FLCCC ALLIANCE EDUCATIONAL CONFERENCE

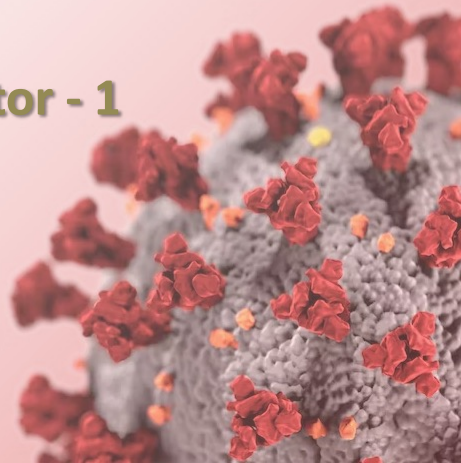
EMERGING APPROACHES TO TREATING SPIKE PROTEIN-INDUCED DISEASES

April 28-29, 2023 • Fort Worth, Texas

Spike Protein and Amyloid Fibrin Microclots

Pathology of the S1 Subunit of the Spike Protein; Microclots; and Local Tissue Hypoxia

Hypofibrinolysis and Plasminogen Activator Inhibitor - 1



Presented By:

Jordan F. Vaughn MD

Figured it out in Spring of 2020

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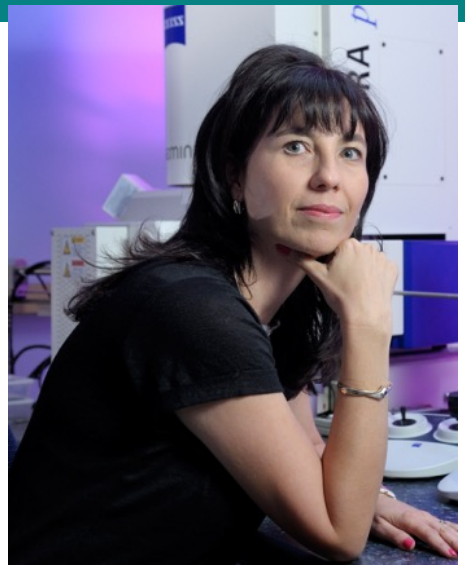
Standing of the Shoulder of and Special Thanks to:



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Standing of the Shoulder of and Special Thanks to

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Overview

- **S1 Subunit of the Spike Protein and Amyloid Fibrin**
- Hypofibrinolysis and Plasminogen Activator Inhibitor – 1 (PAI-1)
- Persistent Spike Symptoms: Long COVID/Vaccine Injury
- Consequences in blocking capillaries
- What can we do about it?

S1 SPIKE PROTEIN ALONE can catalyze fibrinaloid formation

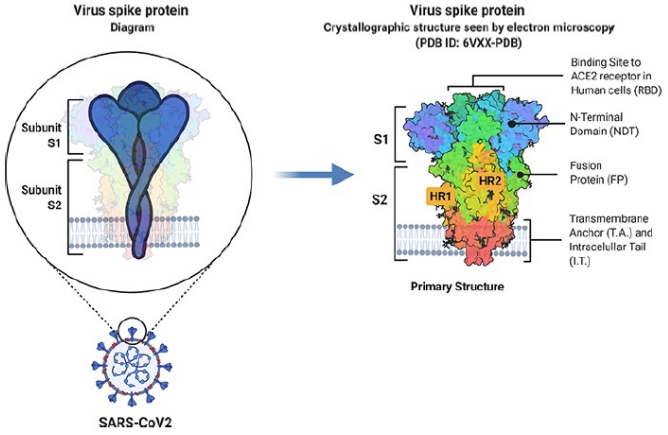
Bioscience Reports (2021) **41** BSR20210611
https://doi.org/10.1042/BSR20210611

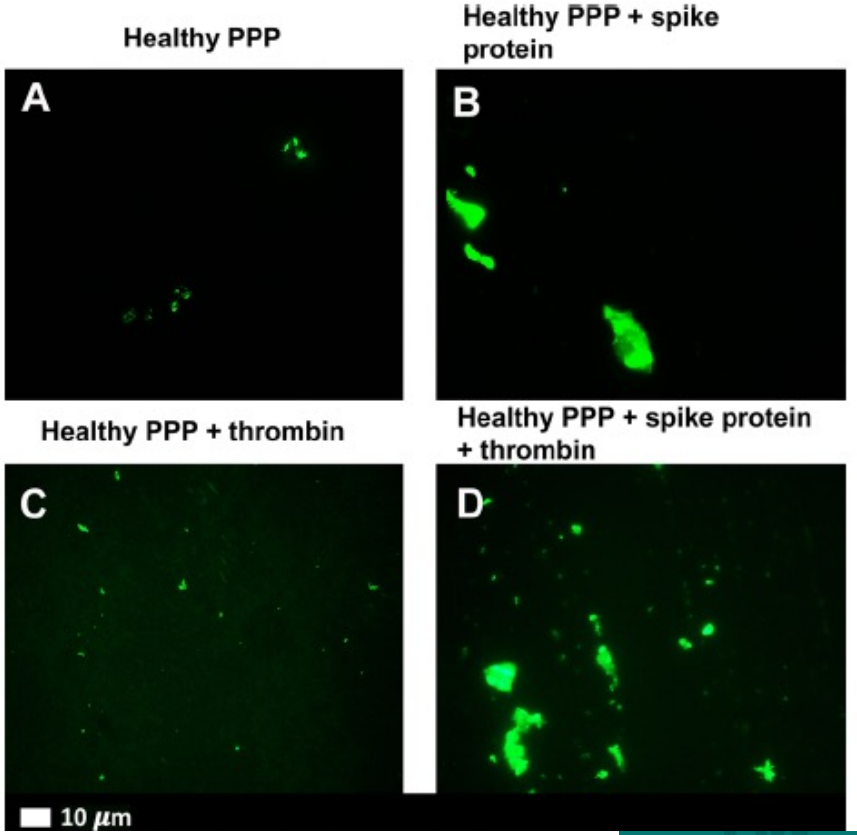
PORTLAND PRESS

Research Article

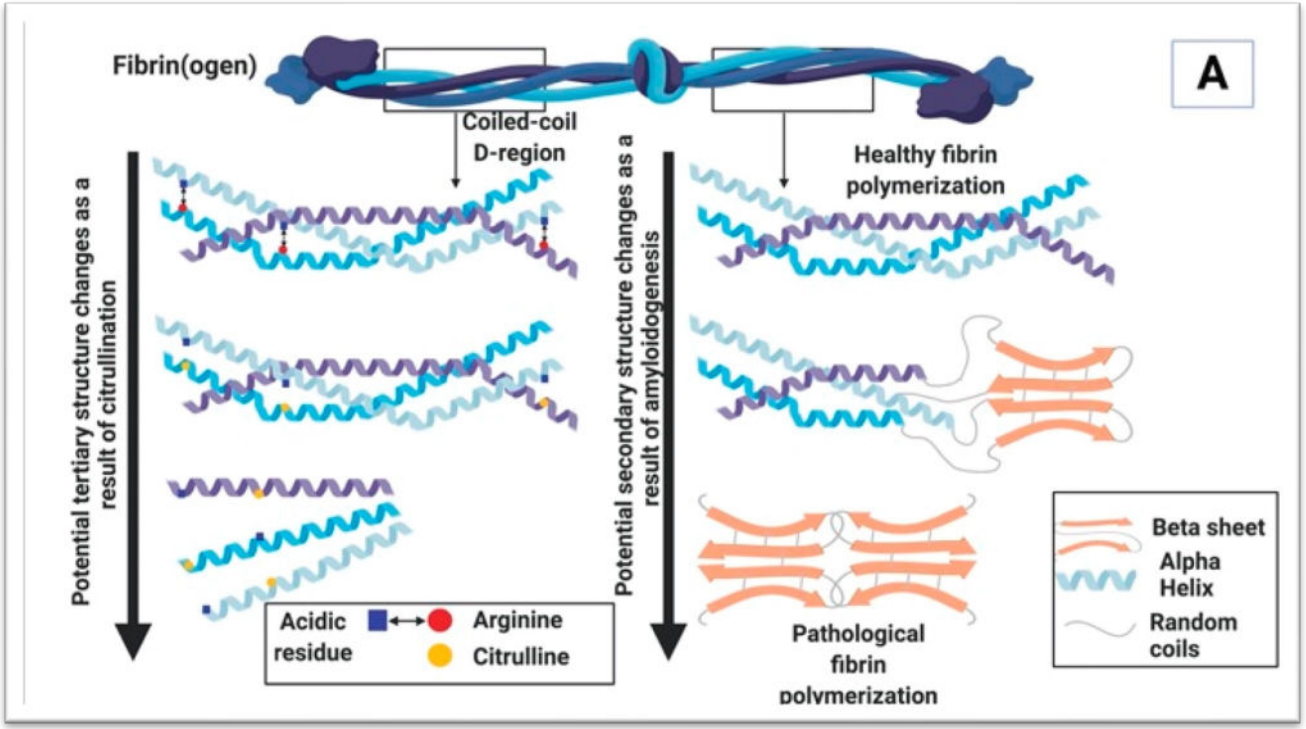
SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19

Lize M. Grobbelaar[1], Chantelle Venter[1], Mare Vlok[2], Malebogo Ngoepe[3,4], Gert Jacobus Laubscher[5], Petrus Johannes Lourens[5], Janami Steenkamp[1,6], Douglas B. Kell[1,7,8] and Etheresia Pretorius[1]





Normal Fibrin vs Amyloid Fibrin (Amyloidogenesis)



Randeria, S.N.; Thomson, G.J.A.; Nell, T.A.; Roberts, T.; Pretorius, E. Inflammatory cytokines in type 2 diabetes mellitus as facilitators of hypercoagulation and abnormal clot formation. Cardiovasc. Diabetol. **2019**, 18, 72.

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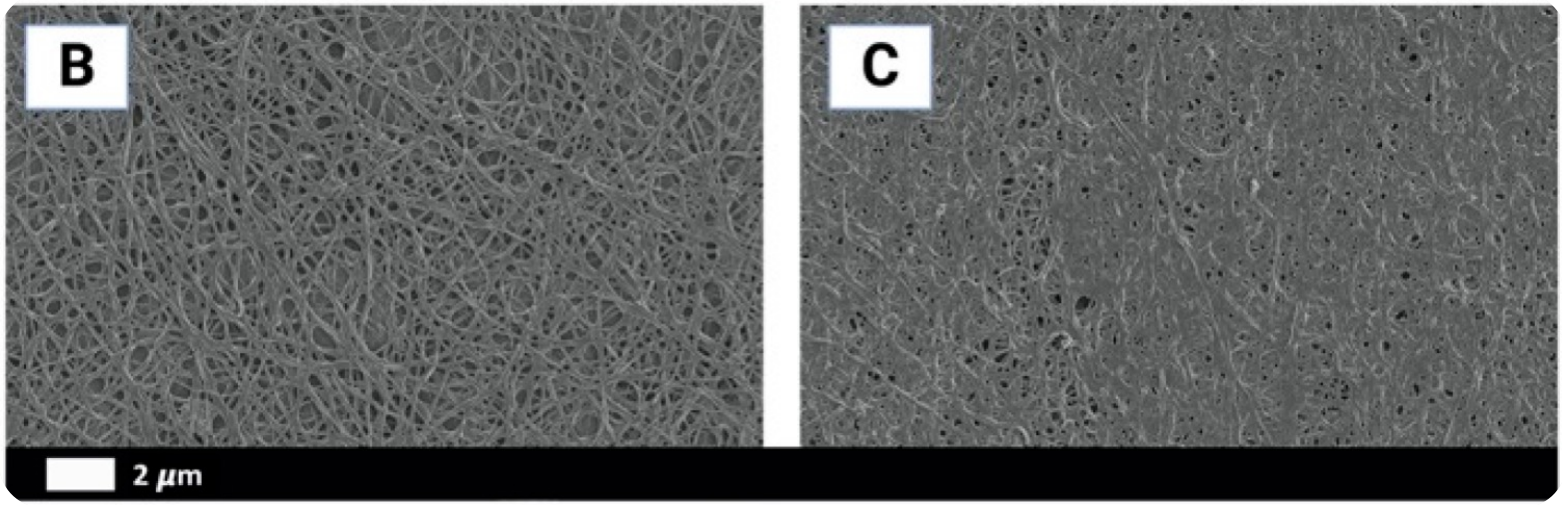
Amyloid Fibrin Structure

Normal Fibrin Structure

Amyloid Fibrin Structure



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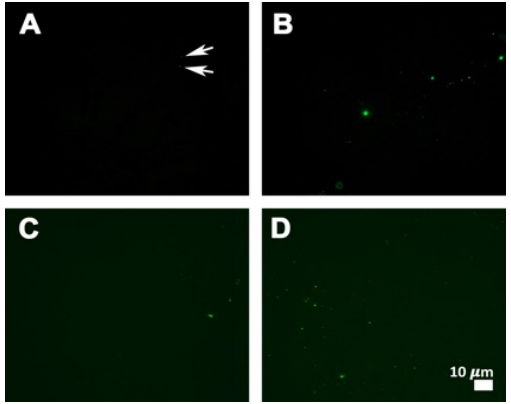
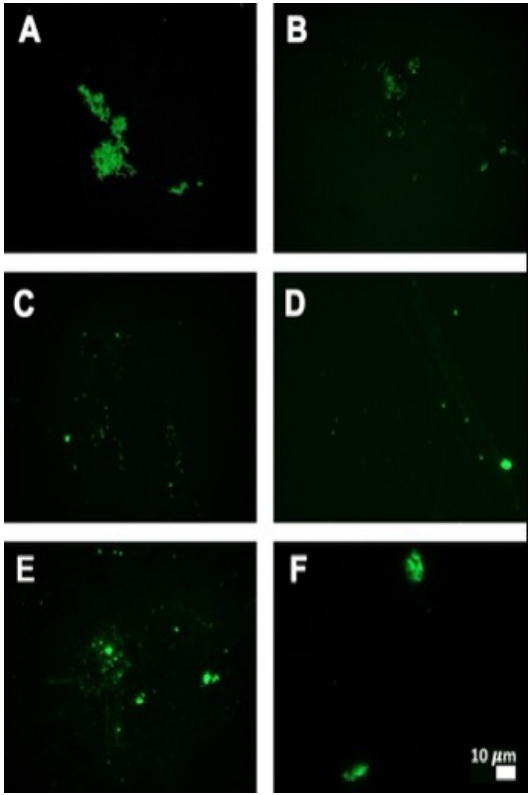
Structural Changes in Fibrin(ogen) in Disease

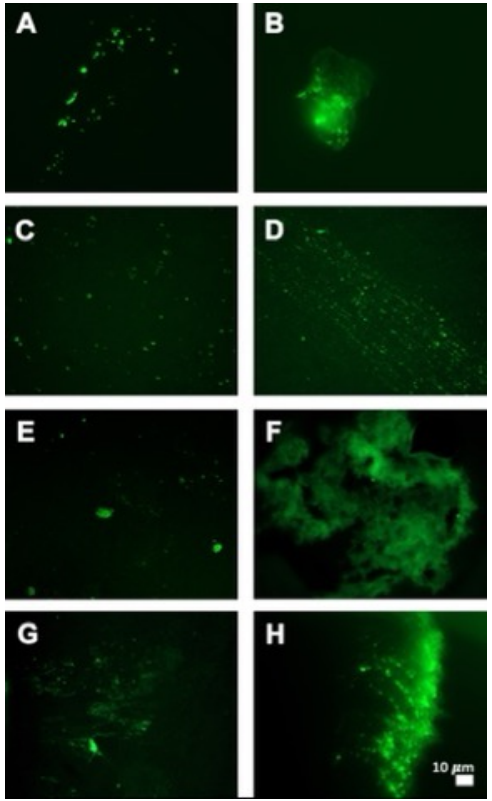
Healthy Plasma



Type 2 DM Plasma



COVID-19 Plasma



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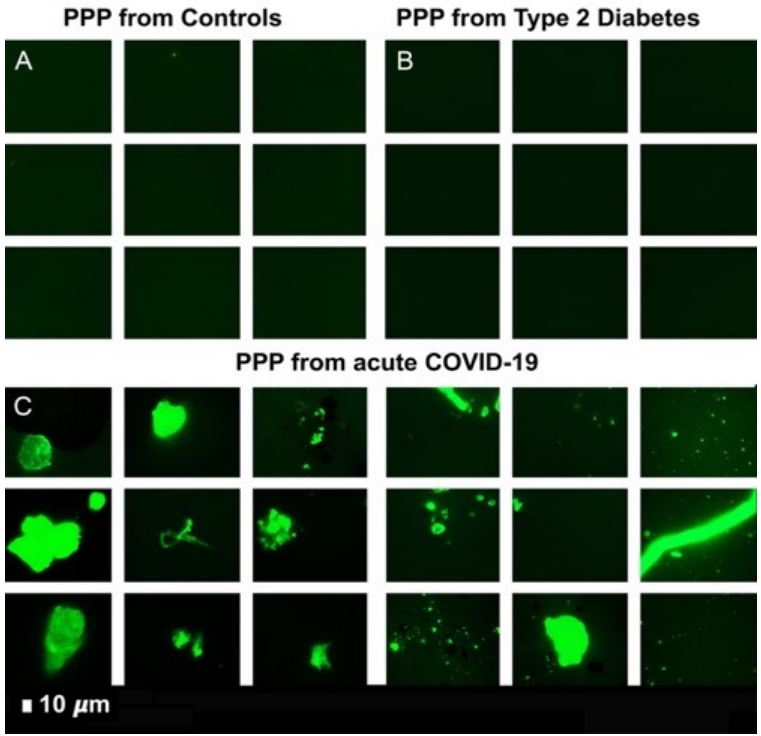
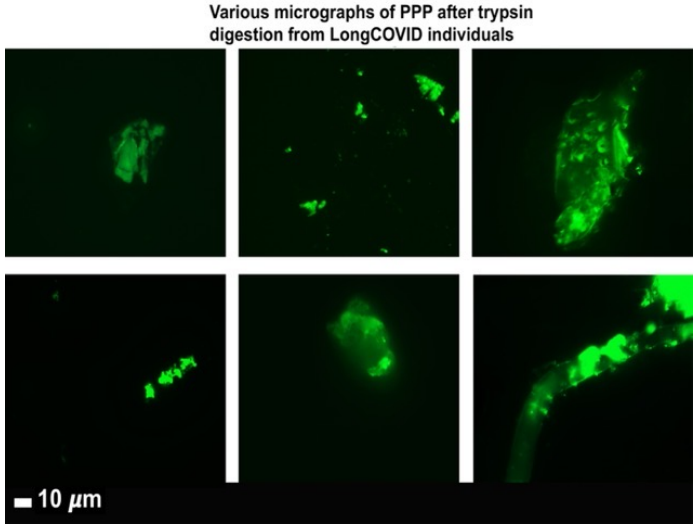
Fibrin that is RESISTANT to Fibrinolysis

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*

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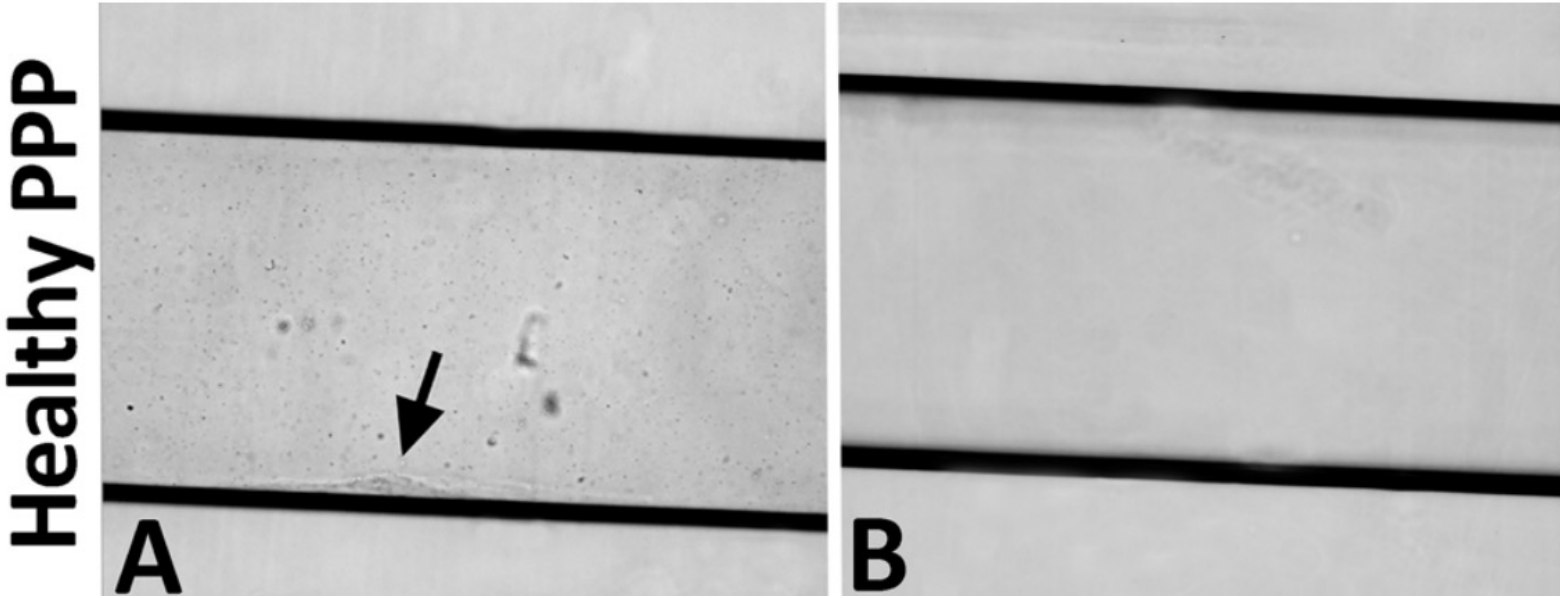
Microfluidic Channel and PPP



Bioscience Reports (2021) 41 BSR20210611 https://doi.org/10.1042/BSR20210611

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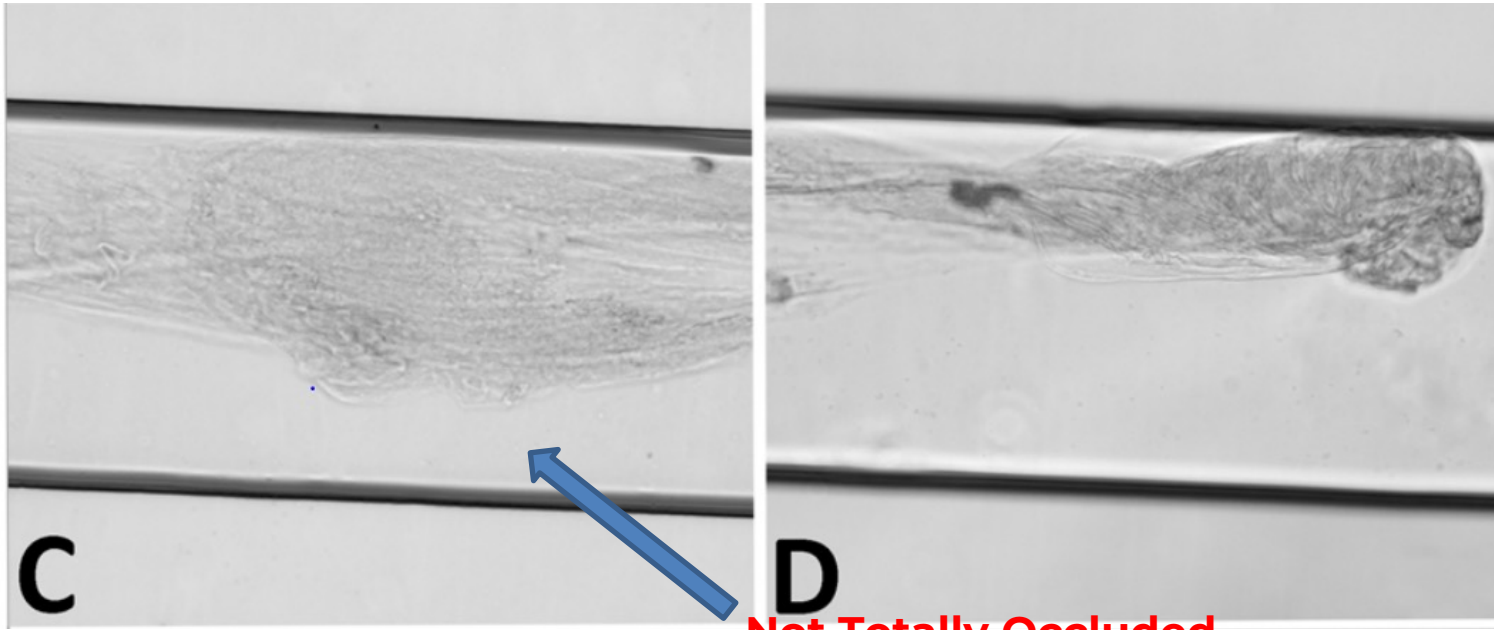
Microfluidic Channel and PPP

COVID-19



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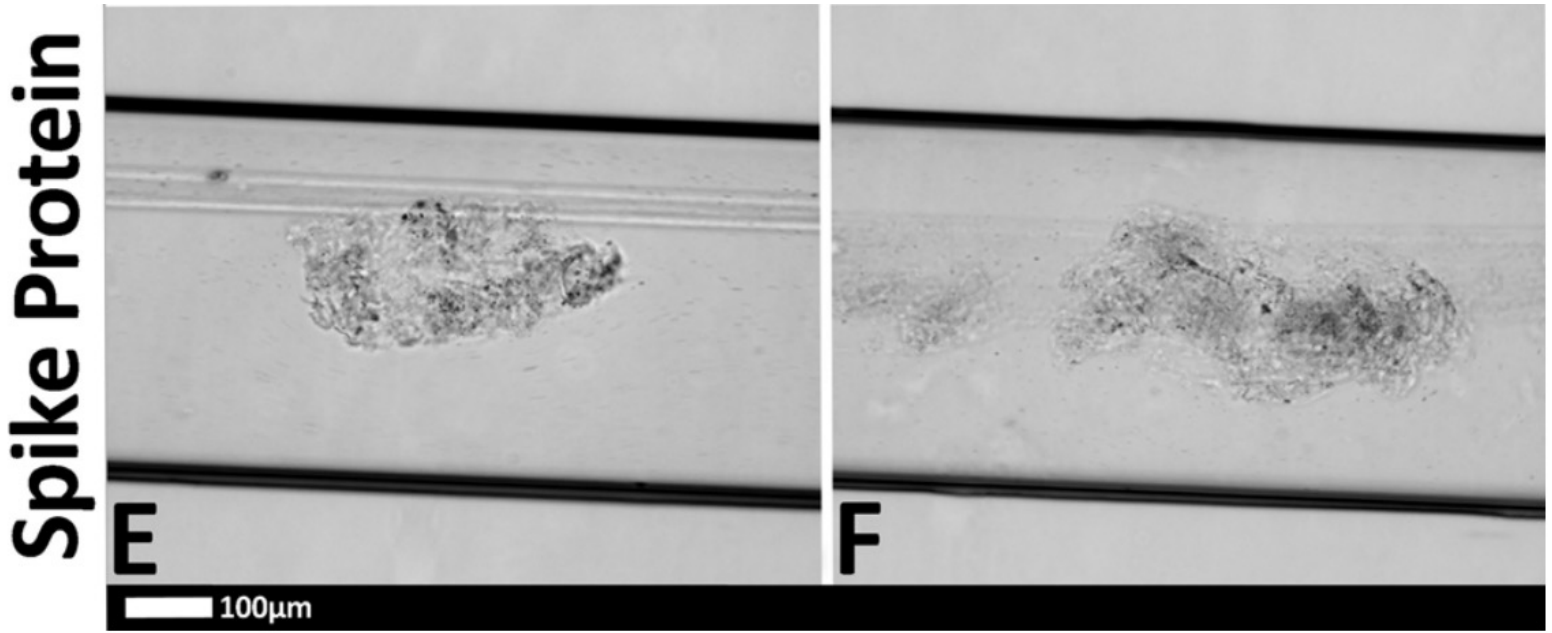
Microfluidic Channel and PPP



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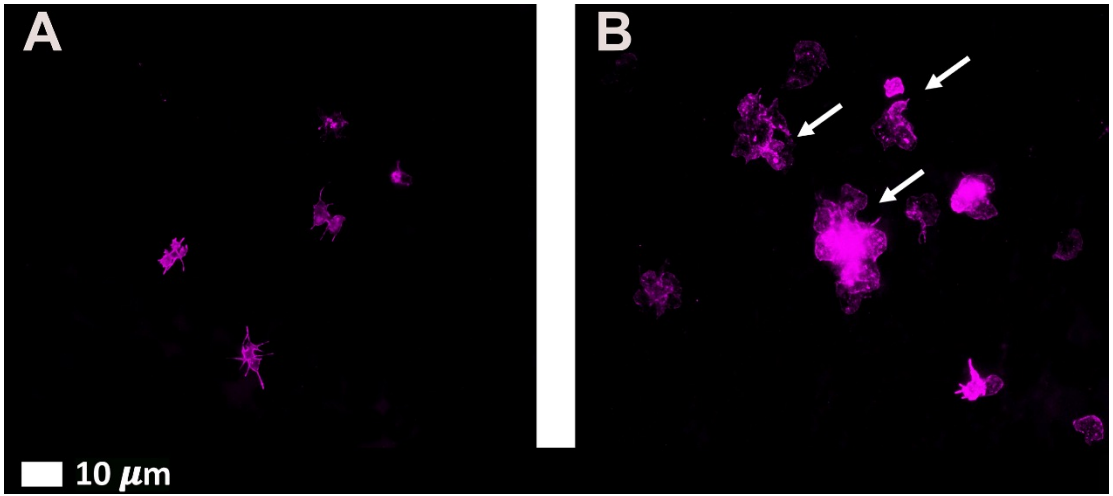
PLATELETS BEFORE AND AFTER EXPOSURE TO SPIKE PROTEIN



Fluorescence microscopy micrographs of platelets, before and after exposure to spike protein(A) Representative platelets from hematocrit incubated with fluorescent marker, CD62P-PE. (B) Representative micrographs showing activated platelets after exposure to spike protein. The white arrows point to hyperactivated activated platelets. White arrows show hyperactivated platelets clumping together.

Biosci Rep Volume 41 Issue 8 2021 BSR20210611 10.1042/BSR20210611

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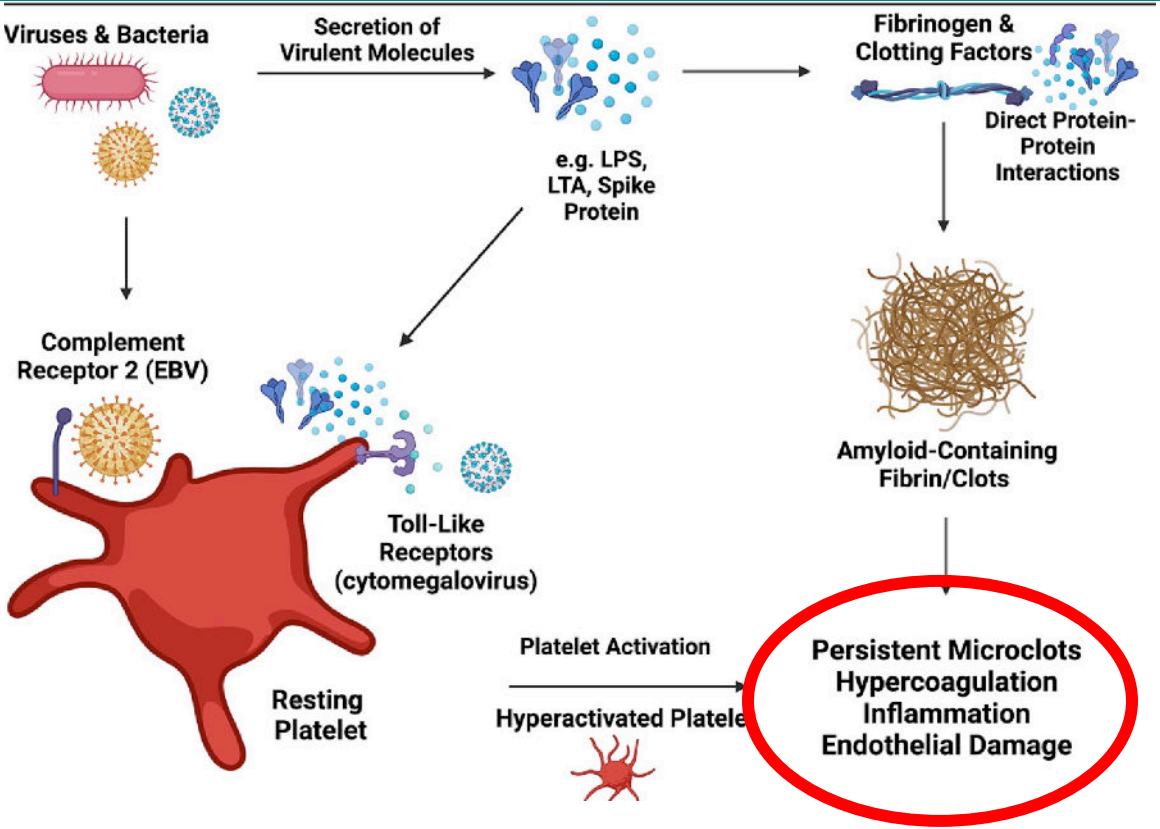
A Nasty Sludge of a Mess



Nunes JM, Kell DB, Pretorius E. Cardiovascular and haematological pathology in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): A role for viruses. Blood Rev. 2023 Mar 20:101075. doi: 10.1016/j.blre.2023.101075. Epub ahead of print. PMID: 36963989; PMCID: PMC10027292.

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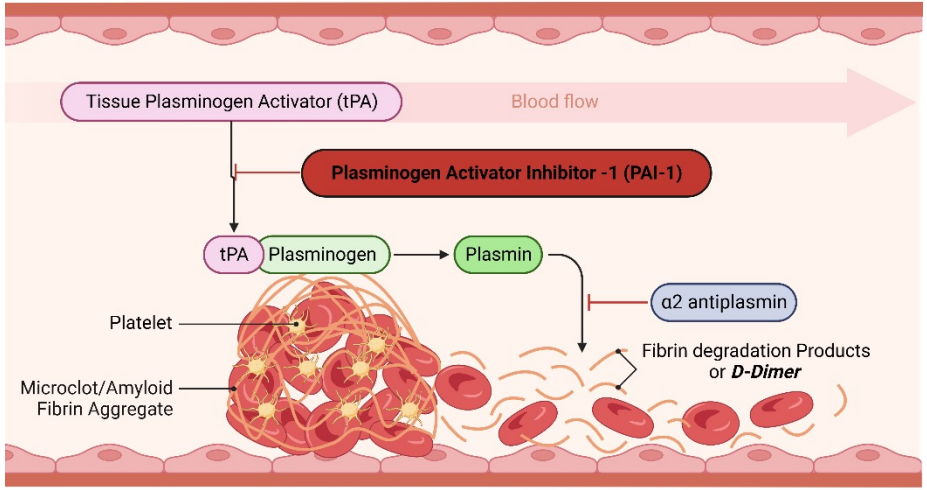
Pre-existing Issues with Fibrinolytic Breakdown?

- **Plasminogen Activator Inhibitor -1 (PAI-1)**
- Levels of **PAI-1** in Acute Covid **EXTREME** elevated and predicted covid severity.
- 90% of PAI-1 is located within Platelets
- **PAI-1** Levels Increased by:
  - Increase Visceral Fat
  - Diabetics/HTN/Age
- **PAI-1** 4G/5G Genetic Polymorphism associated historically associated with:
  - Early CAD, Stroke, Thrombo and Venous Emboli
  - Fertility, Pre-eclampsia, PCOS, Gestational Diabetes
  - **189 of 210** in Long COVID Patients are Hetero or Homozygote for 4g/5g mutation.



Whyte CS et al. The suboptimal fibrinolytic response in COVID-19 is dictated by high PAI-1. J Thromb Haemost. 2022 Oct;20(10):2394-2406. doi: 10.1111/jth.15806. Epub 2022 Jul 21. PMID: 35780481; PMCID: PMC9349442.

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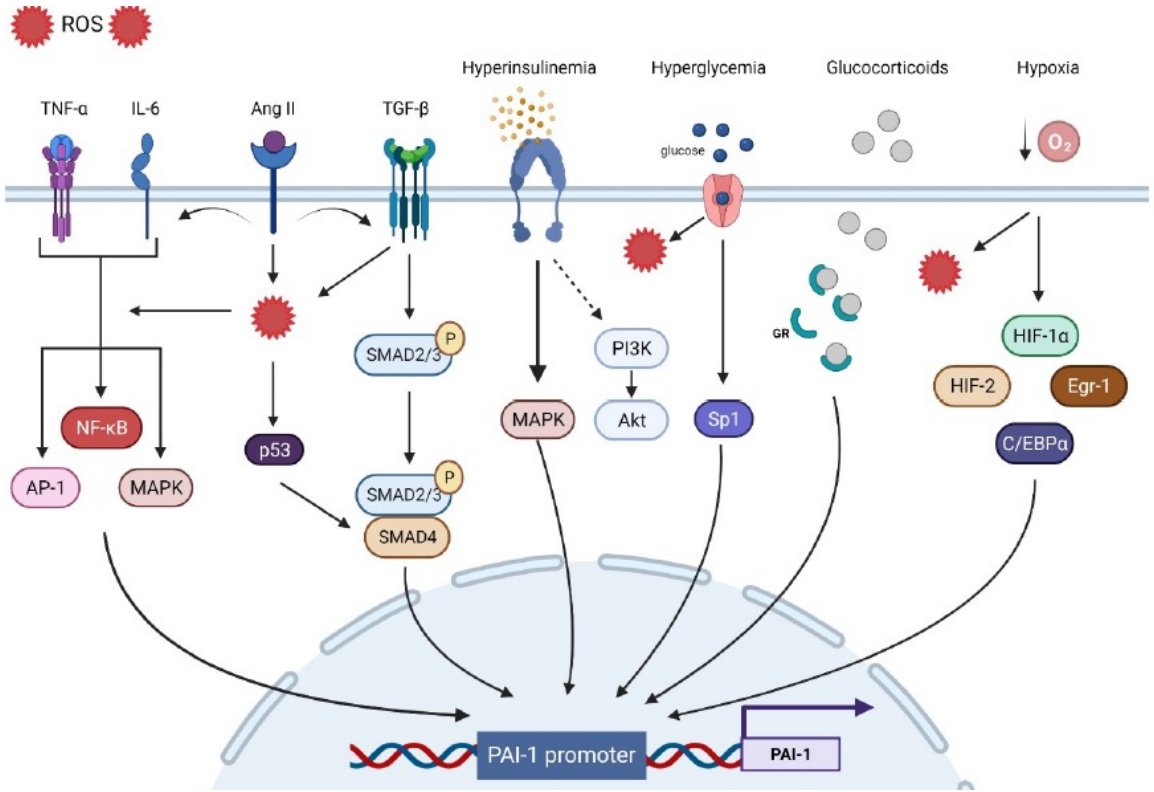
PLASMINOGEN ACTIVATOR INHIBITOR-1



PAI-1 Levels **Increased** by:

- Increase Visceral Fat
- Diabetics/HTN/Age
- Hypoxia
- Cortisol
- Cytokines: IL-6; TNF-a etc

Badran M, Gozal D. PAI-1: A Major Player in the Vascular Dysfunction in Obstructive Sleep Apnea? Int J Mol Sci. 2022 May 15;23(10):5516. doi: 10.3390/ijms23105516. PMID: 35628326; PMCID: PMC9141273..

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PLASMINOGEN ACTIVATOR INHIBITOR-1

- PAI-1 Levels Decreased by:
  - Low Visceral Fat
  - Increased Noradrenaline (EXERCISE/HiiT)
  - Better Sugar Control and Also Metformin AND SGLT-2i (Farxiga/Jardiance/Invokana)
  - Pentoxifylline
  - ACE-I like Ramipril
  - Nattokinase
  - Small Molecules that are not FDA approved Yet.
    - Antagonize PAI-1 or Inhibit PAI-1

Dziewierz A, Zabojszcz M, Natorska J, Ślusarczyk-Dolecka M, Kuleta M, Siudak Z. Dapagliflozin reduces plasma concentration of plasminogen activator inhibitor-1 in patients with heart failure with preserved ejection fraction and type 2 diabetes. Pol Arch Intern Med. 2022 Dec 21;132(12):16383. doi: 10.20452/pamw.16383. Epub 2022 Dec 15. PMID: 36520466.

Microclot Proteomics Analysis

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

Kruger A, Vlok M, Turner S, Venter C, Laubscher GJ, Kell DB, Pretorius E. Cardiovasc Diabetol. 2022 Sep 21;21(1):190. doi: 10.1186/s12933-022-01623-4.
PMID: 36131342; PMCID: PMC9491257.

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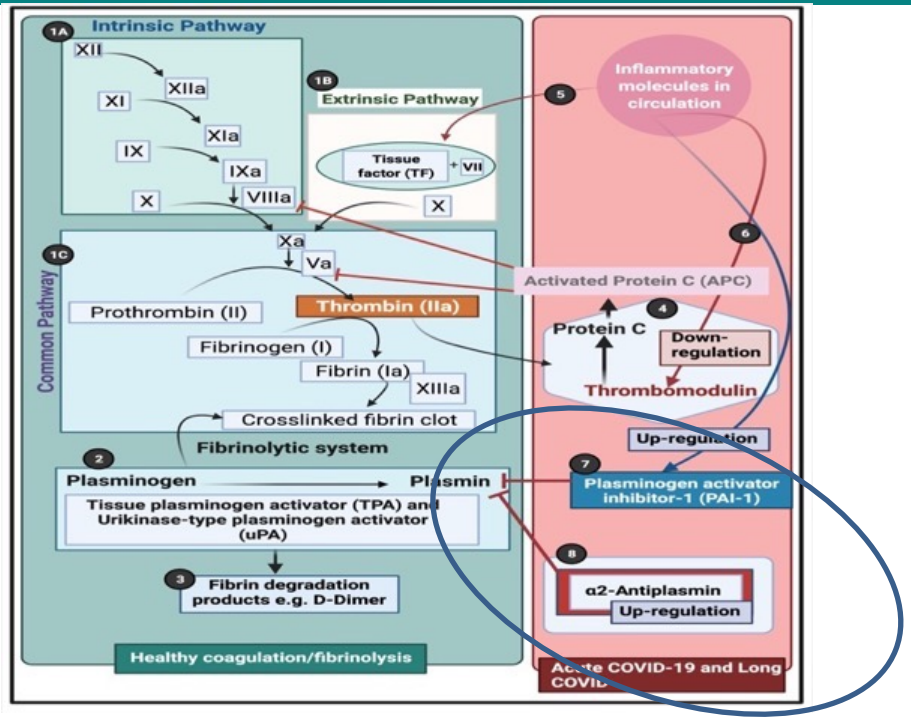
Microclots and Trapped Inflammatory Molecules



PAI-1 and α2-antiplasmin (α2AP) inhibit plasmin and ultimately will prevent sufficient fibrinolysis to happen

Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, Kell DB. Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. Cardiovasc Diabetol. 2021 Aug 23;20(1):172. doi: 10.1186/s12933-021-01359-7. PMID: 34425843; PMCID: PMC8381139.

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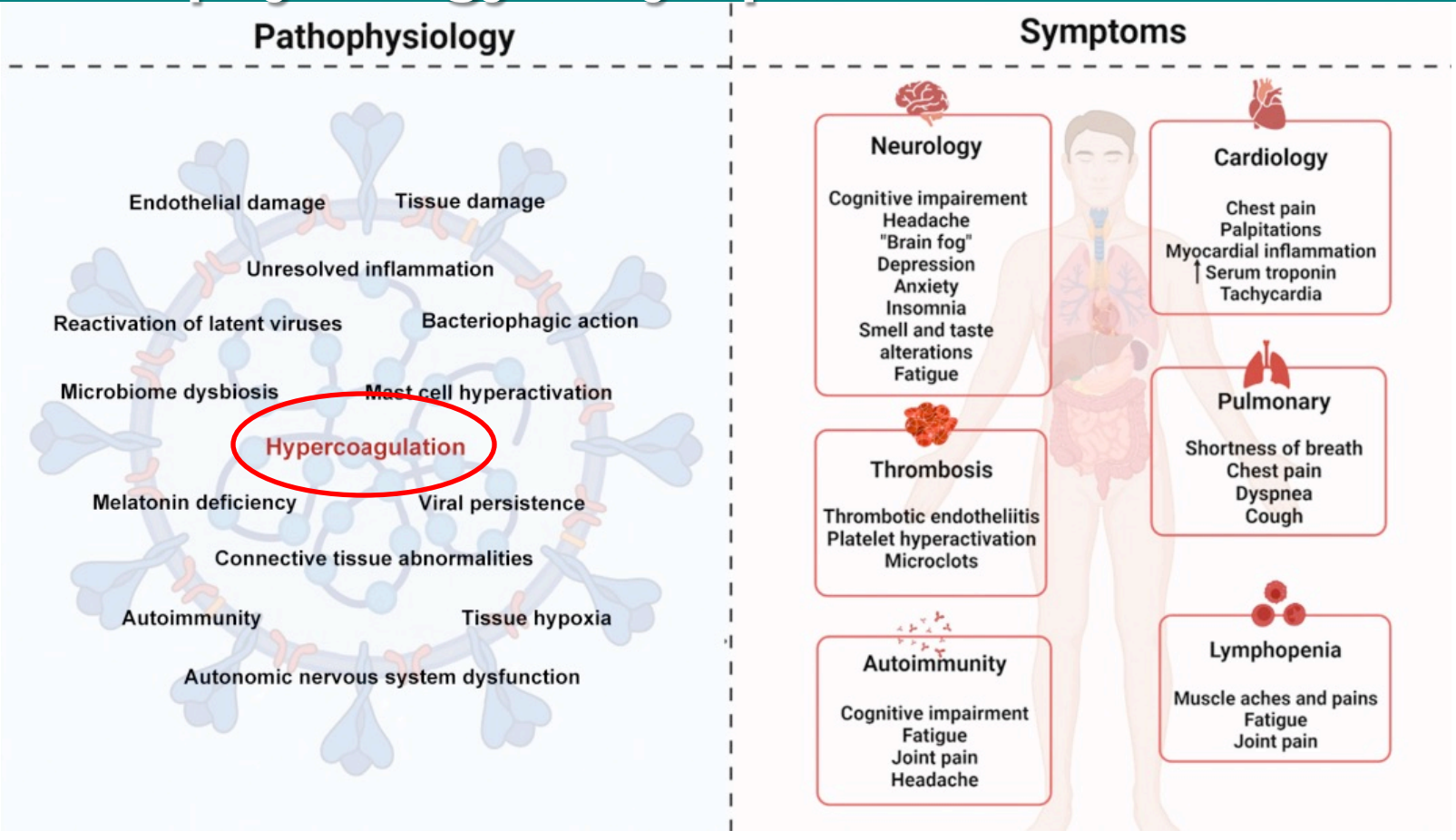
- What can we do about it?

Persistent Spike Protein manifestations: Pathophysiology →Symptoms



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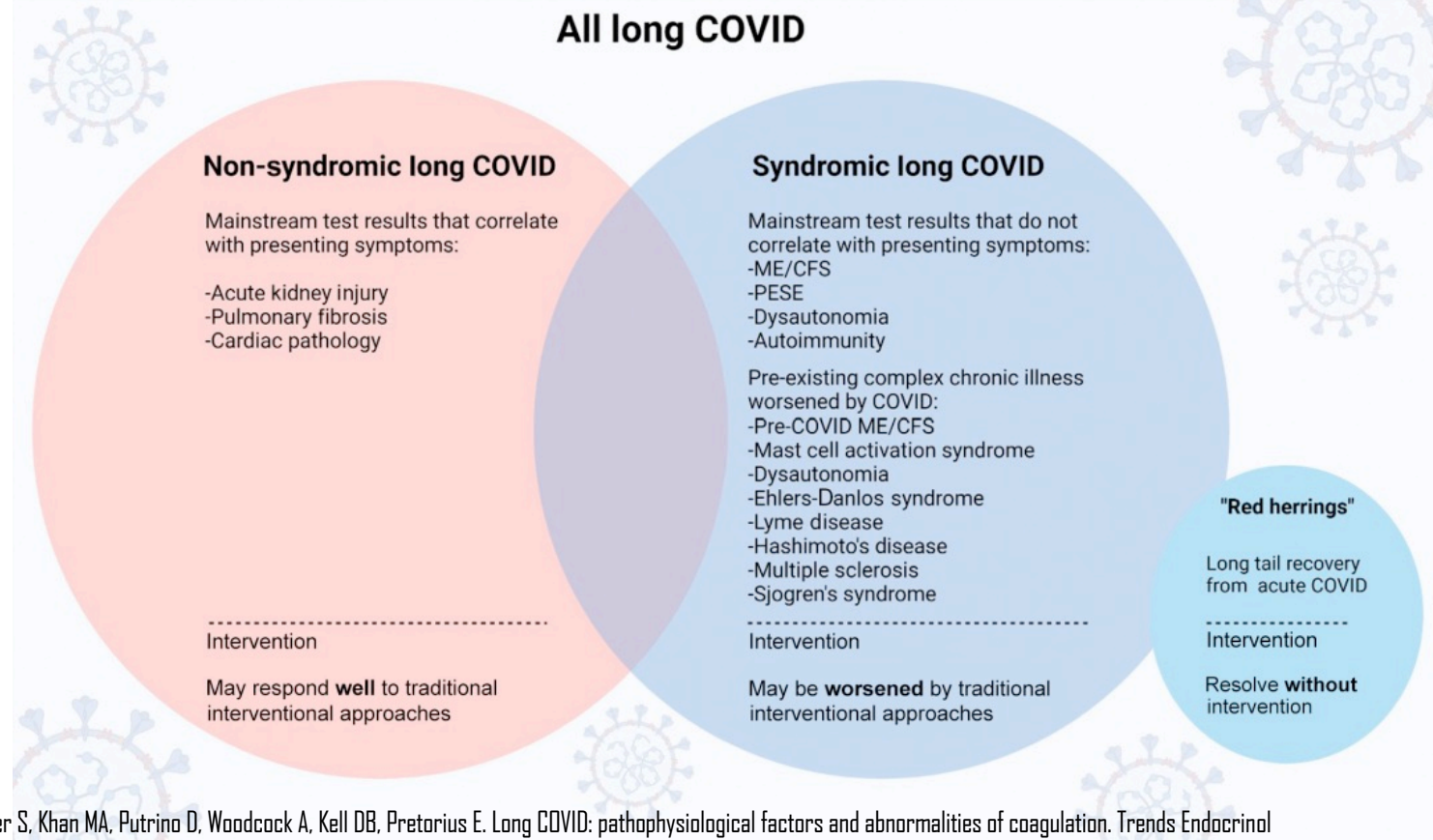
Persistent Spike Protein/Long COVID Subtypes?



Turner S, Khan MA, Putrino D, Woodcock A, Kell DB, Pretorius E. Long COVID: pathophysiological factors and abnormalities of coagulation. Trends Endocrinol Metab. 2023 Apr 18:S1043-2760(23)00055-3. doi: 10.1016/j.tem.2023.03.002. Epub ahead of print. PMID: 37080828; PMCID: PMC10113134.

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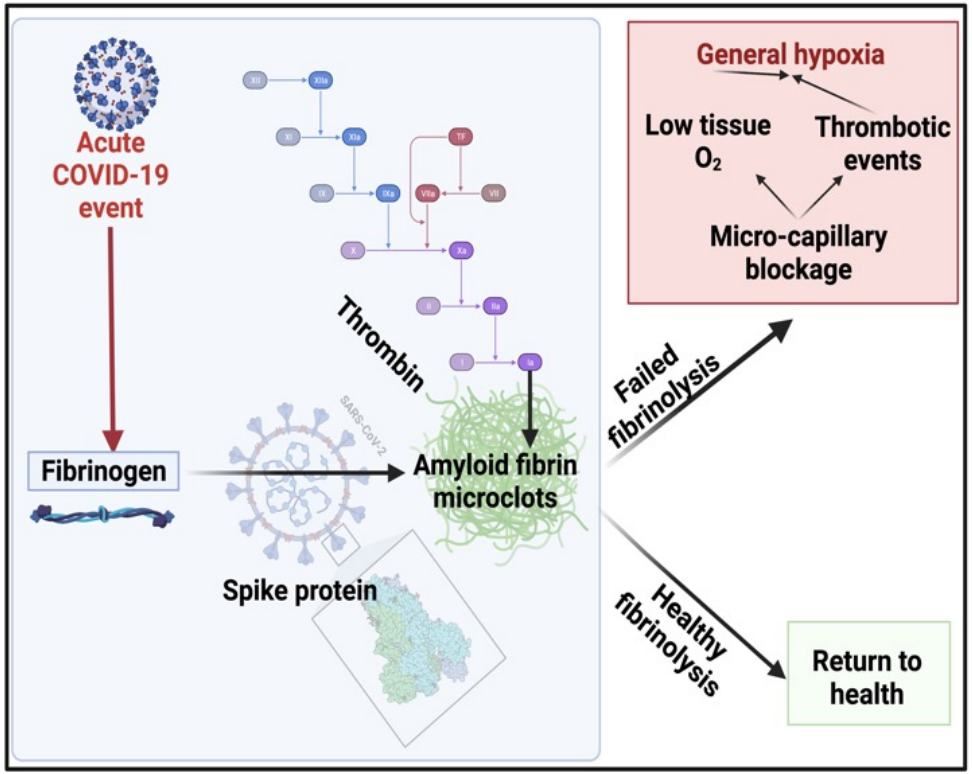
Why Me?



Microclot resolved via the usual **fibrinolytic processes** after acute COVID-19 or, **in Long COVID patients, result in a failed fibrinolytic process**

Douglas B. Kell, Gert Jacobus Laubscher, Etheresia Pretorius; A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications. Biochem J 25 February 2022; 479 (4): 537–559. doi: https://doi.org/10.1042/BCJ20220016

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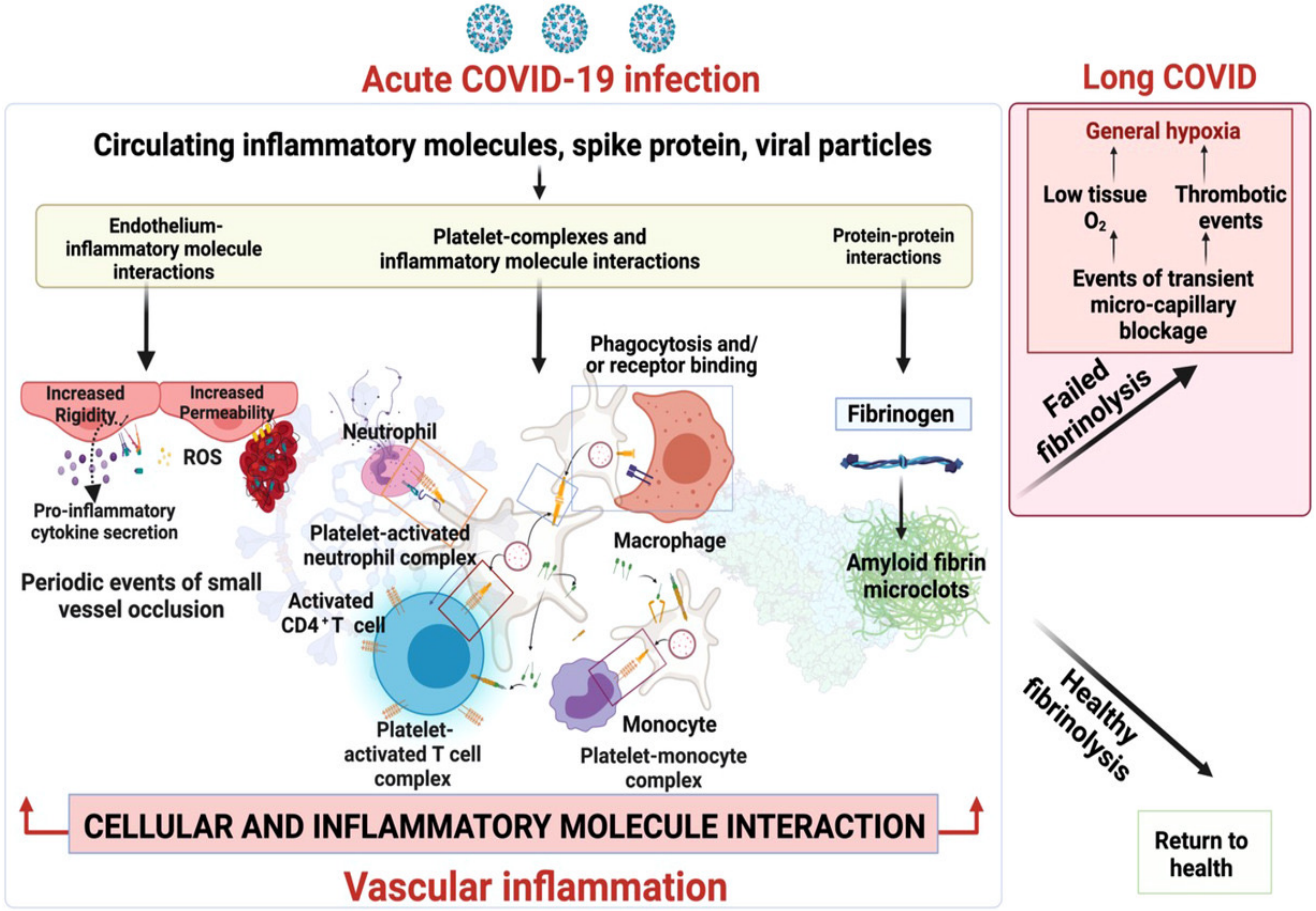
Long COVID: mechanisms, risk factors and recovery



Astin, R., Banerjee, A., Baker, M. R., Dani, M., Ford, E., Hull, J. H., Lim, P. B., McNarry, M., Morten, K., O'Sullivan, O., Pretorius, E., Raman, B., Soteropoulos, D. S., Taquet, M., & Hall, C. N. (2023). Long COVID: mechanisms, risk factors and recovery. Experimental Physiology, 108, 12– 27. https://doi.org/10.1113/EP090802

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Microclots and Symptoms of Long COVID

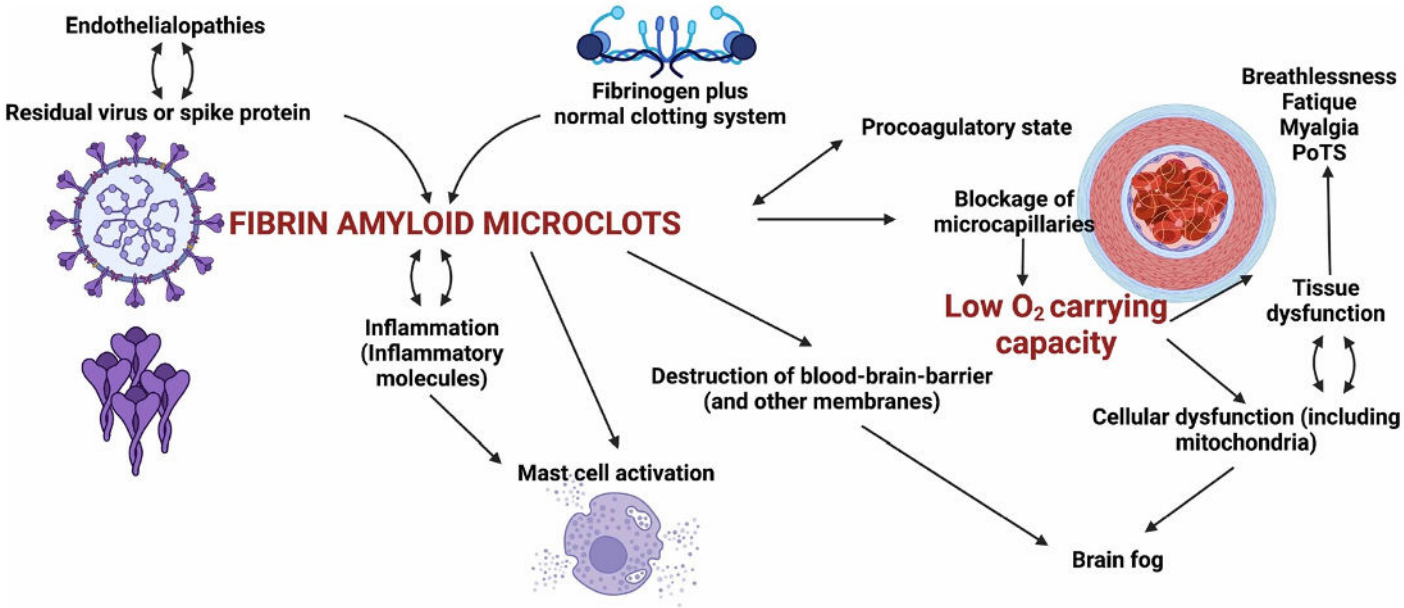

Figure 10. Some of the sequelae of fibrinaloid microclot formation in the symptomology of Long COVID.
Many others, such as a role for auto-antibodies, are not shown.

Douglas B. Kell, Etheresia Pretorius; 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* 31 August 2022; 479 (16): 1653–1708. doi: https://doi.org/10.1042/BCJ20220154

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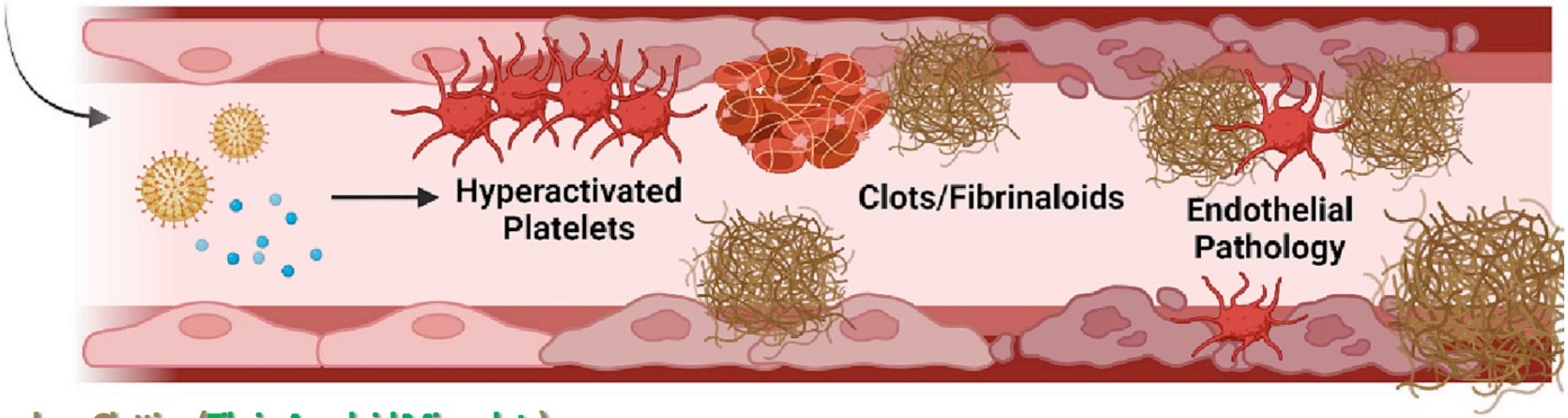
- What can we do about it?

Endothelial Pathology leads to Tissue Hypoxia



Anomalous Clotting (Fibrin Amyloid Microclots)

+

Hyperactivated Platelets

+

Endothelial Damage and Dysfunction From Spike

→ Vessel Damage/Subtotal Occlusion

↓

Local Tissue Hypoxia and Hypoperfusion

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Gert J Laubscher, M Asad Khan, Chantelle Venter et al. Treatment of Long COVID symptoms with triple anticoagulant therapy, 21 March 2023, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-2697680/v1]

CONSEQUENCES OF MICROCAPILLARY BLOCKAGE BY MICROCLOTS

- RBC cannot penetrate to tissues
- Ischemia
- Hypoxia
- Fatigue
- Damage to any tissue undergoing hypoxia
- → Ischemia-reperfusion injury

Microcapillary blockage by MICROCLOTS:
Areas now use the Dissolved Oxygen(PP Oxygen) in Serum not Red Blood Cells.

Partial pressure of oxygen in humans

Table 1. References values of PtO^2 measurements using different techniques

PtO_2 (mmHg)	Organ and Tissue	Reference
108 mmHg	Alveoulus	Guyton [4]
30 mmHg	Brain	Meixensberger [51], Hoffman [52], Ortiz-Prado [3]
30 mmHg	Vestibular System (Balance)	
30.6 mmHg	Cornea	Bonanno [64]
28.9 mmHg	Skeletal Muscle fibers	Beerthuizen [58], Carreau [53]
29.6 mmHg	Myocardium	
22 mmHg	The Eye	Bonanno [64]
8 mmHg	Skin epidermis	Wang [35], Carreau [53]
24 mmHg	Dermal papillae	
55 mmHg	Liver	Leary [56]
72 mmHg	Superficial cortex of the kidney	Muller [57], Carreau [53]
90 ± 5 mmHg	Arterial PO^2	Mah and Cheng [20], Guyton [4]
40 ± 5 mmHg	Venous PO^2	Mah and Cheng [20], Guyton [4]

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Ortiz-Prado E, Dunn JF, Vasconez J, Castillo D, Viscor G. Partial pressure of oxygen in the human body: a general review. Am J Blood Res. 2019 Feb 15;9(1):1-14. PMID: 30899601; PMCID: PMC6420699.

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- **What can we do about it?**

Combined triple treatment of fibrin amyloid microclots and platelet pathology in individuals with Long COVID/ Post-Acute Sequelae of COVID-19 (PASC) can resolve their persistent symptoms

Etheresia Pretorius (resiap@sun.ac.za)
Stellenbosch University https://orcid.org/0000-0002-9108-2384

Treatment of Long COVID symptoms with triple anticoagulant therapy

Gert J Laubscher
Mediclinic Stellenbosch
M Asad Khan
Directorate of Respiratory Medicine, Manchester University Hospitals https://orcid.org/0000-0003-1838-9002

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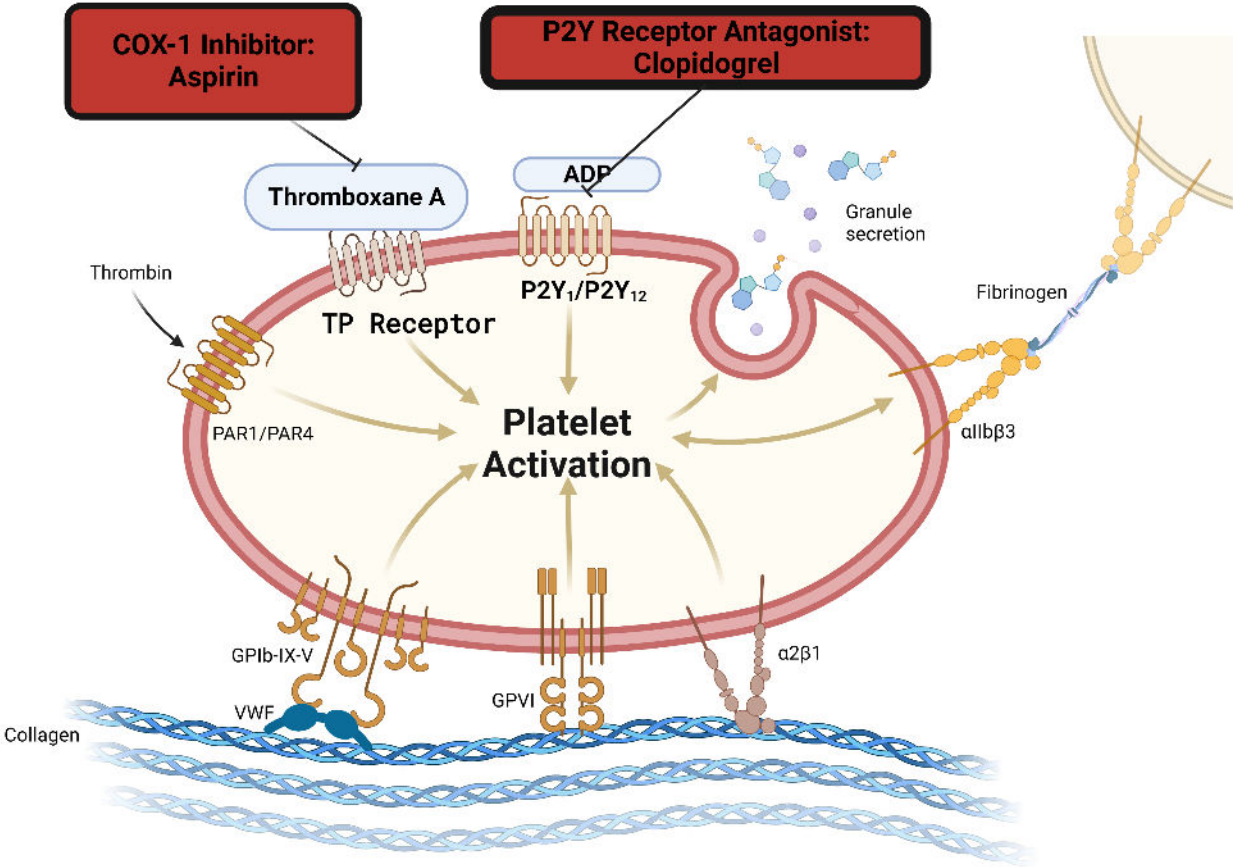
Why Triple Therapy?
Remember they are Resistant NOT Impervious

- Aspirin stops Platelets from Sticking to Each Other
- Plavix Stops Platelets from Sticking to Endothelium
- Direct oral Anticoagulant (DOAC) Stops Precipitation of Fibrin from Fibrinogen out of Plasma to Serve as Mortar in Microclot Complex
- Famotidine for Stomach Protection.

Triple Treatment: Dual Anti-platelet



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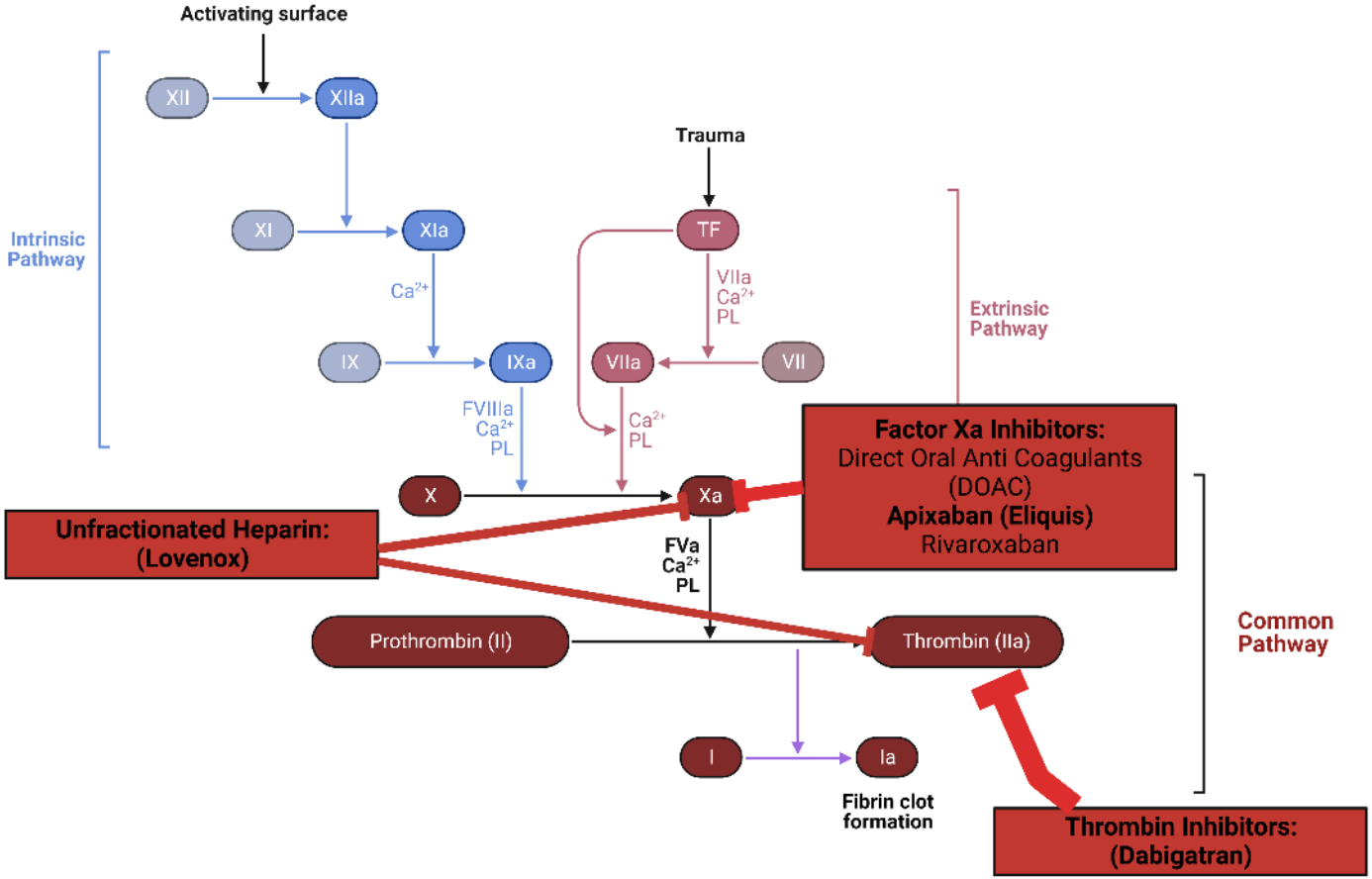
Triple Treatment: Anticoagulation



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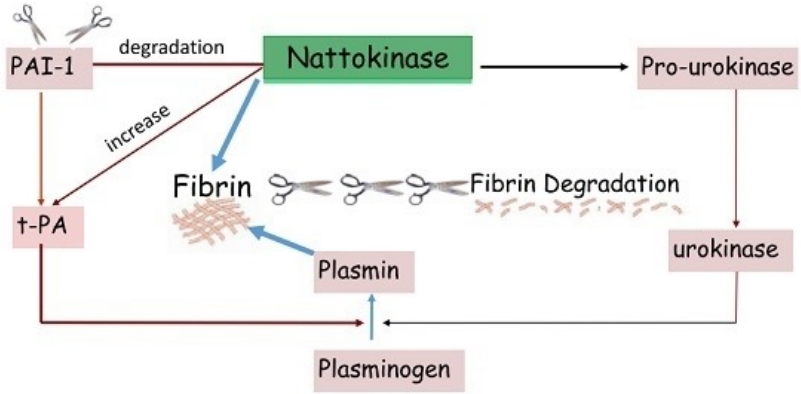
Natural Supplements:

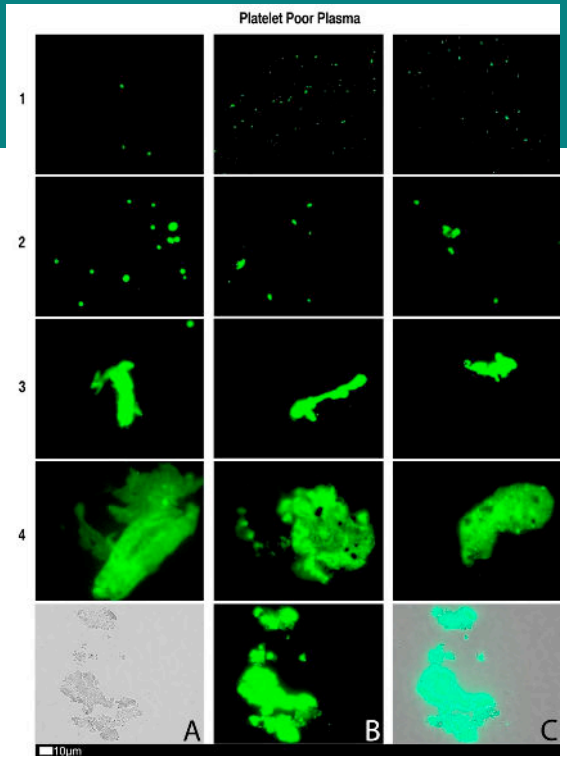
- Natural Proteolytic Enzymes
  - Nattokinase:
  - Not only degrades fibrin directly but also increases the release of tPA with a subsequent increase in the formation of plasmin.
  - Enhances fibrinolysis through cleavage and inactivation of PAI-1.
  - Serrapeptase
  - Bromelain
- Other Natural Agents with Potential
  - N-acetyl cysteine
  - Curcumin
  - Lactoferrin



Weng, Y.; Yao, J.; Sparks, S.; Wang, K.Y. Nattokinase: An Oral Antithrombotic Agent for the Prevention of Cardiovascular Disease. *Int. J. Mol. Sci.* **2017**, *18*, 523. https://doi.org/10.3390/ijms18030523

Assessing for Patients:

- Obviously, Using Dual Antiplatelet and DOAC in combination has risk beyond other Treatments in the very safe FLCCC Protocol.
- Despite these risk, in the patients that have microclots in vasculature treatment is life altering.
- A good history of Spike protein related interactions and resultant symptoms is important.
- ImmunoFluor of PPP makes diagnosis and progress easier!
- Hopeful for Flow Cytometry being Validated soon
- Younger, otherwise healthy prior to COVID or Vaccination are easiest.
  - Unvaccinated High Functioning Young people including some college athletes were my first few patients to utilize the triple therapy.
  - Easy Objective History of Decline with spike exposure and no chronic disease states.
  - Easy to avoid skydiving, ATV usage, gutter cleaning, (in men anything wife would say is stupid).
  - Women of Menstruating Age require close monitoring around Cycle.
  - Utilize Mast Cell Stabilizers and Anti-histamines Liberally to control symptoms. Younger fertile women seem to be very sensitive to treatment.
- Older and Patients with Multiple Chronic Conditions More Difficult to Parse Spike Disease.
  - Older is age is Heterogenous thank goodness! A 1yo is a 1yo but a 65yo is not a 65yo.
  - More extensive history is needed and closer following on therapy.
  - I usually seem them weekly.



Microclot in PPP Grading Stages

Stage 1 Minimal Microclots
Stage 2 Mild Microclots
Stage 3 Moderate
Stage 4 Significant and Widespread

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Underlying Principles to this Treatment:

- Be a Physician: Listen and CARE!!!!
  - Two C's of Medicine:
    - CARE about your Patient!
    - Be CURIOUS about them specifically and the disease they are suffering from!
- Informed Consent is Fundamental
- Start on Core Therapy Initially!
  - Avoid Too many Supplements or adjuvants it will just confuse you both.
    - Time for these is at least a month after core Triple Therapy
- Transition to Natural Fibrinolytics → Lifelong Risk Modification?
- Find a Pharmacy that understands what you are doing!.

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Treatment Expectations:

- Response: If selected right patient results are incredibly for patient.
- Learned from Treating 800+ people:
  - First 1-2 weeks on Therapy Old Symptoms may come back, worsen, or new ones appear!
  - Symptoms appearing from Antiplatelet and DOAC therapy are discomforting but in my clinical experience a sign that:
    - Picked the correct patient.
    - Discussing this ahead of time is a way to help stave off worries and confirm to the patient that 'something' positive is happening. (Avoids Anxiety to Patient and Calls for Physician
  - Only one GI Bleed in 84yo with history of AVM intestine. (Stopped Triple and received 1 unit of blood)
- **Length of Treatment**: Short Long COVID versus Long Long COVID
  - Seems to depend on how long since infection or vaccine and the Immune Systems Status:
  - Easiest and Shortest: (4-6 weeks)
    - Young, previously healthy, unvaccinated, Long COVID
  - Hardest and Longest: (4 months to 6months or more?)
    - Older, chronic disease (esp autoimmune), multiple jabs and boosters, post covid.

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THANK YOU