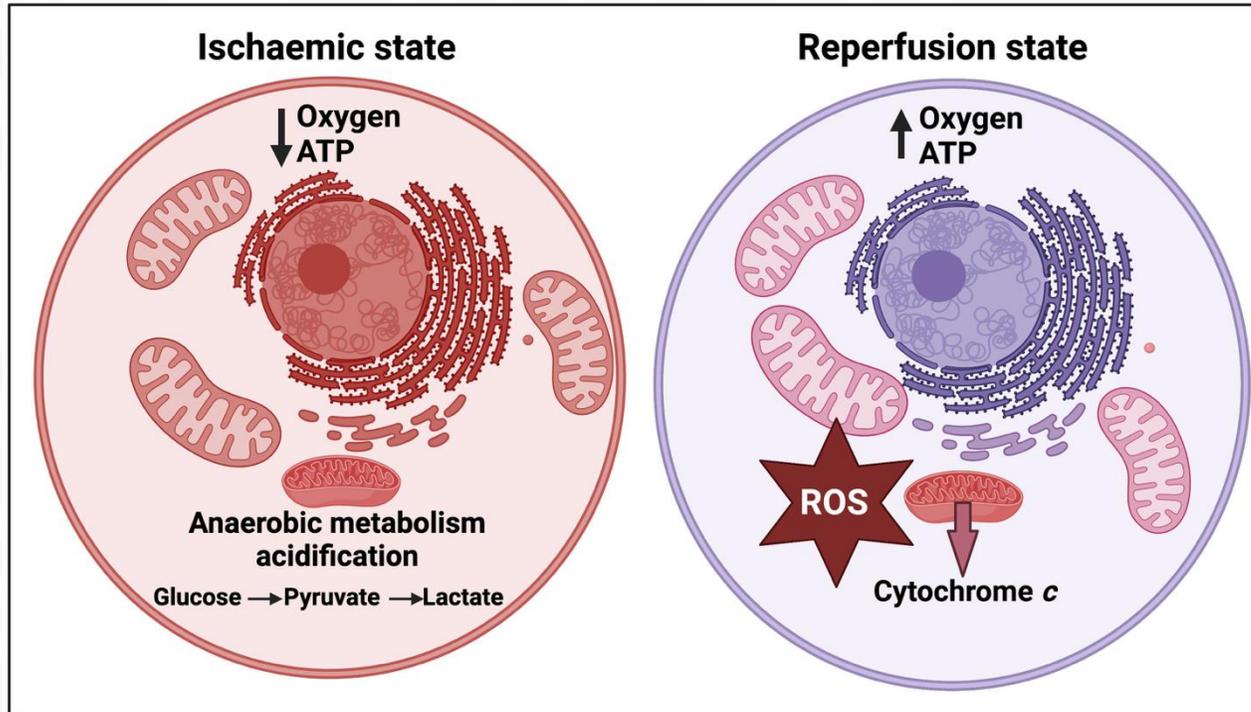
Plasminogen	2.3	0.0007
Fibrinogen β chain	2.8	0.007
Coagulation factor XIII B	2.7	0.01
Fibrinogen α chain	3.1	0.0002
Complement component C6	7.5	0.01
α 2 antiplasmin (α 2AP)	9.2	0.0003
Complement factor 1	25	0.0009

Long COVID and trapped inflammatory molecules

α 2-antiplasmin (α 2AP) inhibit plasmin and ultimately will prevent sufficient fibrinolysis from happening



Ischaemia–reperfusion (I–R) injury



- **What is Ischaemia–reperfusion (I–R) injury?**
- It is initiated via bursts of reactive oxygen species produced during the reoxygenation phase following hypoxia,
- This is a well-known process in a variety of acute circumstances.
- We argue that I–R injury also underpins elements of the pathology of Long COVID. I
- Ischaemia may be initiated via fibrin amyloid microclot temporary blockage of capillaries, for instance as exercise is started;
- Reperfusion is a necessary corollary when it finishes

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.



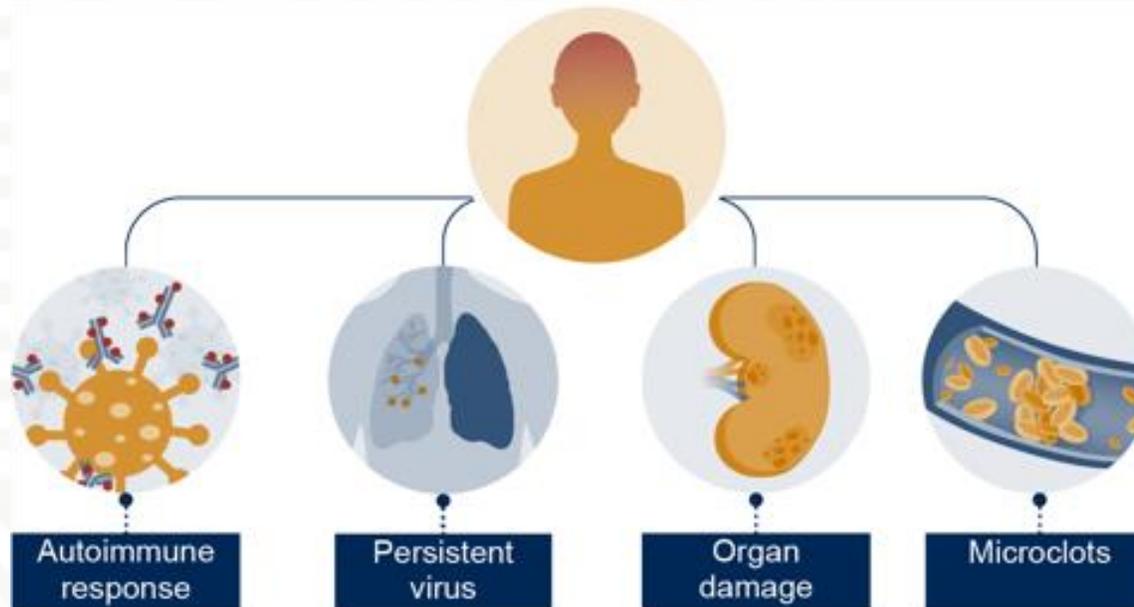
Science, Technology Assessment,
and Analytics

SCIENCE & TECH SPOTLIGHT:
LONG COVID

MARCH 2022

WHY THIS MATTERS

Long COVID has potentially affected up to 23 million Americans, pushing an estimated 1 million people out of work. The full magnitude of health and economic effects is unknown but is expected to be significant. The causes of long COVID are not fully understood, complicating diagnosis and treatment. The condition raises policy questions, such as how best to support patients.



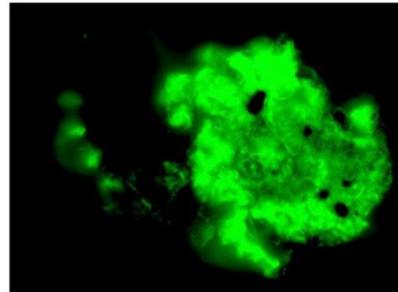
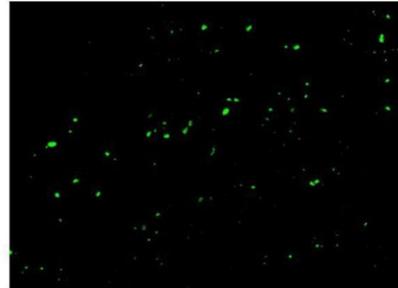
Source: GAO analysis of medical literature. | GAO-22-105666

NEWS FEATURE: NATURE

24 August 2022

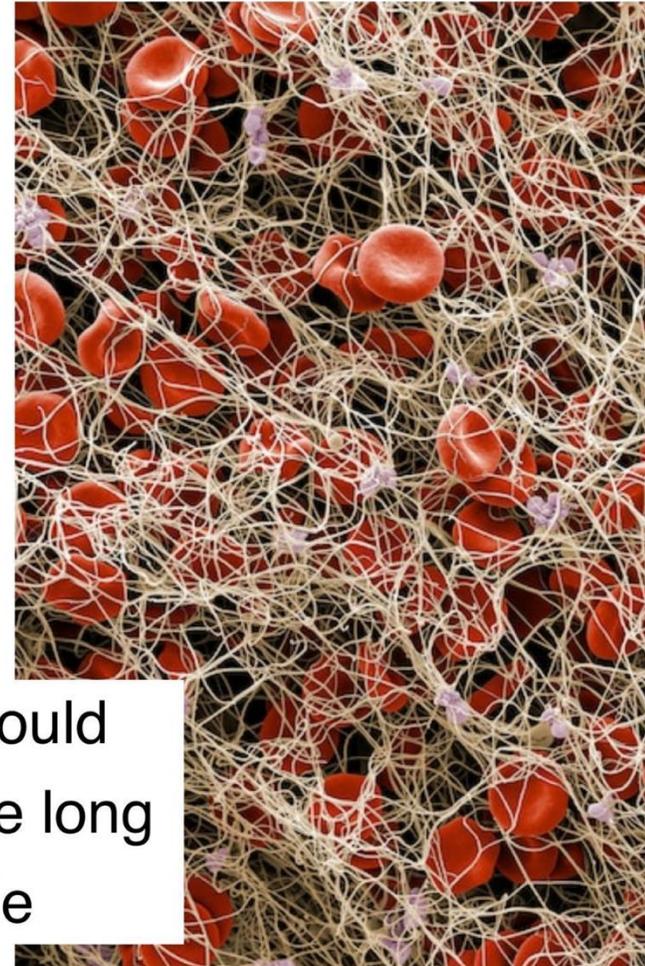
Could tiny blood clots cause long COVID's puzzling symptoms?





Fluorescence microscopy images reveal microclots circulating in healthy blood (left) and a severe one in a long COVID patient (right). The COVID clots are more resistant to a natural clot-busting process called fibrinolysis and don't break down easily.

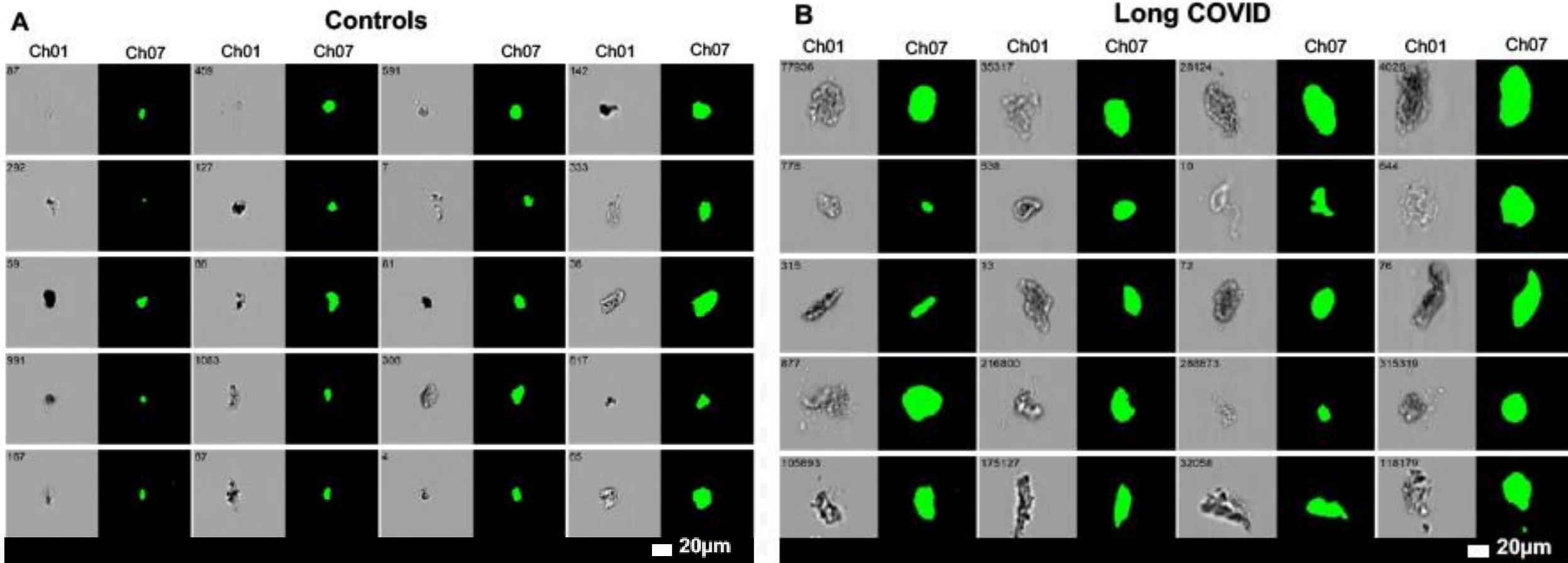
MICROGRAPH BY CHANTELE VENTER
AND RESIA PRETORIUS



‘Microclots’ could help solve the long COVID puzzle

<https://www.nationalgeographic.com/magazine/article/microclots-pots-long-covid-treatment-diagnosis>

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.

Microclots: more than just tiny aggregates but rather complexes

- 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 (inflammatory molecules of interest for a trial)***
 - ***activity***
 - ***biochemical characteristics***
- We cannot ignore the role of hyperactivated platelets and the numerous inflammatory molecules in circulation
- Ultimately platelet and clotting pathologies are driving thrombotic endothelialitis

Proteomic evidence for amyloidogenic cross-seeding of inflammatory molecules into microclots

- Inflammatory molecules in circulation
- Proteomics analysis
- These proteins (e.g., von Willebrand factor, periostin, adiponectin, α -2-antiplasmin) have **high amyloidogenicity scores** and can integrate into microclots

<http://biongram.biotech.uni.wroc.pl/AmyloGram/>

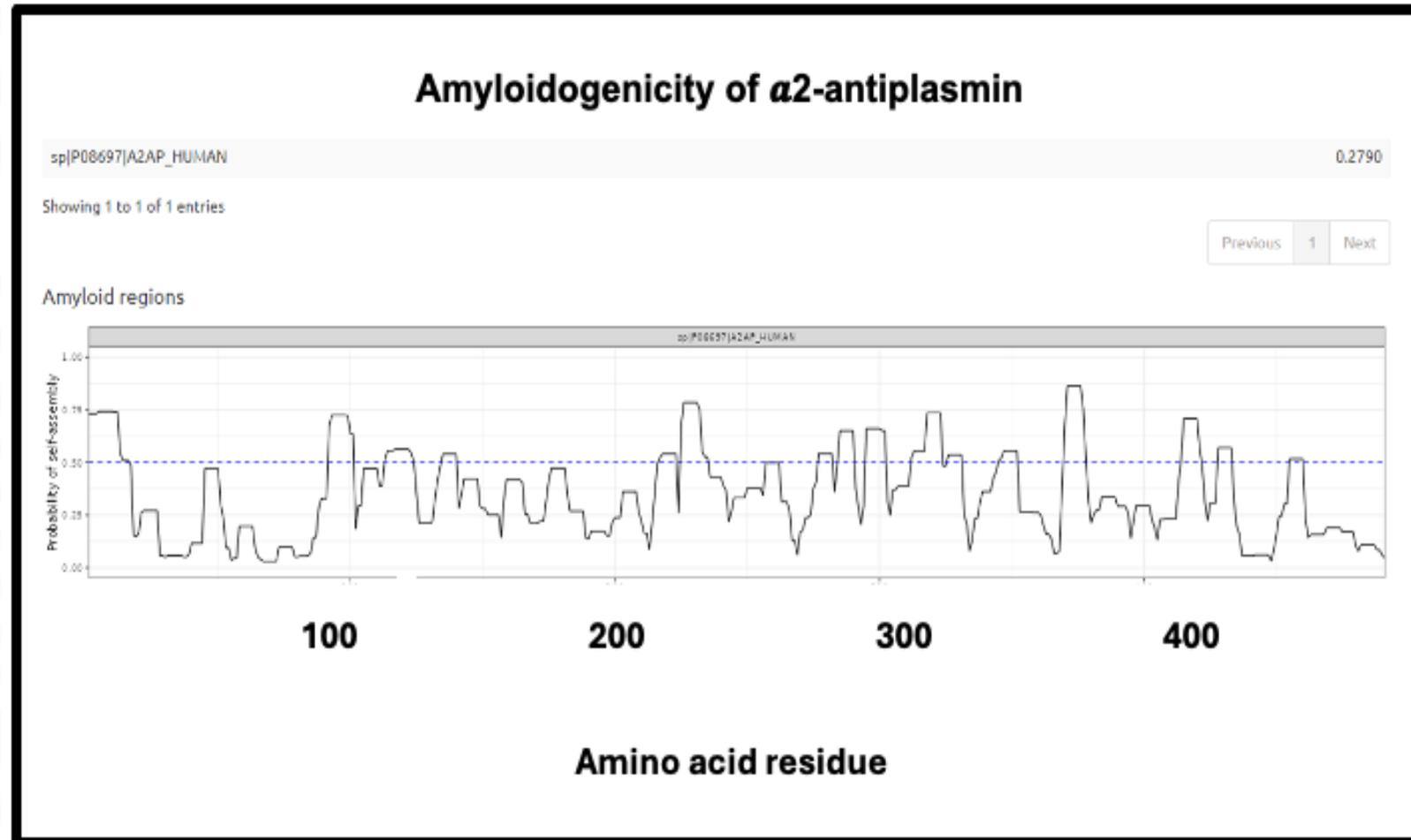
- A process of cross-seeding: structural association

α -2-antiplasmin (SERPINF2) is highly amyloidogenic

X-axis: amino acid residue number along the sequence of the protein

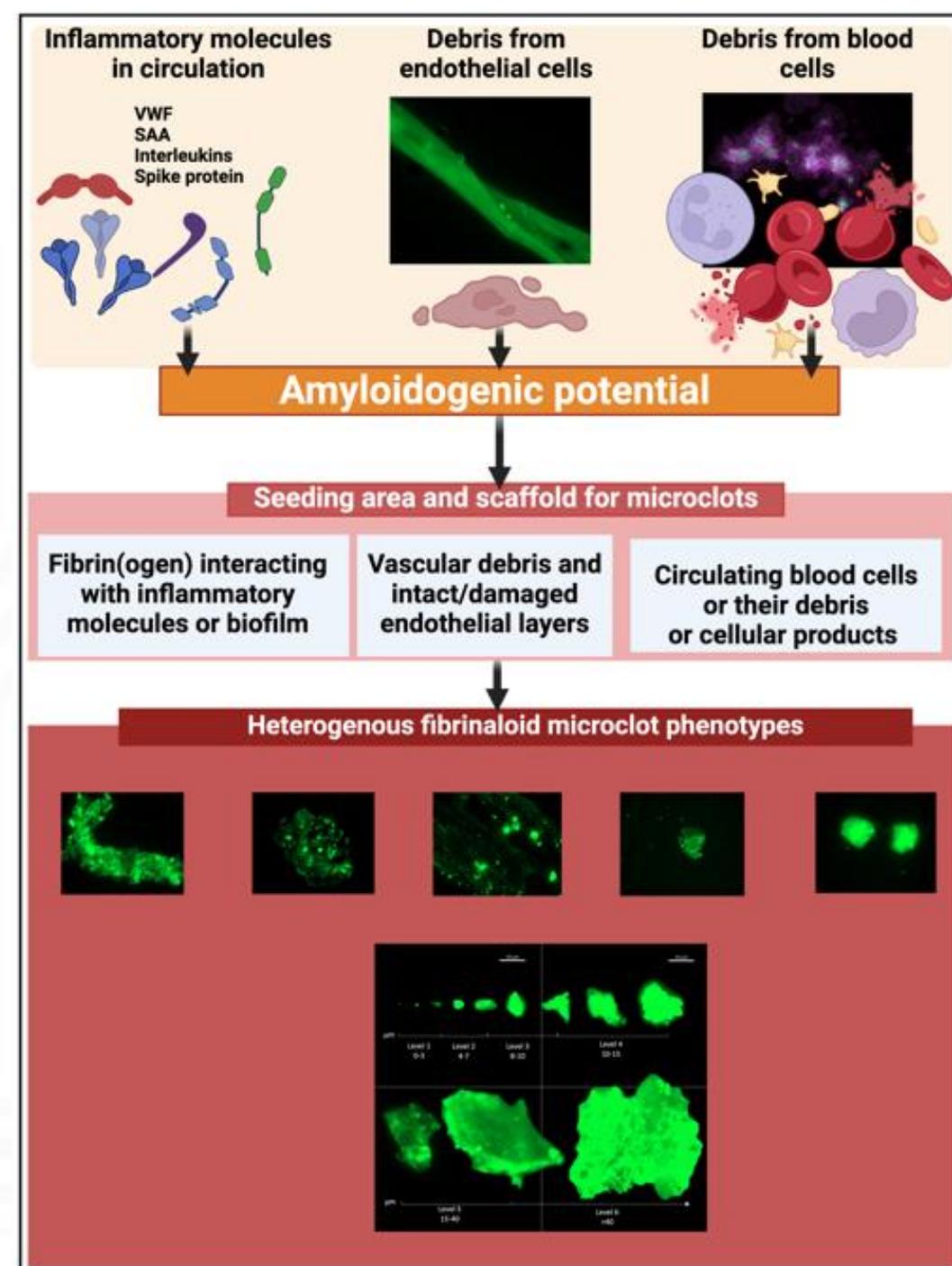
y-axis: amyloidogenicity score, which is essentially the probability that a given sequence segment can form amyloid structures

Scores above 0.75 are considered highly amyloidogenic

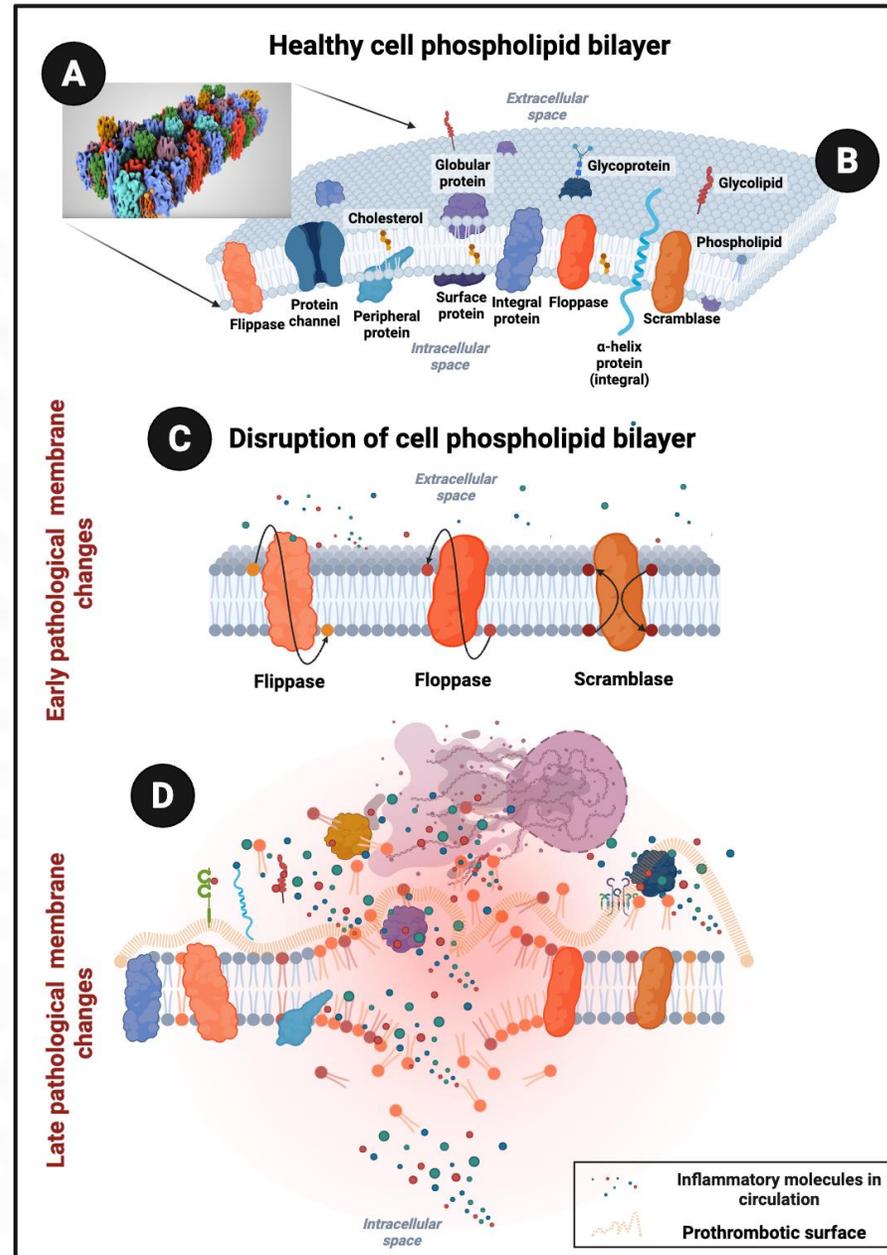


Heterogenous fibrinaloid microclot complex phenotypes

- **Seeding onto debris** from damaged blood vessels or blood cell membranes
- Fibrinaloid microclot forming **immune complexes**
- Fibrin(ogen) aggregating: **fibrinaloid microclot**
- Heterogenous inflammatory molecule aggregates and fibrin(ogen) **seeding onto blood cells/endothelial cells**



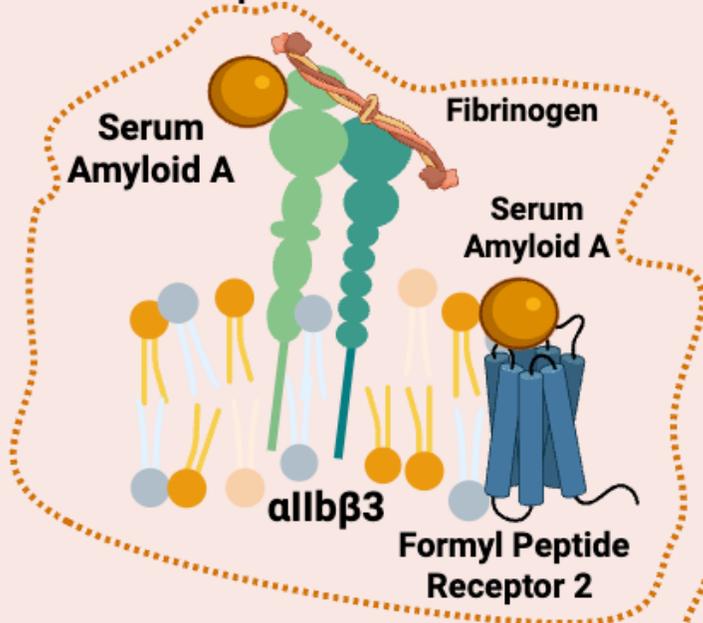
Seeding onto cellular debris: the development of a prothrombotic surface



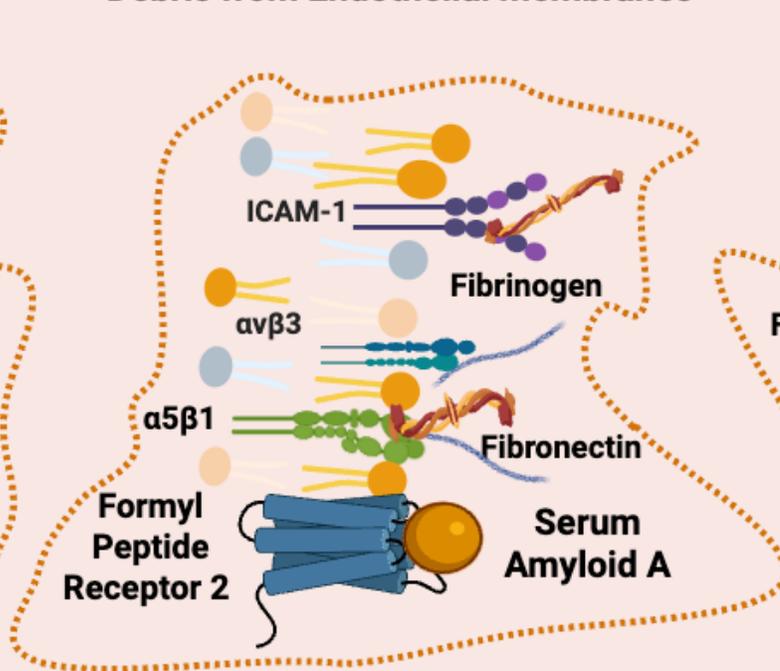
Cellular debris as prothrombotic seeding areas

A procoagulant and amyloidogenic surface to seed fibrinogen and other pro-clotting molecules

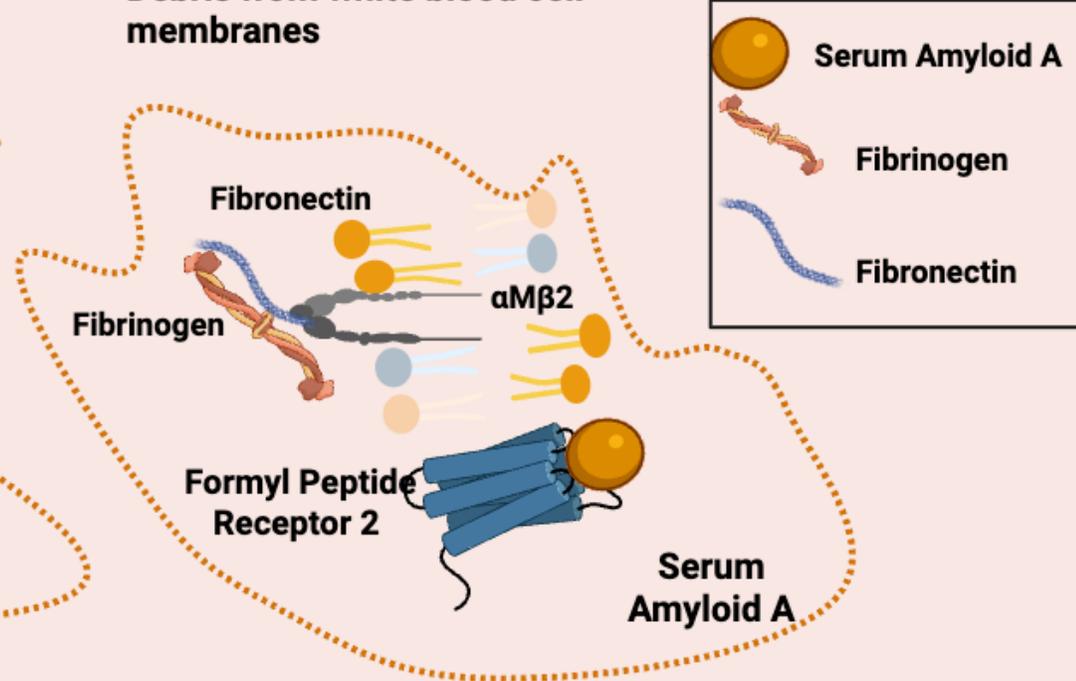
Debris from platelet membranes



Debris from Endothelial membranes



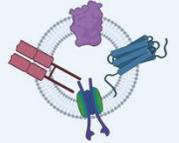
Debris from white blood cell membranes



Cellular debris as prothrombotic seeding areas

From white blood cells, platelets and endothelial cells to prothrombotic amyloidogenic microparticles: inflammation and an immune hypo-responsiveness

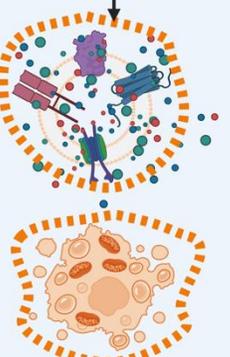
Healthy cell



- No PS flip
- Proteins receptors like fibrinogen Integrin $\alpha\text{IIb}\beta\text{3}$ (GPIIb/IIIa) are in a low affinity binding state

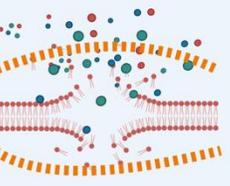
Cell damage

Prothrombotic surface



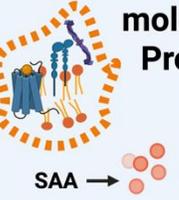
- A prothrombotic membrane
- PS flip initiated
- Membrane proteolysis
- Proteins receptors like fibrinogen Integrin $\alpha\text{IIb}\beta\text{3}$ (GPIIb/IIIa) switch to high affinity binding state
- "Eat me" signals muted
- Immune hypo-responsiveness

Cell membranes deteriorate to debris



- Prothrombotic surface enhanced by inflammatory molecule binding
- Increased stay in circulation
- Immune hypo-responsiveness

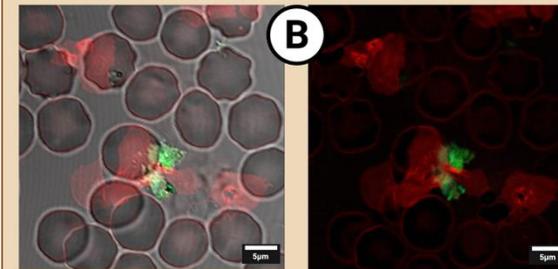
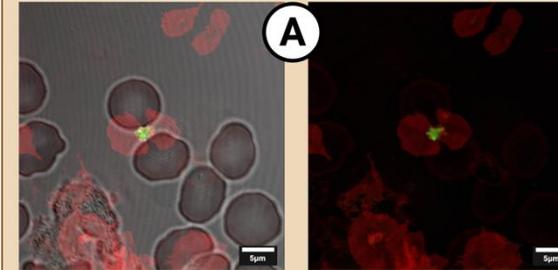
Prothrombotic fragments with misfolded molecules and fibrin(ogen) AND Prothrombotic Microparticles



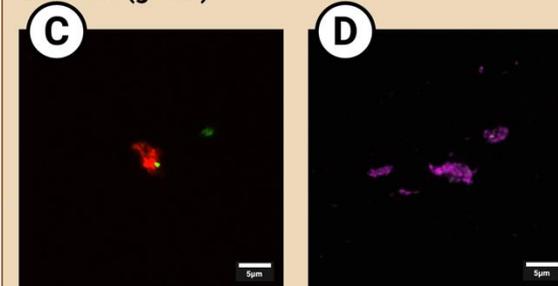
- Proteins receptors like fibrinogen Integrin $\alpha\text{IIb}\beta\text{3}$ (GPIIb/IIIa) associated with ligands like fibrinogen and SAA
- Prothrombotic microparticle formation

SAA →

Confocal microscopy examples



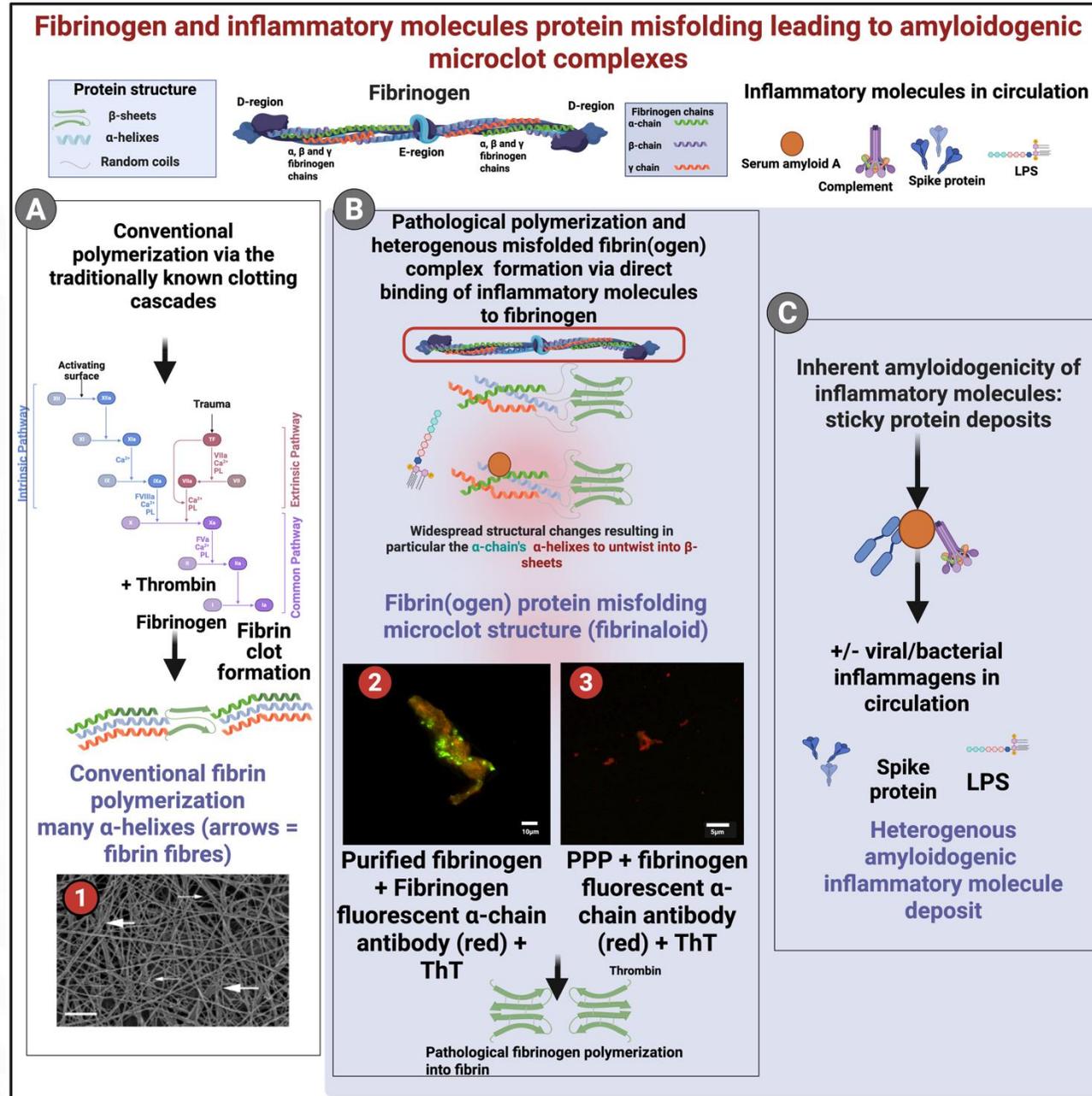
Whole Blood: Brightfield and fluorescence in confocal: RBCs and membrane of platelets stained with cell mask (red); microclot stained with ThT (green)



Microclot complex in PPP: Membrane debris: cell mask (red); microclot stained with ThT (green)

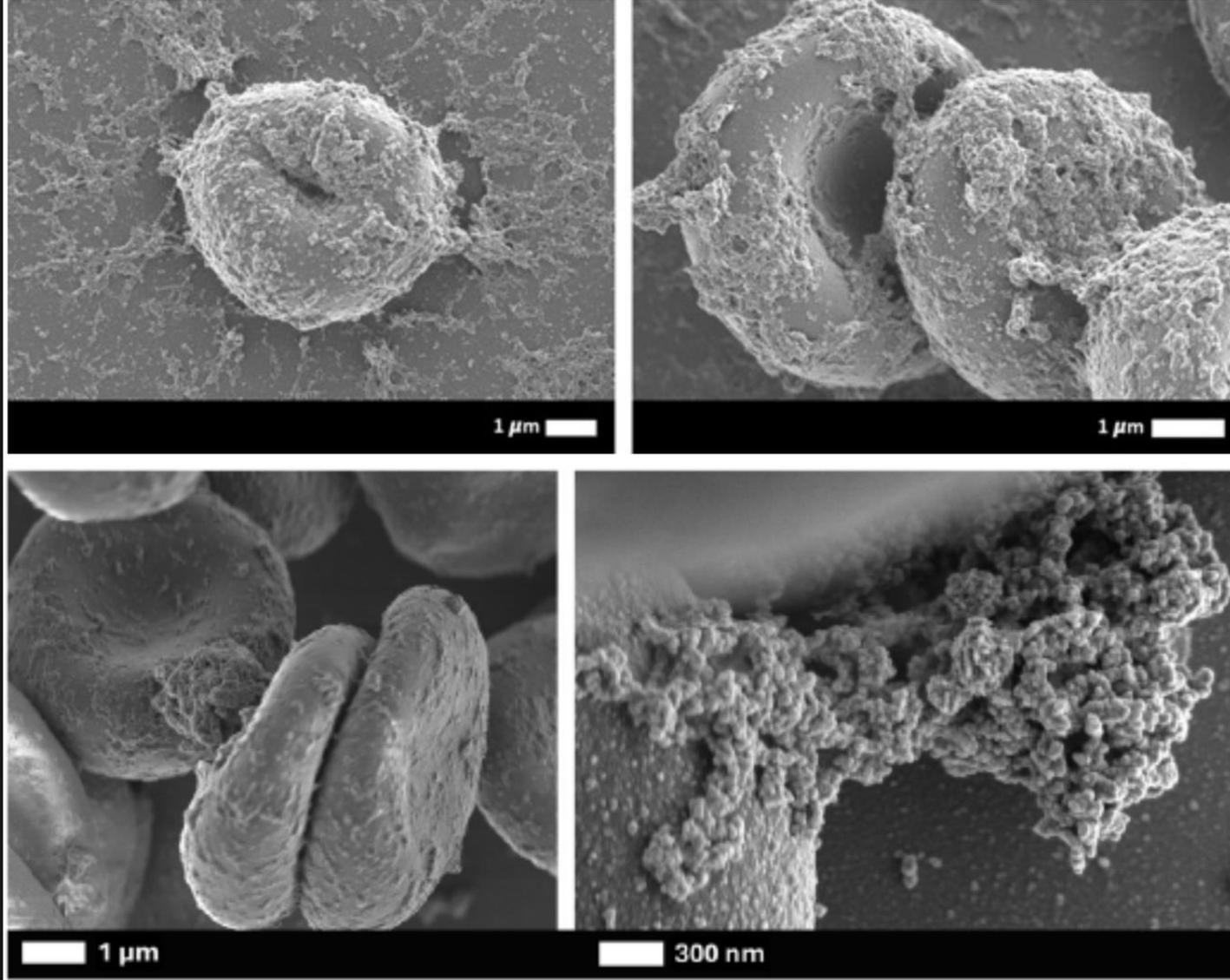
Microclot complex in PPP: Fibrinogen antibody (α -chain) (pink); ThT signal (green)

Heterogenous fibrinogen and inflammatory molecule phenotype

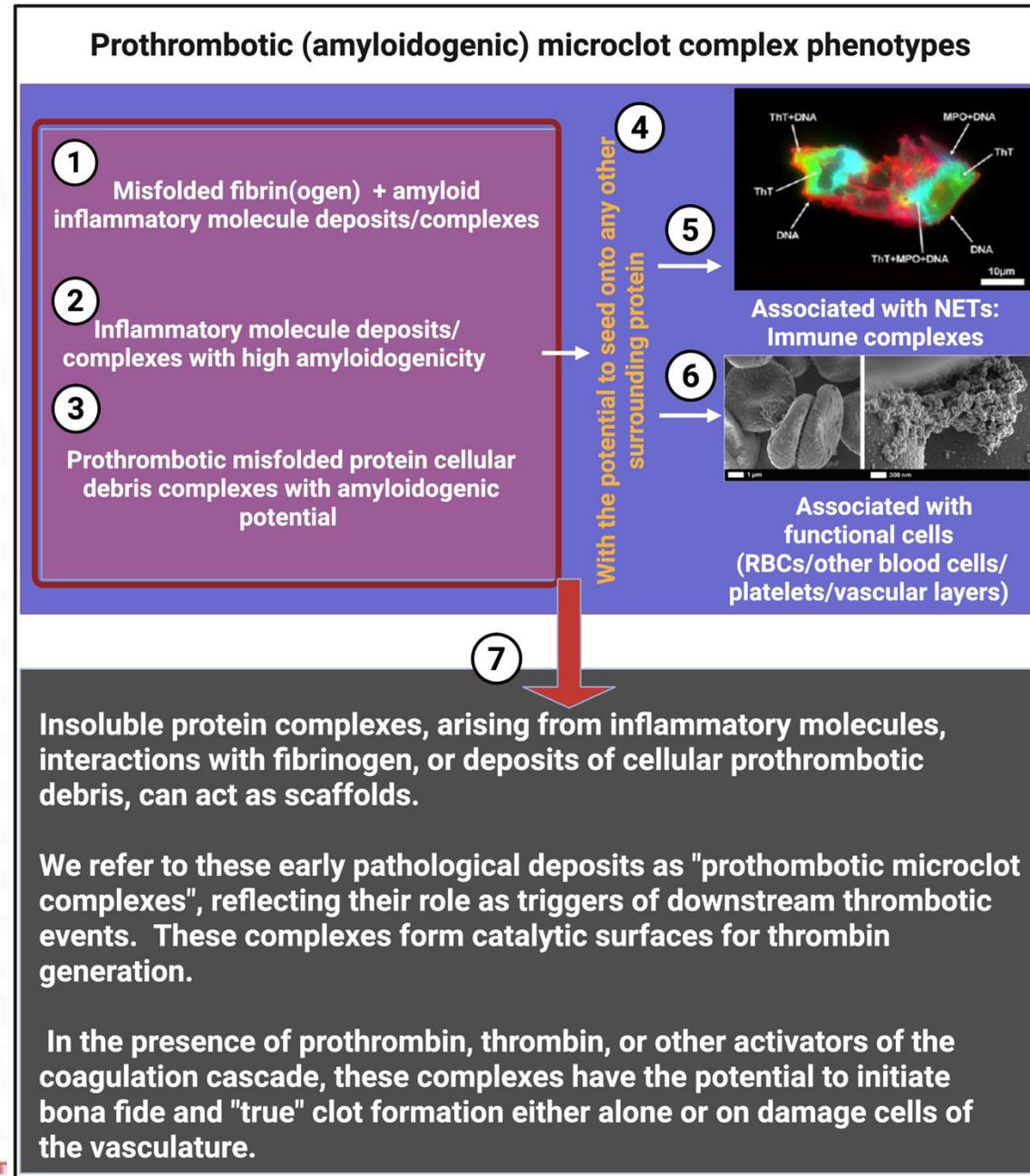


Microclot complexes seeding on intact cells

Amyloidogenic microclot complexes seeding onto intact cells in the vasculature: seeding onto red blood cells



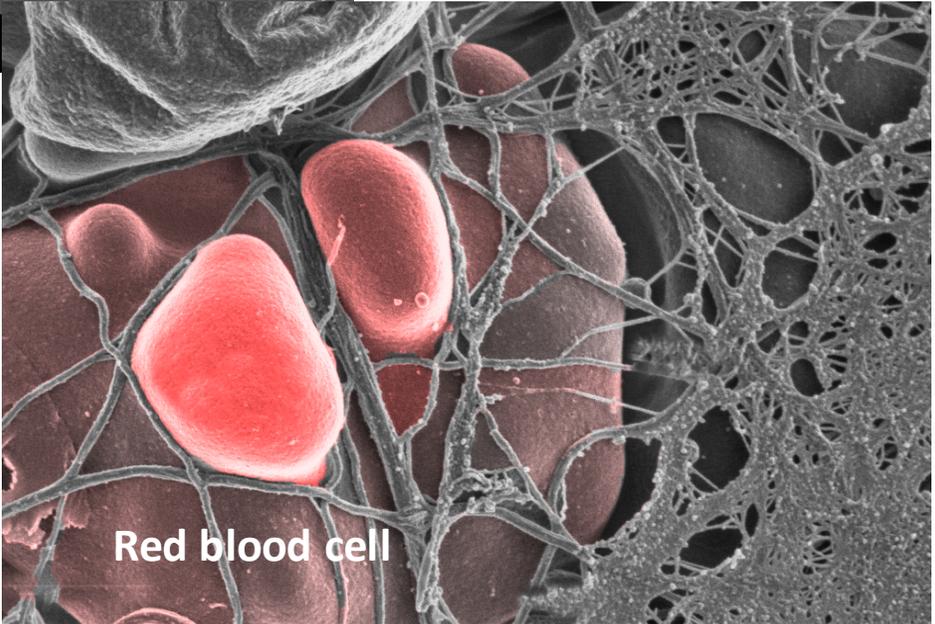
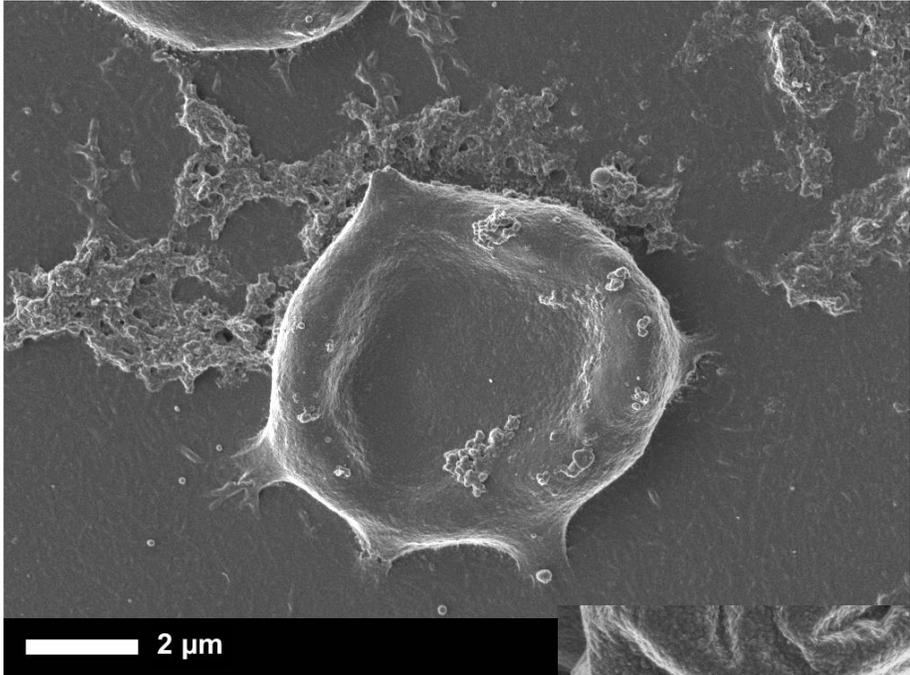
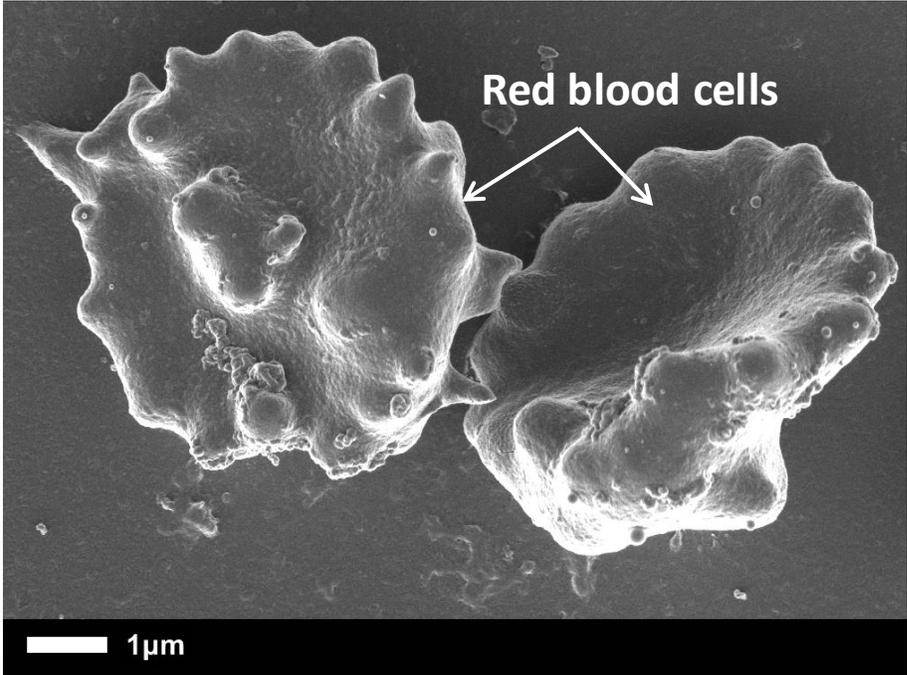
Microclot complex phenotypes



Microclot complex phenotypes

- Insoluble protein complexes arise from:
 - Inflammatory molecules
 - Fibrinogen
 - Deposits of cellular prothrombotic debris
 - Debris act as **scaffolds**
 - We call them “**prothrombotic microclot complexes**” because they trigger downstream thrombotic events
 - They form **catalytic surfaces** that assemble prothrombinase and generate thrombin
 - In the presence of prothrombin or other activators, they can initiate **bona fide “true” clot formation**
 - Collectively, these precursor complexes are termed **ThT-positive fibrinaloid microclot complexes (FMCs)**

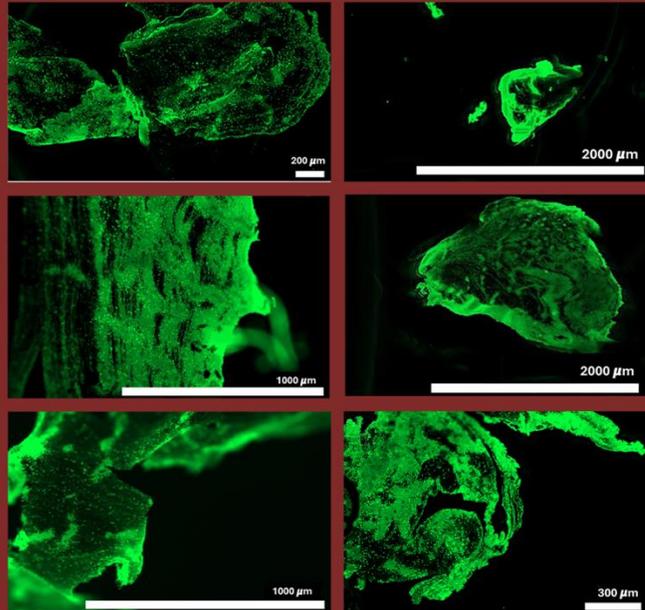
Microclots seeding on RBCs: thrombotic stroke



Thrombo-embolic-ischemic stroke

Thrombotic stroke: *Stroke*

Amyloid Presence in Acute Ischemic Stroke Thrombi: Observational Evidence for Fibrinolytic Resistance



Background

Thrombolytic therapy fails in approximately one-third of acute ischemic stroke (AIS) cases.

Fibrin can adopt an amyloid-like configuration, termed *fibrinaloid*, which is resistant to fibrinolysis.

It was unknown whether large AIS thrombi also exhibit this amyloid feature.

Methods

Thrombi were retrieved from AIS patients undergoing mechanical thrombectomy.

Samples were wax-embedded, sectioned, de-waxed, and stained with *thioflavin T (ThT)*, a standard dye that fluoresces upon binding amyloid structures.

Fluorescence microscopy was used to visualize amyloid presence.

Results / Conclusions

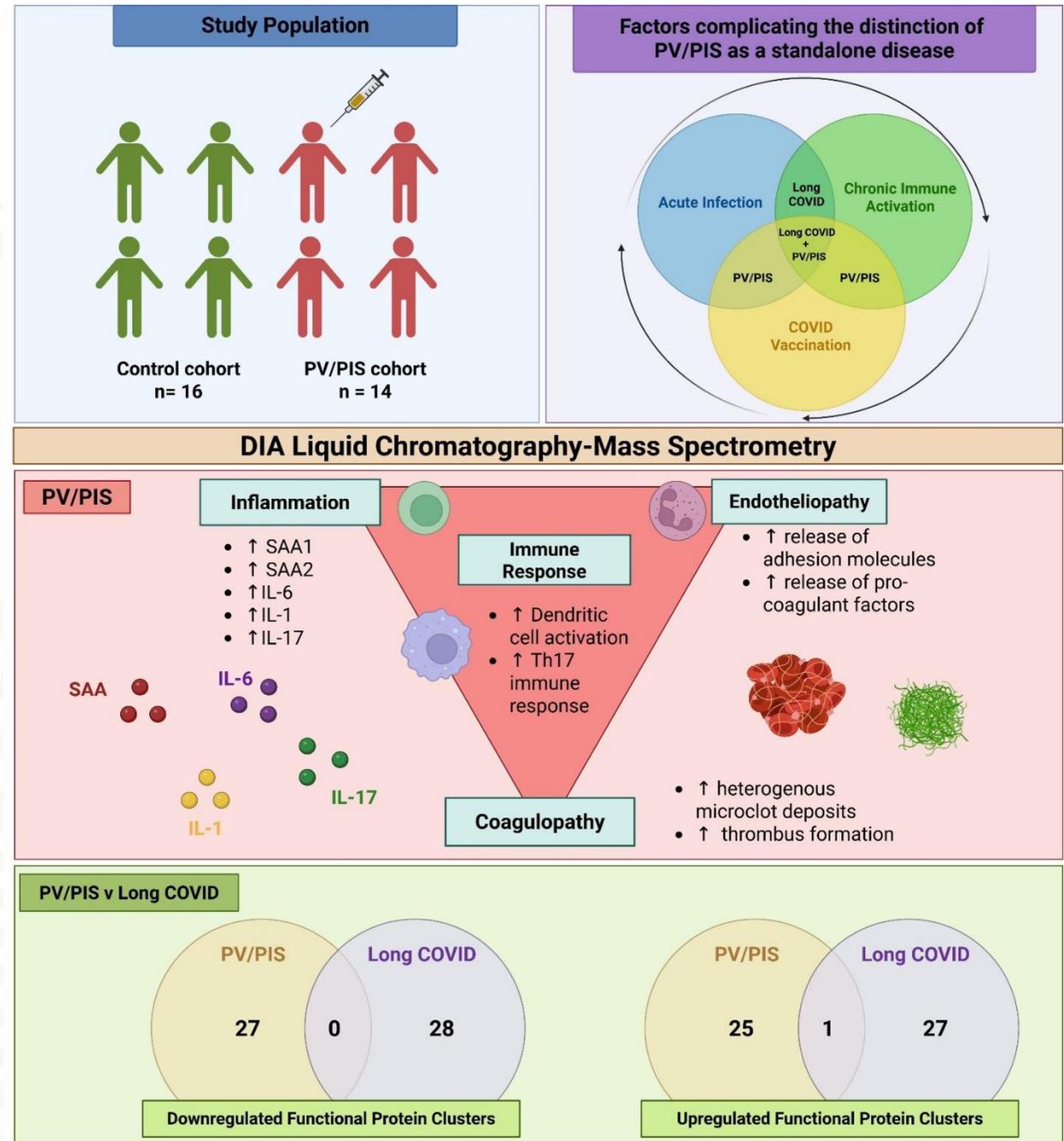
All analyzed AIS thrombi exhibited *robust ThT fluorescence*, indicating high amyloid content.

These amyloid-rich structures may explain resistance to fibrinolytics. Findings support further research into *amyloid-targeted stroke therapies*.

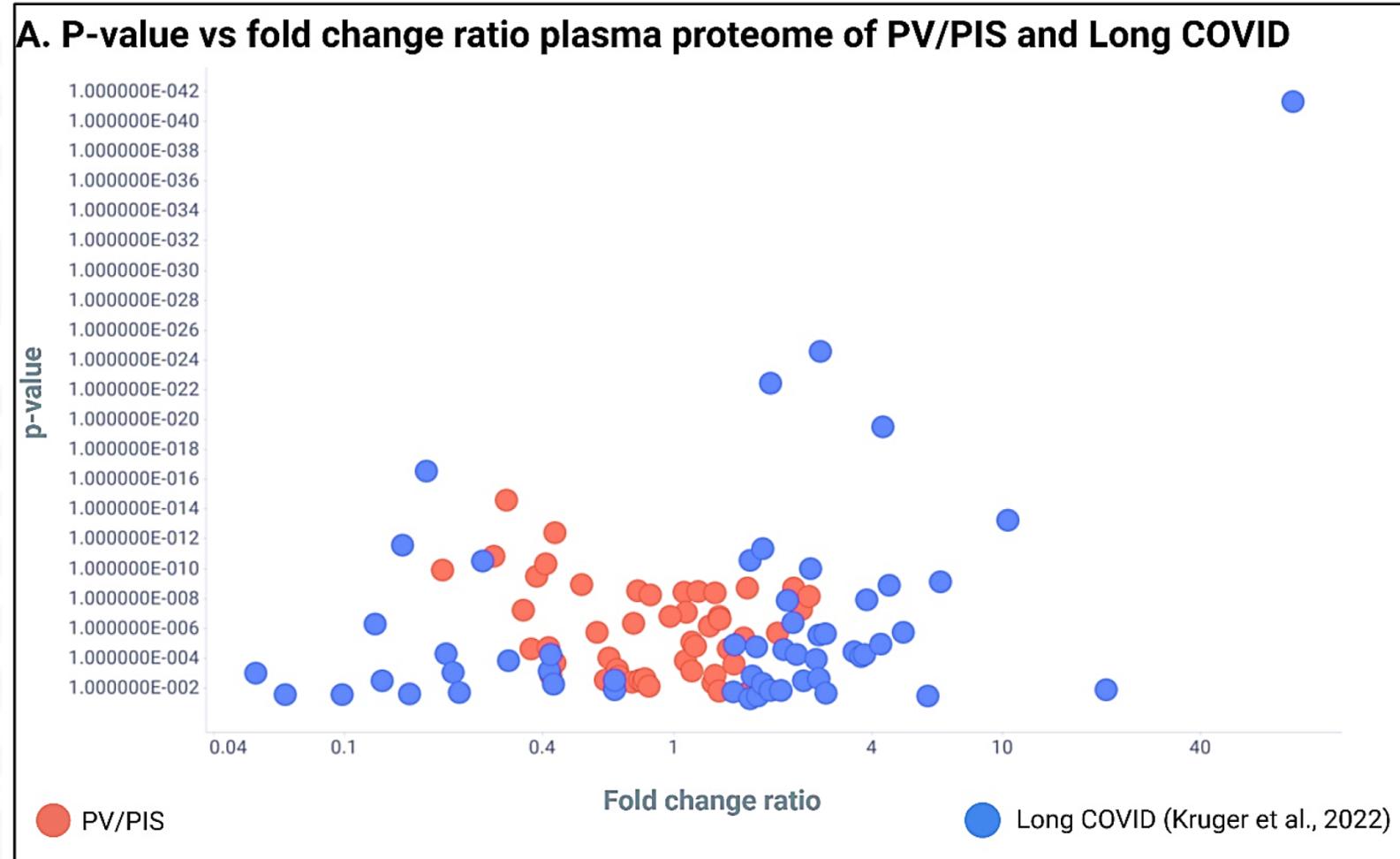
Latest research: preprint

Proteomic signatures of Post-Vaccination/Post-Infection Syndrome (PV/PIS): Insights into immune dysregulation and coagulopathy

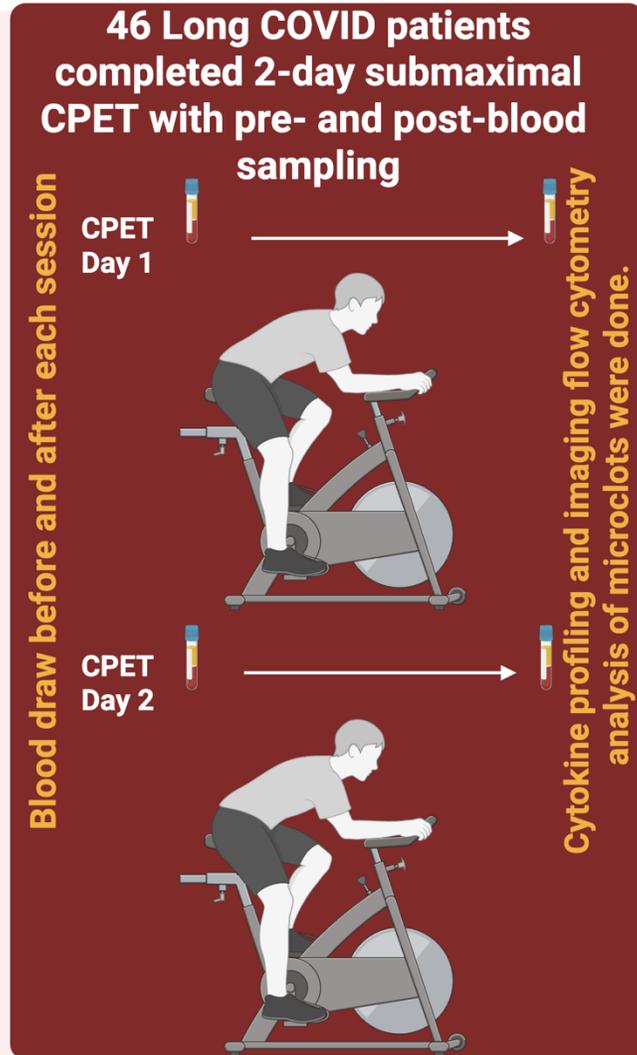
Maxine Waters¹, Mare Vlok², Elouise E. Kroon³, Maritha J. Kotze⁴, Kelebogile E. Moremi^{4,5}, Sunday O. Oladejo⁶, Kanshukan Rajartnam⁶, Jean M. Nunes¹, Chantelle Venter¹, Chantelle J. Scott^{4,6}, Douglas B. Kell^{1,7,8} and Etheresia Pretorius^{1,7*}



Proteomic signatures



Exercise-induced changes in microclotting and cytokine Levels



Key findings

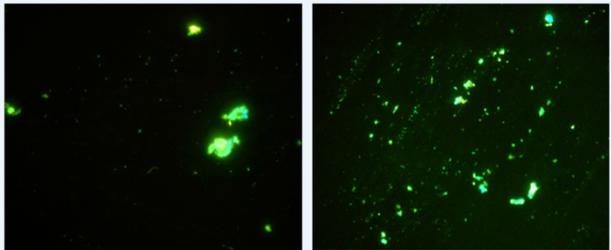
CPET induced the fragmentation of large microclots (100–3000 μm^2) and caused an increase in small microclots (<30 μm^2) after repeated exertion. Fragmentation correlated with increases in cytokines associated with pro- and anti-inflammation, as well as vascular injury.

Cytokines

IL-6 ↑
EGF ↑

IP-10 ↓
RANTES ↓

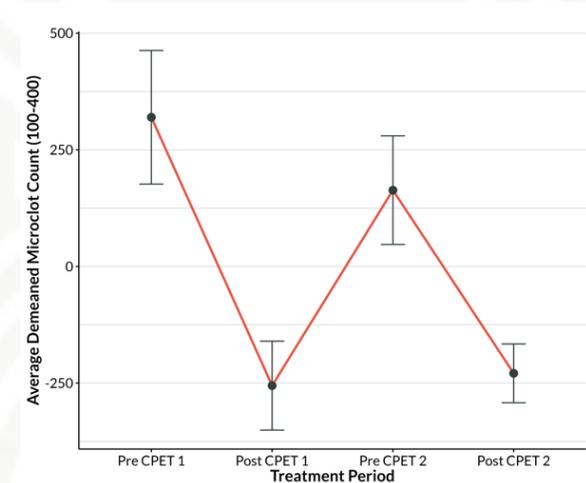
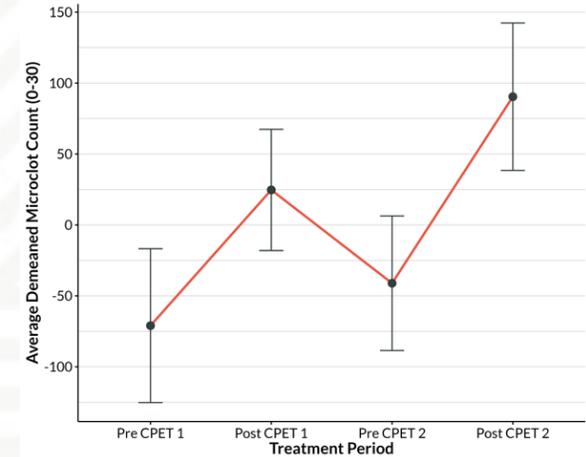
Microclot size shifts due to exercise



Fragmentation correlated with increases in cytokines associated with pro- and anti-inflammation, as well as vascular injury.

Conclusion

Fragmentation of persistent microclots after exercise may drive inflammation and impair oxygen utilization in Long COVID. These findings **caution against unmonitored rehabilitation** and underscore the need for targeted vascular repair strategies.



Exercise-induced changes in microclotting and cytokine levels

Our *paper challenges the rationale for graded exercise therapy* in Long COVID
Underscores the need for exercise to be carefully considered

MOST IMPORTANTLY: the *effects of exercise should be objectively monitored* and
patient managed carefully

Where microclots are located and how they induce pathology in long-covid



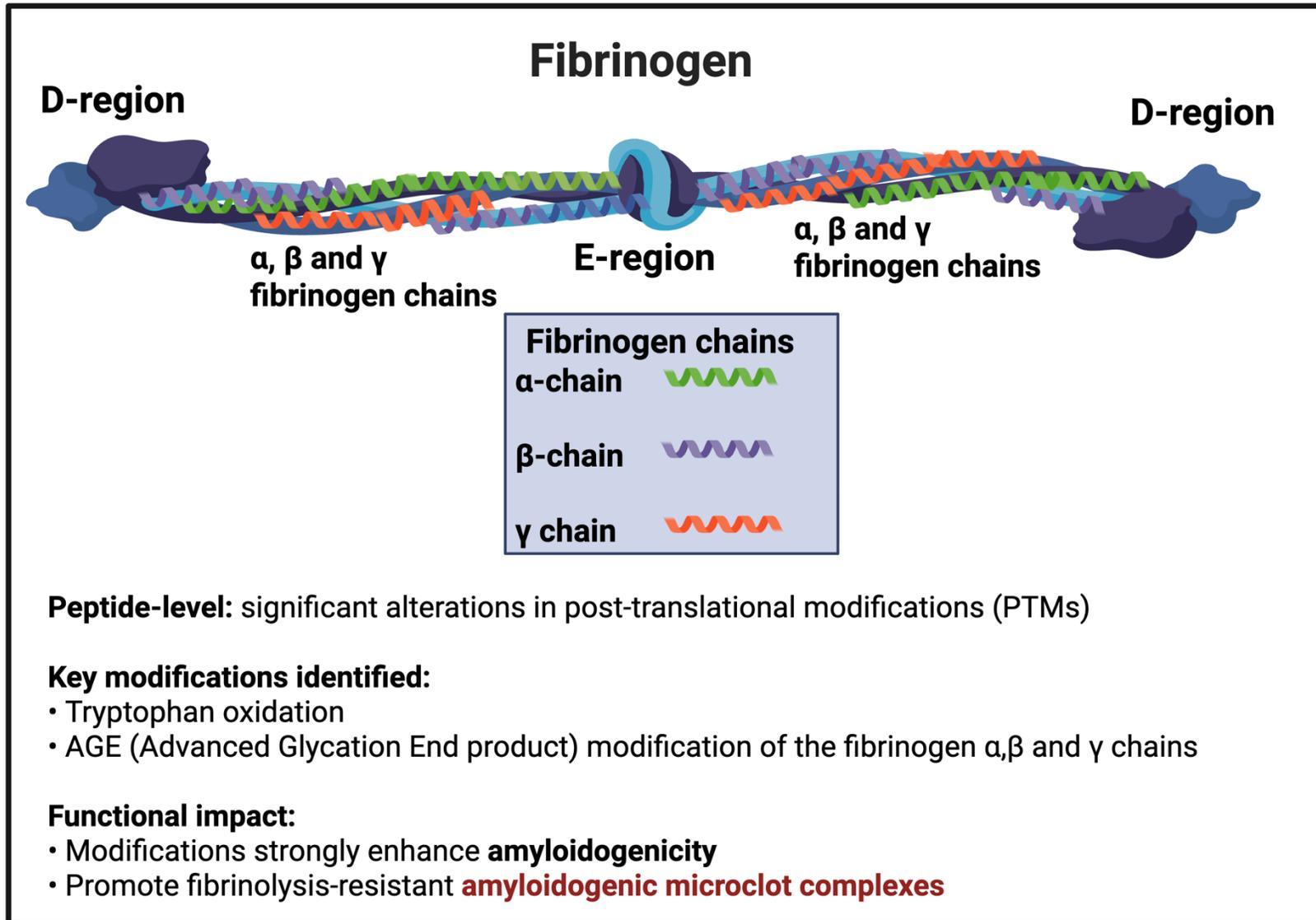
Jeroen den
Dunnen

Resia Pretorius

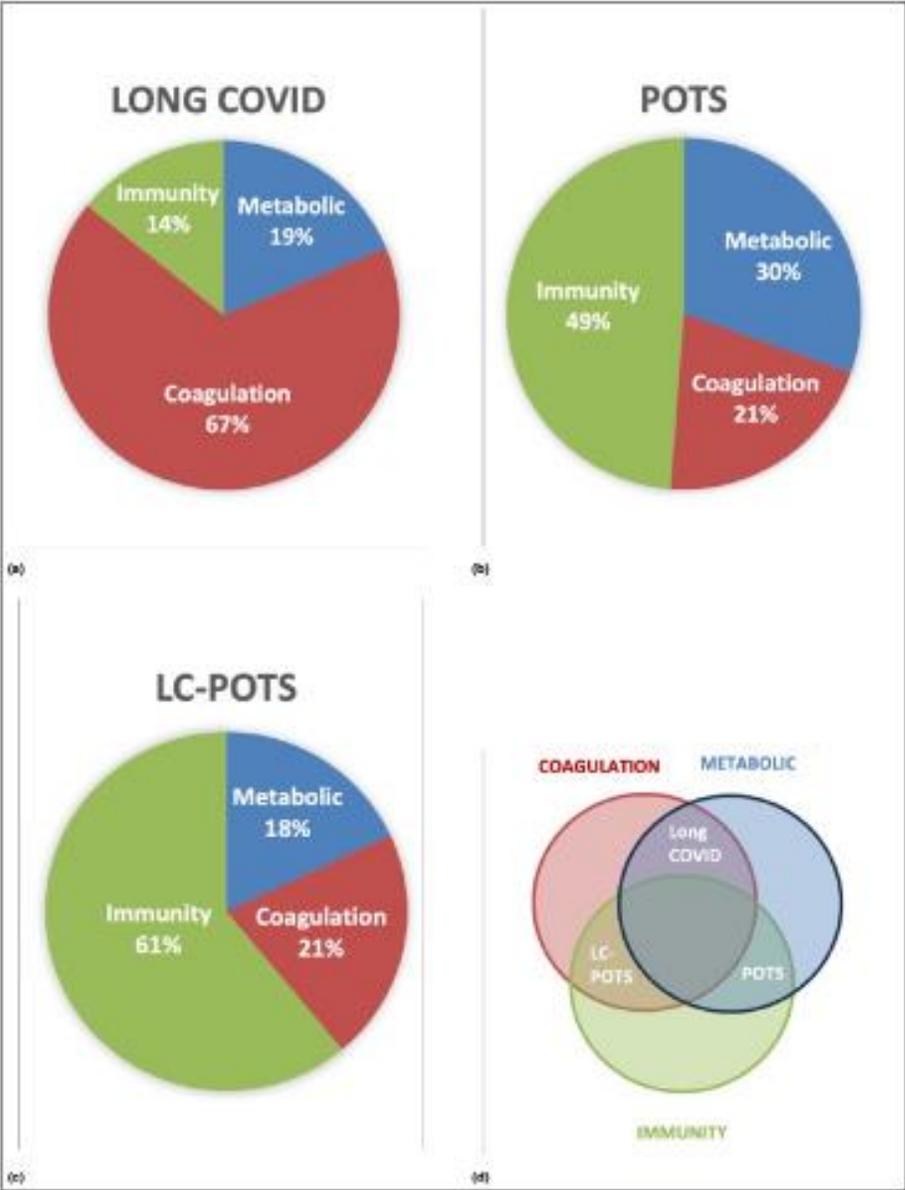
Denise Visser

Brent Appelman

Our latest findings: fibrinogen changes



Our latest findings: fibrinogen changes



Sonar images from the carotid artery (Fritz van Schalkwyk and team)

Fritz van Schalkwyk

- MHSCT Cardiology and vascular studies

Dr Johan Jordaan

- PhD in Cardiothoracic surgery
- University of the Free State

Dr Lezelle Botes

- PhD in Cardiothoracic surgery
- Central University of the Free State

Prof Muriel Meiring

- PhD in Haematology
- University of the Free State



Nano-tweezer experiments

With Physicists at Stellenbosch
University:

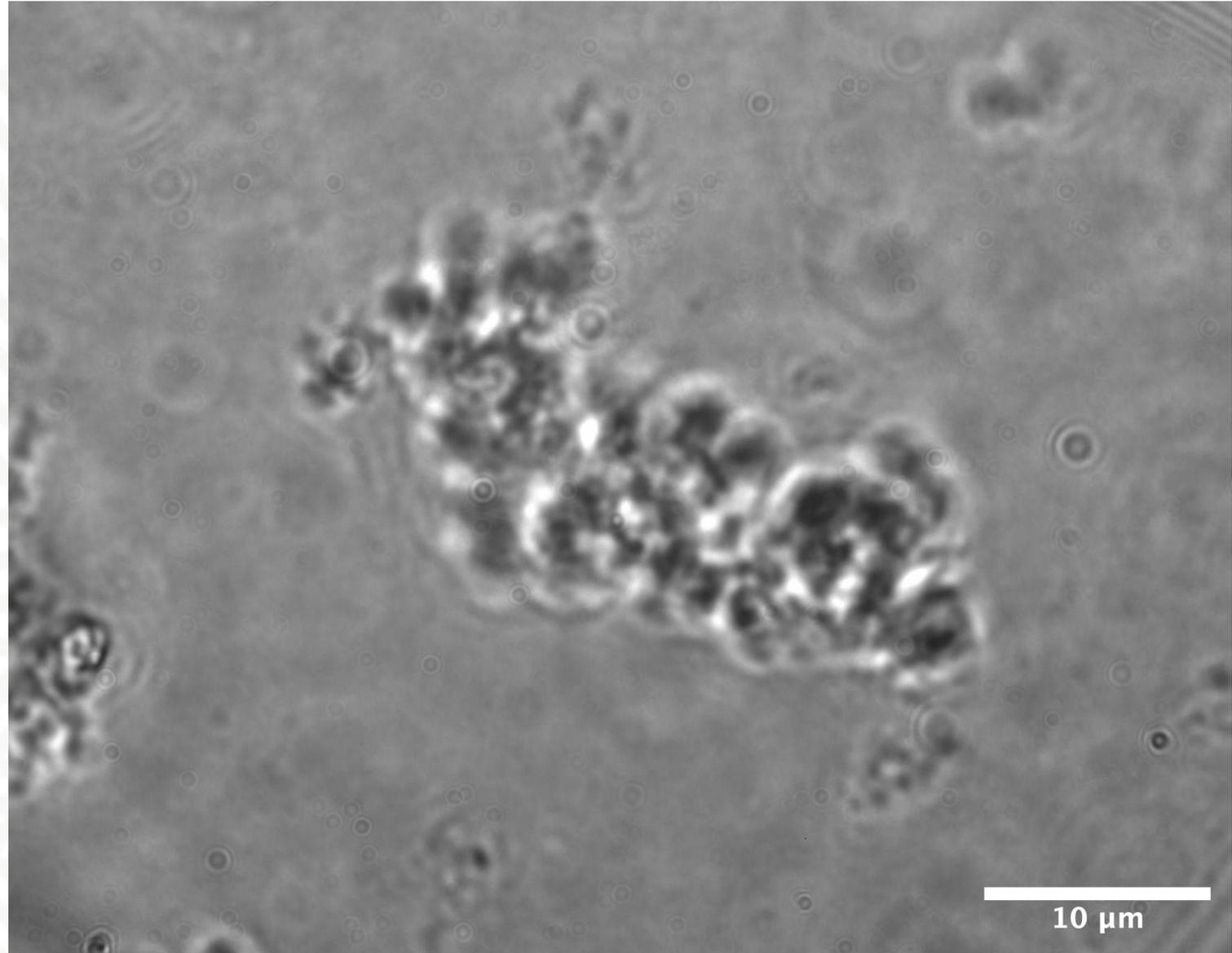
Pieter Neethling lab

and

Le Roi Du Plessis

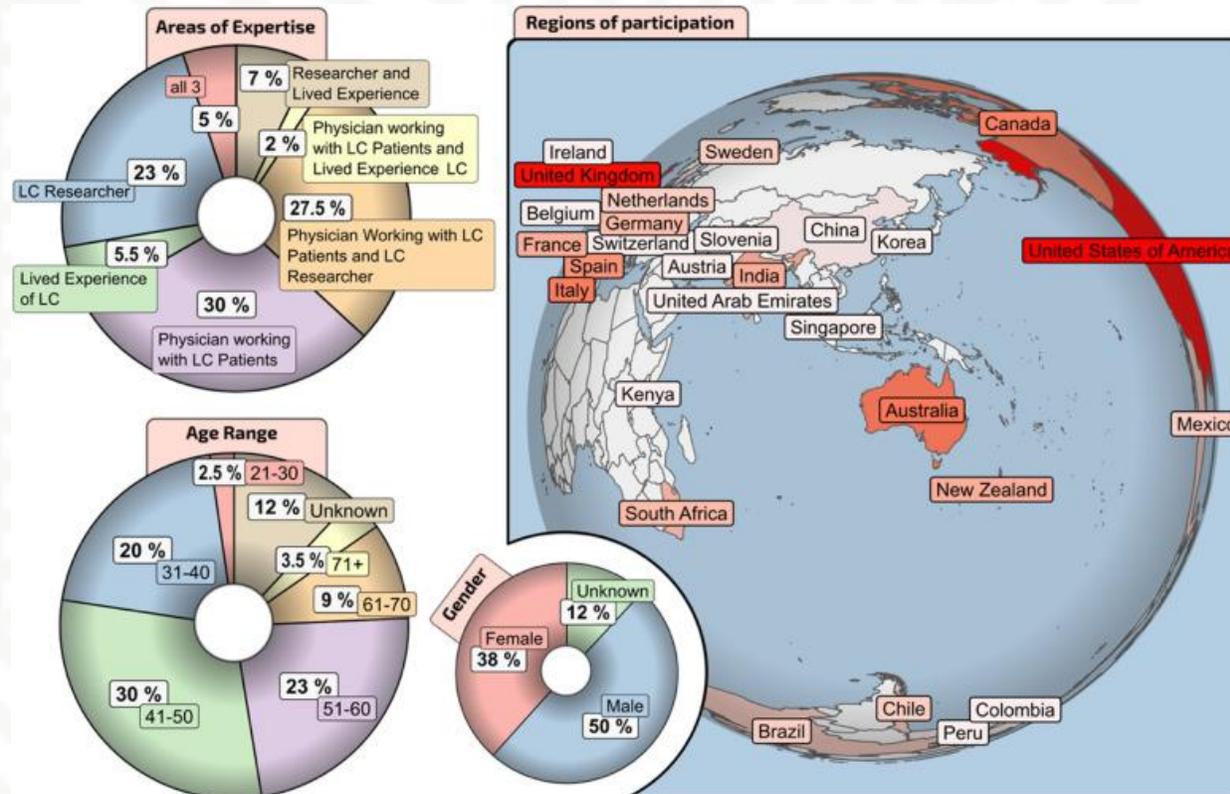
Willie Perold (SU Engineering)

Jason Vermaak (SU Engineering):
pending HREC clearance



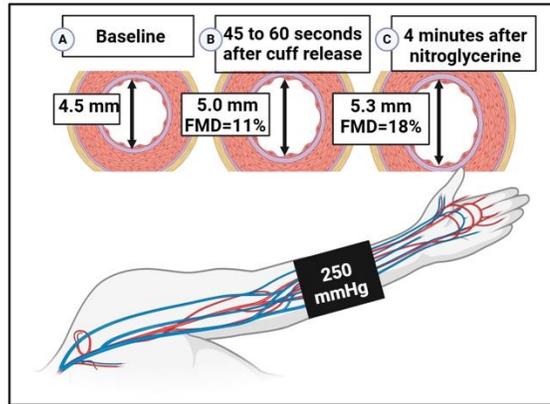
A Delphi written by the World Health Network (WHN)

EWING, A. G., JOFFE, D., BLITSHTEYN, S., BROOKS, A. E. S., WIST, J., BAR-YAM, Y., BILODEAU, S., CURTIN, J., DUNCAN, R., FAGHY, M., GALLAND, L., PRETORIUS, E., SALAMON, S., BUONSENSO, D., HASTIE, C., KANE, B., KHAN, M. A., LAL, A., LAU, D., MACINTYRE, R., MCFARLAND, S., MUNBLIT, D., NICHOLSON, J., OLLILA, H. M., PUTRINO, D., ROSARIO, A. & TAN, T. 2025. Long COVID clinical evaluation, research and impact on society: a global expert consensus. *2025 Ann Clin Microbiol Antimicrob*, 24, 27.

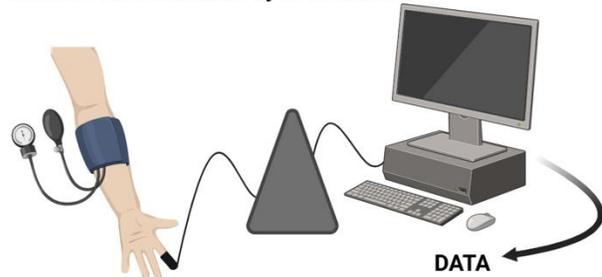


Hypercoagulation (microclot formation/platelet hyperactivation) and endothelial damage

- 1) **Flow Mediated Dilatation (FMD):**
Useful to confirm endothelial dysfunction.

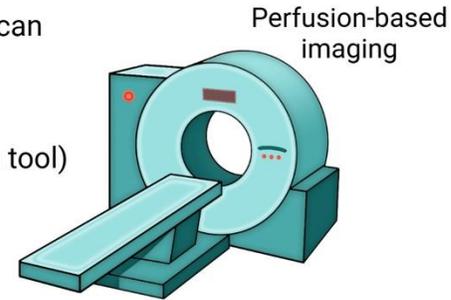


- 2) **Peripheral arterial tonometry and finger thermal monitoring:**
For example: The EndoPAT® device and E4-diagnose device (Polymath Company.)
Useful to confirm endothelial dysfunction.

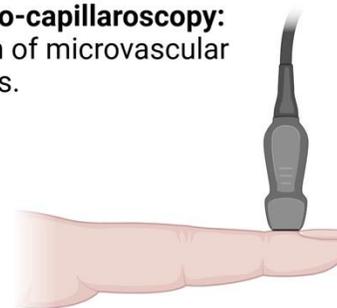


- 3) **Radiology:**
Useful to detect **vasculopathic** and **thrombotic** phenomena.

- SPECT / VQ scan
- DECT
- MRI (research tool)



- 4) **Nailfold video-capillaroscopy:**
Identification of microvascular abnormalities.



Most popular article award

Eberhard F. Mammen 2025 Awards—Most Popular Articles

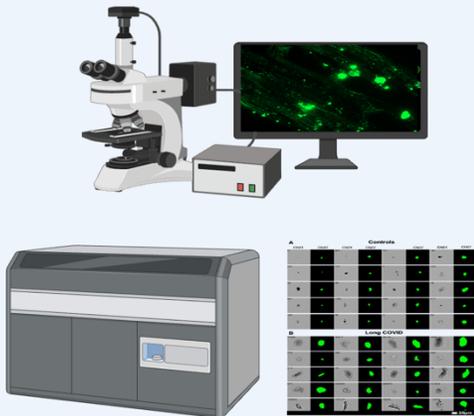


From left to right, top to bottom: Arneaux Kruger, David Joffe, Graham Lloyd-Jones, Muhammed Asad Khan, Špela Šalamon, Gert J. Laubscher, David Putrino, Douglas B. Kell, Etheresia Pretorius.

Nailfold (video) capillaroscopy

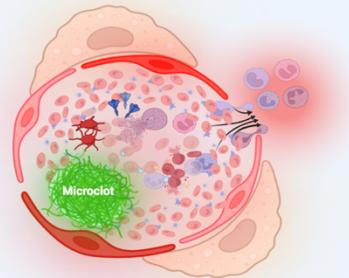
On the utility of nailfold (video)capillaroscopy in detecting the effects of fibrinoid microclots in diseases involving blood stasis

Structural assays for fibrinoid microclot complexes:
fluorescence microscopy and
imaging flow cytometry

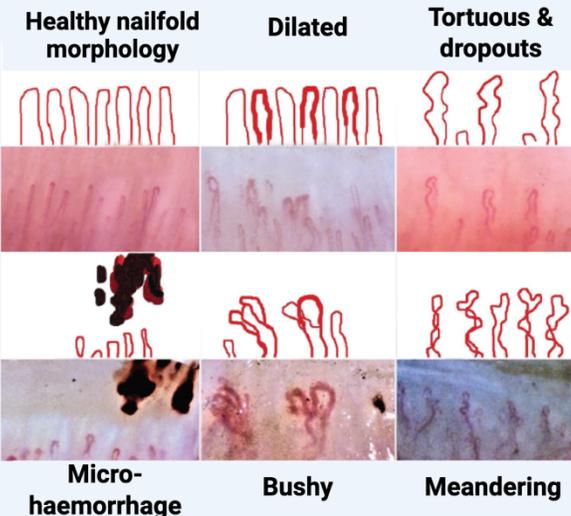


From fibrinoid microclot complexes to persistent symptoms

Hypoxia
Oxidative stress
Vascular damage
Blood stasis

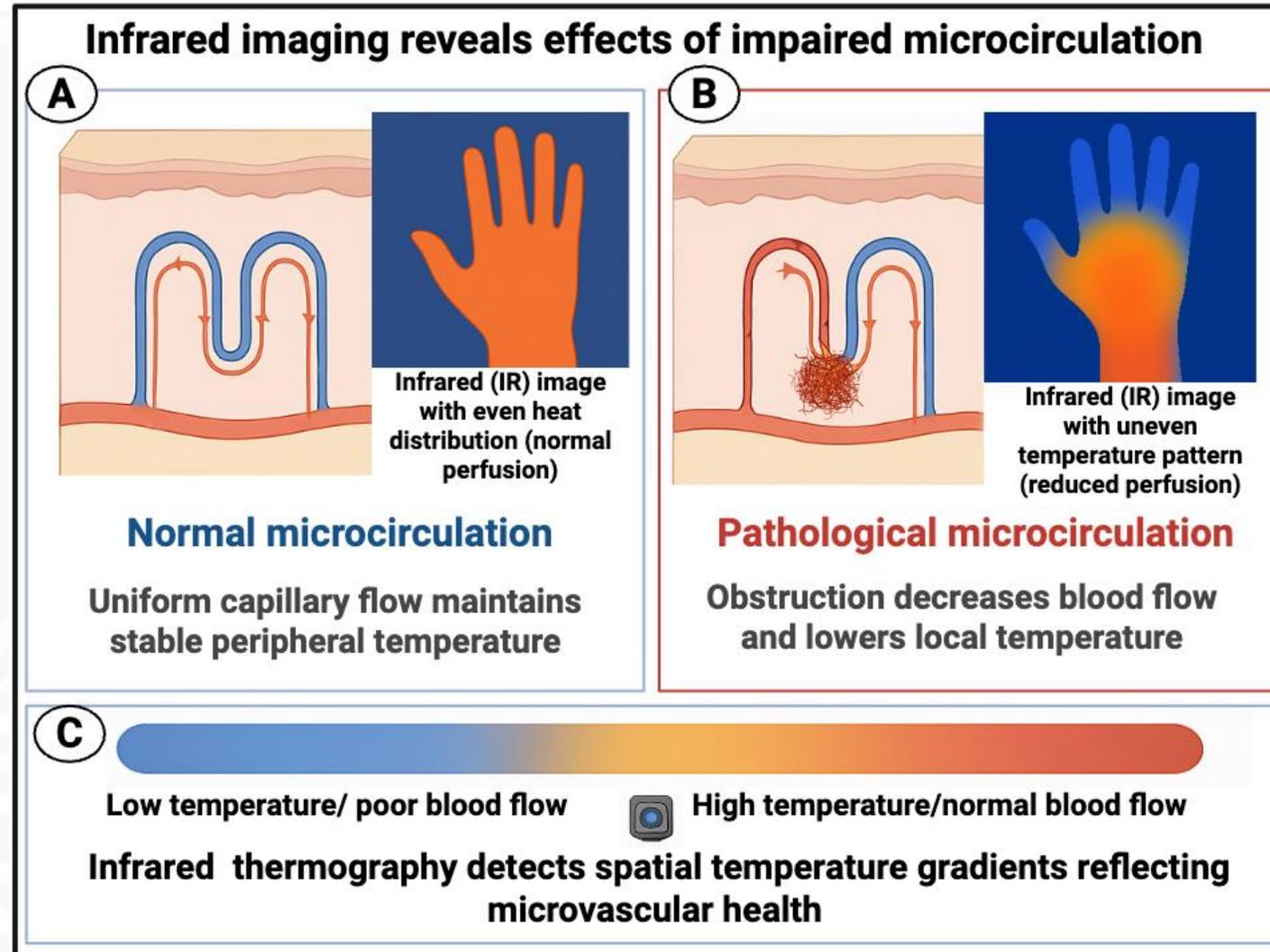
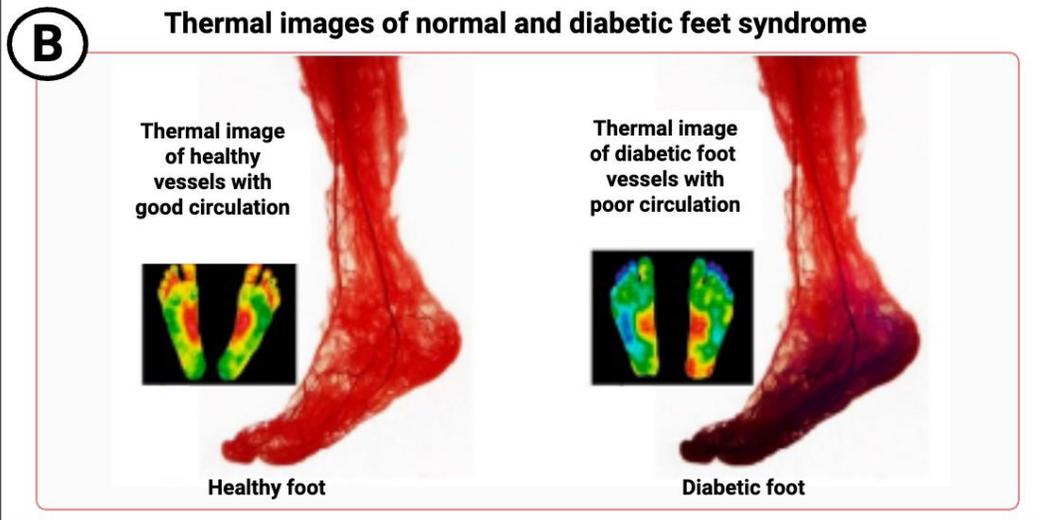
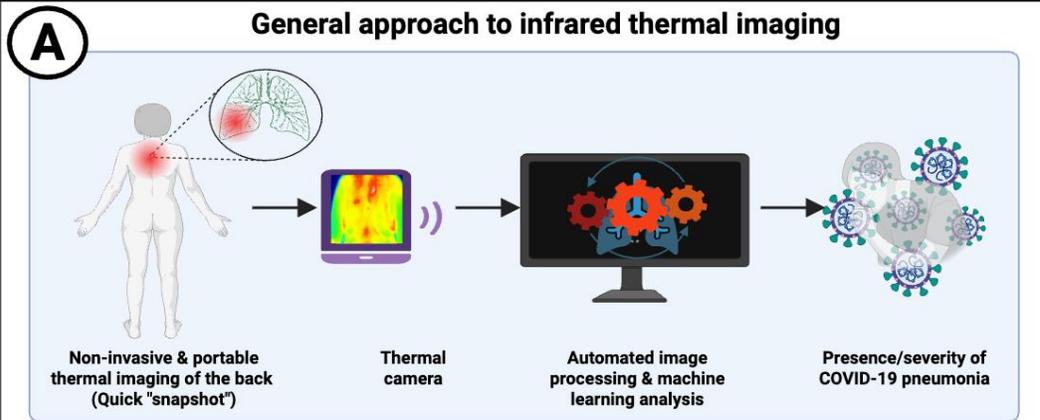


Functional assay:
nailfold (video)capillaroscopy

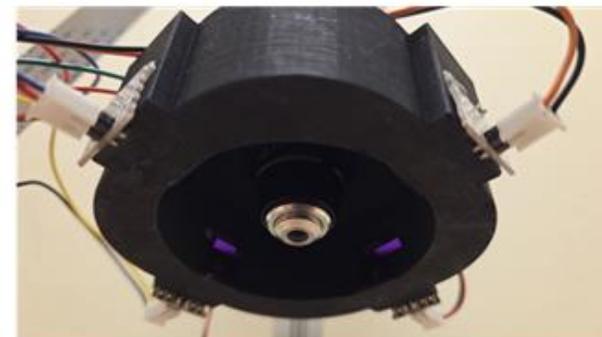
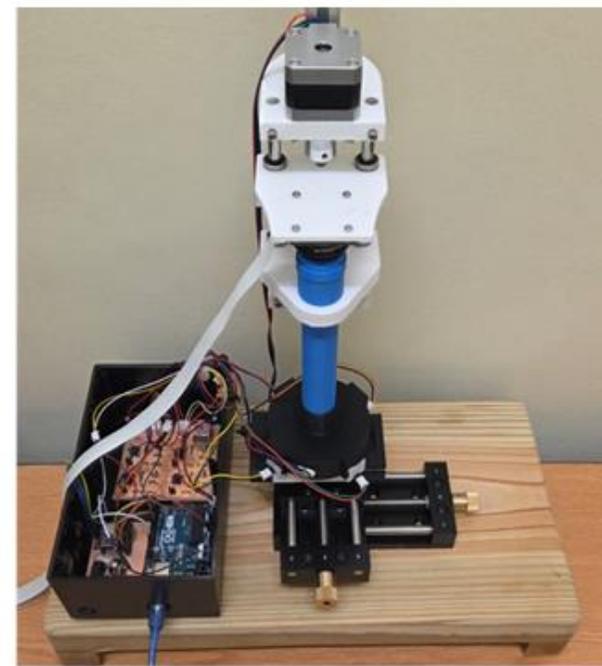
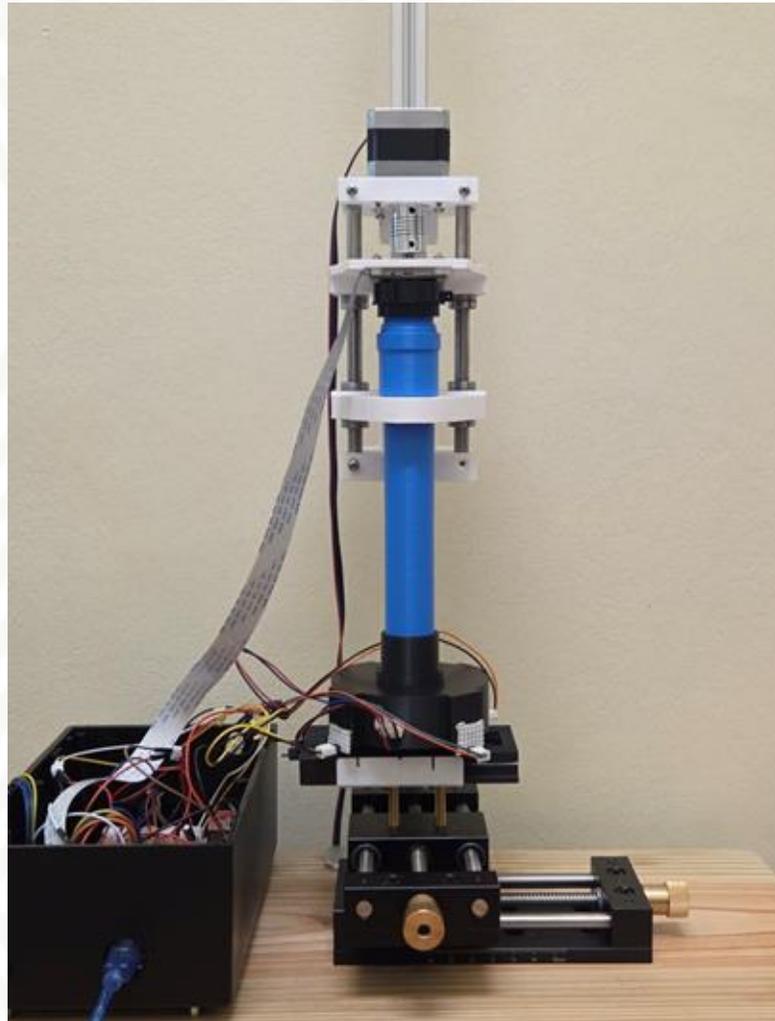


Fibrinoid microclots impede microcirculation. Combining microclot imaging with nailfold (video) capillaroscopy provides complementary structural and functional insights across multiple inflammatory and vascular diseases

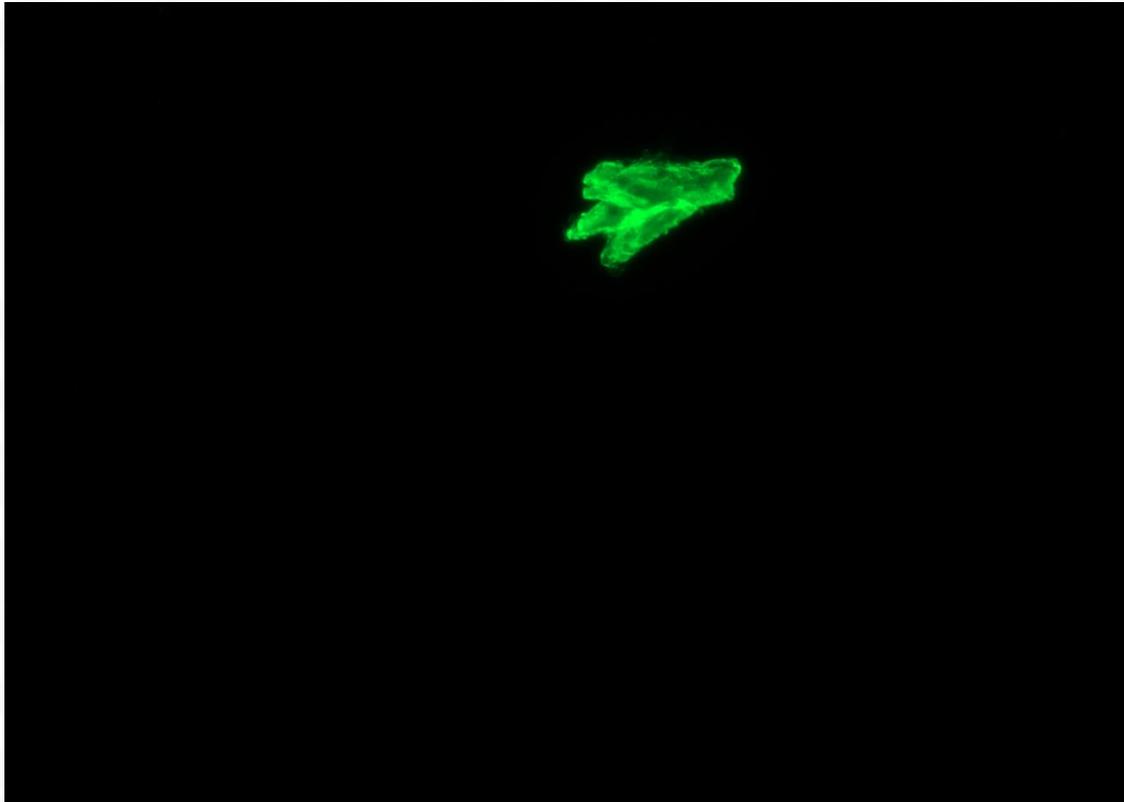
Infrared imaging



Development of a Point-of-Care Microclot Detection and Characterization Device (MDCD)



MDCD validation: MDCD vs Zeiss microclot Image comparison ThT staining

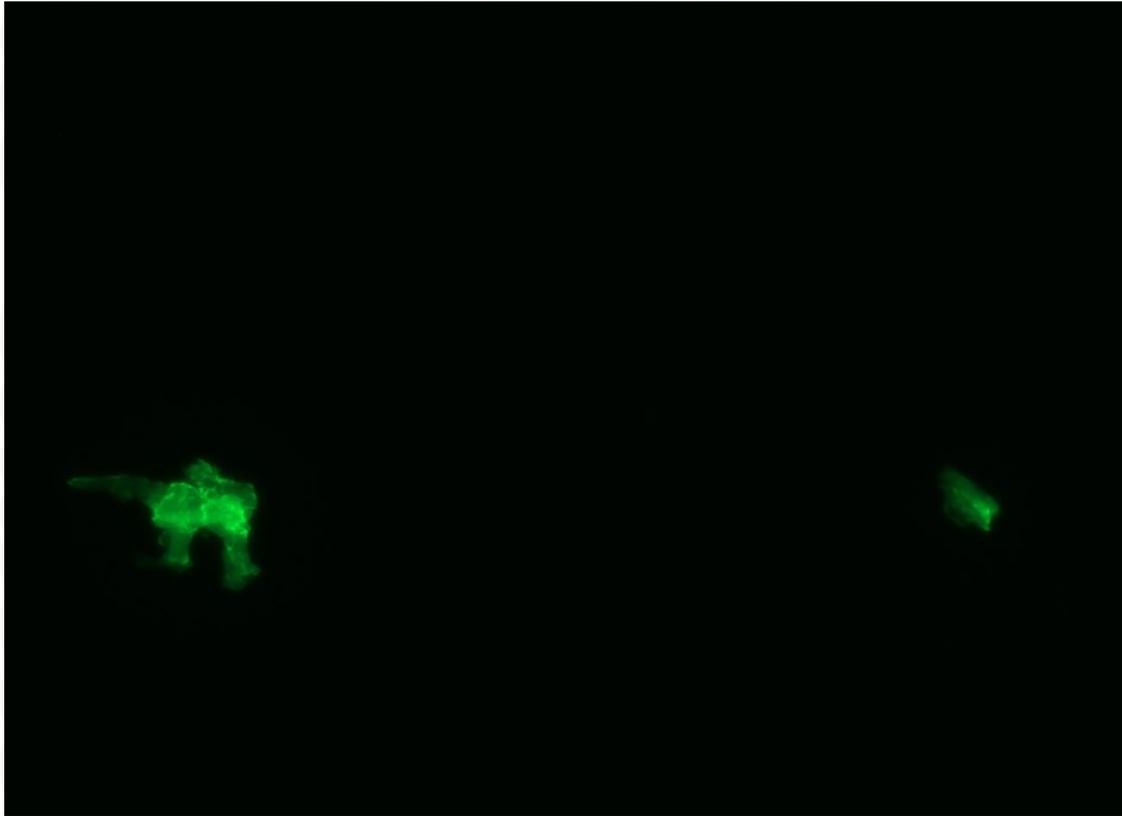


Zeiss



MDCD

PAC1 FITC staining MDCD validation



Zeiss



MDCD

Microclots, their content and diagnosis for hypercoagulation: a measurable biomarker in clinical trials

Microclot Testing a new diagnostic frontier

Our patented diagnostic technology detects abnormal microclots in the blood, indicating poor vascular health, inflammation, and an increased risk for thrombotic endothelialitis. This pioneering test has been used to detect and aid in the treatment of microclots in thousands with Long Covid, a debilitating condition impacting millions worldwide and deeply affecting global health, society, and the economy.

[License Microclot Technology](#)



iNUS.laboratories



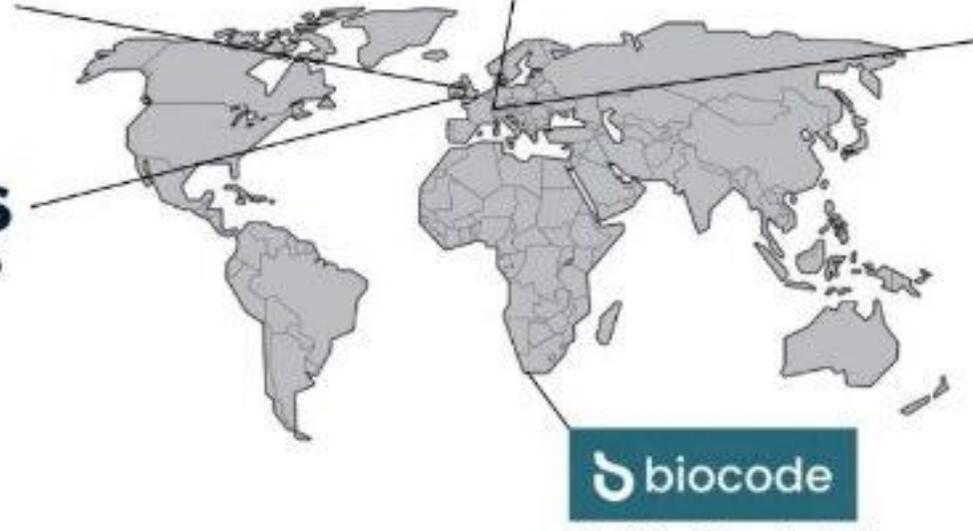
Simon Abrams, PhD

Yannick Kok, PhD



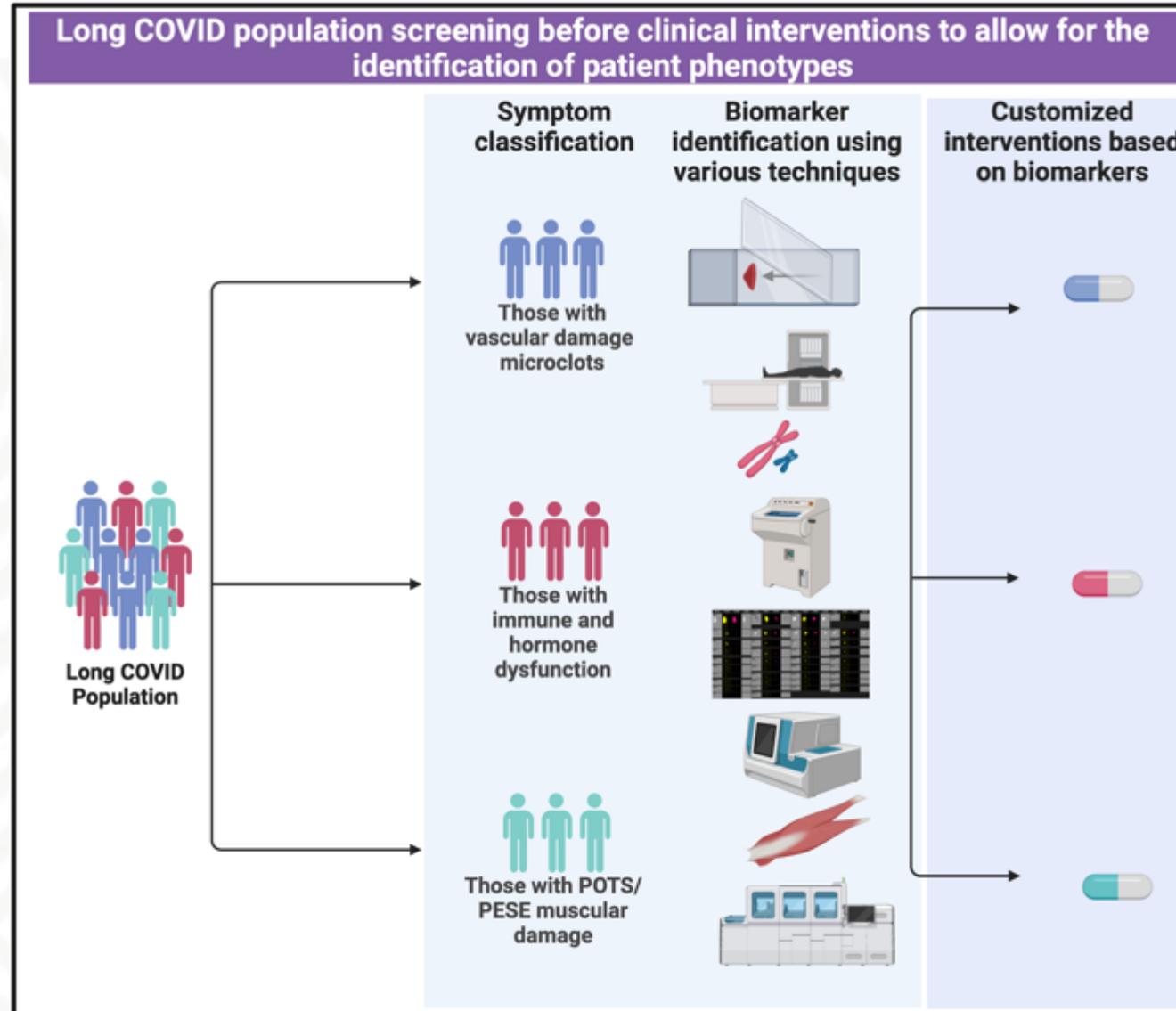
Felix Scholkmann, PhD

SANTERSUS
Andrew Aswani, MD PhD



 biocode

What is needed in Long COVID and post-viral research?







**Thank you
Enkosi
Dankie**



Stellenbosch

UNIVERSITY
IYUNIVESITHI
UNIVERSITEIT

forward together
sonke siya phambili
saam vorentoe