

# The occurrence of hyperactivated platelets and fibrinoid microclots in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

**Massimo Nunes**

Stellenbosch University

**Arneaux Kruger**

Stellenbosch University

**Amy Proal**

PolyBio Research Foundation

**Douglas Kell**

University of Liverpool <https://orcid.org/0000-0001-5838-7963>

**Etheresia Pretorius** (✉ [resiap@sun.ac.za](mailto:resiap@sun.ac.za))

Stellenbosch University <https://orcid.org/0000-0002-9108-2384>

---

## Article

**Keywords:** Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), platelets, fibrinoid microclots, hypercoagulability

**Posted Date:** June 8th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1727226/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

We have previously demonstrated that platelet poor plasma (PPP) obtained from patients with LongCovid/Post-Acute Sequelae of COVID-19 (PASC) is characterized by a hypercoagulable state reflected in hyperactivated platelets and the presence of considerable numbers of fibrin(ogen) amyloid microclots or fibrinaloid microclots. Due to substantial overlap in symptoms and aetiology between PASC and ME/CFS, we investigated whether coagulopathies, platelet hyperactivation and/or fibrin amyloid formation differed between individuals exhibiting ME/CFS and gender- and age-matched healthy controls. ME/CFS patients were statistically far more hypercoagulable as judged by thromboelastography of both whole blood and platelet-poor plasma. The area of plasma images containing fibrinaloid microclots was commonly more than 10-fold greater in untreated platelet-poor plasma from individuals with ME/CFS than in that of healthy controls. A similar difference was found when the plasma samples were treated with thrombin. Using fluorescently labelled PAC-1, which recognizes glycoprotein IIb/IIIa, and CD62P, which binds P-selectin, we observed massive hyperactivation and spreading of platelets in samples from individuals with ME/CFS. Using a quantitative scoring system, this was found to have a score of  $2.72 \pm 1.24$  vs 1.00 (activation with pseudopodia formation) for healthy controls. We conclude that ME/CFS is accompanied by substantial and measurable changes in coagulability, platelet hyperactivation, and fibrinaloid microclot formation. However, fibrinaloid microclot load was not as prevalent as was previously noted in PASC. Fibrinaloid microclots, in particular can provide a ready explanation, via (temporary) blockage of microcapillaries and hence ischaemia, for many of the symptoms, such as fatigue, seen in patients with ME/CFS. The discovery of these biomarkers pointing to significant and systemic endothelial inflammation, represents an important development in ME/CFS research. It also points at novel treatment strategies using known drugs and/or nutraceuticals that target systemic vascular pathology and endothelial inflammation.

## Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating, multisystem disease that currently lacks a definitive diagnostic biomarker, an effective treatment, and a clear and widely accepted aetiological explanation. Symptoms of the condition include pathological fatigue (not influenced by exertion) that goes unresolved with rest and sleep; other symptoms include sleep perturbations, joint and muscle pain, intolerance towards exercise, headaches, gastrointestinal issues, flu-like symptoms, and cognitive impairments<sup>1,2</sup>. Furthermore, the prevalence figures of ME/CFS are blurred by inconsistent case definitions, self-reporting assessments, and a large proportion of undiagnosed patients<sup>2,3,4</sup>. Regardless of the inconsistent definitions, it has been estimated that there are 1.5 million individuals suffering from ME/CFS (low-end value) in the USA alone<sup>5</sup>. Alarming, the latest predictions suggest that the prevalence of ME/CFS might face a period of rapid growth, possibly by as much as a factor of six<sup>6</sup>.

Most cases of ME/CFS begin with a viral infection or involve multiple exposures to viral and/or bacterial pathogens over time<sup>7,8,9,10</sup>. Viruses implicated in initiating or exacerbating the ME/CFS disease process include human herpes virus (HHV)-6, HHV-7, Epstein-Barr virus (EBV), cytomegalovirus (CMV), enteroviruses, human parvovirus B19, and coxsackie B virus<sup>11</sup>. With regards to bacteria, gut dysbiosis and gram-negative LPS have been suggested to play a role in ME/CFS pathology<sup>12,13,14</sup>. Patients have also met the diagnostic criteria for ME/CFS after infection with bacterial pathogens including *Coxiella burnetii* (Q fever) or bacteria in the genus *Brucella* (brucellosis)<sup>15,16,17</sup>.

More recently, 10–30% of patients infected with the SARS-CoV-2 virus driving the COVID-19 pandemic are developing chronic symptoms that overlap greatly with those of ME/CFS. These patients are being given the diagnosis LongCovid or Post-Acute Sequelae of COVID-19 (PASC)<sup>18</sup>. The overlap between PASC and ME/CFS symptoms is so profound that many PASC patients meet the diagnostic criteria for ME/CFS after 6 months of ongoing symptoms<sup>19,20,21,22,23,24</sup>.

We have demonstrated that LongCovid/PASC platelet poor plasma (PPP) contains large anomalous fibrin/amyloid deposits (microclots) and hyperactivated platelets<sup>25</sup>. The microclots are relatively resistant to fibrinolysis even after trypsinization. When solubilized in the laboratory via a second trypsinization step, they contain inflammatory molecules and clotting factors including  $\alpha(2)$ -antiplasmin ( $\alpha 2AP$ ), various fibrinogen chains, and serum amyloid A (SAA). This pathology may lead to significant endothelial inflammation, (temporary) capillary blockage and hypoxia<sup>26</sup>.

Due to the substantial overlap in LongCovid/PASC and ME/CFS symptoms and aetiology, it is very likely that coagulation-based pathology may also contribute to non-SARS-CoV-2 onset ME/CFS (cases initiated or exacerbated by other pathogens). Despite some inconsistencies in the literature<sup>27,28</sup>, there is precedent for hypercoagulability and platelet activation in ME/CFS. Brewer *et al.* found that ME/CFS individuals presenting with active HHV-6 infection exhibited a state of hypercoagulability<sup>29</sup>, although over 80% of subjects involved in the study possessed hereditary risk factors for thrombosis, thereby skewing the precision of this interpretation. Another study demonstrated a hypercoagulable state with platelet activation, and suggested that fibrin deposits in microcirculatory vessels (perhaps fibrinoid microclots?) can adhere to the endothelial lining and play a part in the manifestation of ME/CFS symptoms, possibly by impairing oxygen and nutrient delivery to tissues<sup>30</sup>. More recently, platelet activity and markers have been implicated in ME/CFS, although the authors indicated a loss of significance after statistical corrections<sup>31</sup>. Endothelial abnormalities have also been noted in ME/CFS<sup>32,33</sup>. For example, endothelial cells exposed to plasma from ME/CFS individuals exhibited functional defects<sup>34</sup>.

There are clear molecular mechanisms by which viral/bacterial infection and/or chronic inflammatory may contribute to coagulation-based sequelae in ME/CFS. Platelets can sense and bind to both viruses and bacteria via a variety of platelet receptors, and dozens of viral and bacterial products stimulate platelets, modulating their function<sup>35,36,37,38</sup>. If these infections or associated inflammation do not resolve, perpetual platelet stimulation by the pathogens or their products may lead to platelet

hyperactivation and clotting sequelae. Pro-inflammatory processes and chronic inflammation alone can also prompt the coagulation system to take on a hypercoagulable state<sup>39, 40, 41, 42, 43, 44, 45</sup>.

In this study we investigate if amyloid fibrinoid microclots are present in ME/CFS plasma, and to what extent. We also measure platelet activity and characterize the constituent nature of identified clots, as well as the viscoelastic properties of blood, which can determine the state of coagulability (hypocoagulable vs normocoagulable vs hypercoagulable)<sup>46, 47</sup>.

## Methods

### Ethical Statement

Ethical clearance for the study was obtained from the Health Research Ethics Committee (HREC) of Stellenbosch University (South Africa) (N19/03/043, project ID #9521). For the volunteers who provided blood samples, the experimental objectives, risks, and details were explained to volunteers and informed consent were obtained prior to blood collection. Strict compliance to ethical guidelines and principles of the Declaration of Helsinki, South African Guidelines for Good Clinical Practice, and Medical Research Council Ethical Guidelines for Research were kept for the duration of the study and for all research protocols.

### Sample Demographics and Blood Collection

Blood samples were obtained from healthy individuals (n=15; 9 females, 6 males) to serve as controls for comparison. Healthy volunteers were only included if they did not smoke, did not suffer from cardiovascular disease or any coagulopathies, not pregnant or taking contraceptives, or did not suffer from Long COVID. ME/CFS patients (n=25; 20 females; 5 males) were recruited via the ME/CFS Foundation of South Africa, and only included in this study if they had not previously been infected with the COVID-19 virus. Participants had to have been diagnosed for longer than 6 months, and were still asked to complete the International Consensus Criteria (ICC) questionnaire<sup>48</sup> to gain an understanding of their perspective of disease severity. Blood was collected in citrated tubes. Whole blood (WB) was used for viscoelastic studies, after which the samples were centrifuged at 3000×g for 15 min at room temperature to collect platelet poor plasma (PPP). Platelets were identified in the hematocrit, after PPP was removed and stored at -80 °C for later analysis.

### Viscoelastic Analysis

Clotting properties of both WB and PPP samples were measured by using the Thrombelastograph® (TEG®) 5000 Hemostasis Analyzer (Haemoscope Corp). Analysing WB can allow for the detection of clotting abnormalities influenced by blood as a whole, while TEG® of PPP allows one to assess the contribution of only the clotting proteins without cellular components (such as erythrocytes and platelets)<sup>49</sup>. 20µL of 0.01M calcium chloride (required to initiate coagulation in blood drawn within citrate tubes)

was added to the TEG® cup, followed by 340µL of either WB or PPP. The test was promptly started and left to run until the maximal amplitude of the clot had been reached.

### **Fibrinoid Microclot Analysis inside Platelet Poor Plasma (PPP)**

PPP was used to study microclots presence in participants with ME/CFS, and compared to those present in healthy participants. PPP samples were incubated with the fluorescent probe, Thioflavin T (ThT) (Sigma-Aldrich, St. Louis, MO, USA), at a final concentration of 5 µM and for a period of 30 min, prior to viewing with a fluorescence microscope. ThT binds to open hydrophobic areas on fibrinogen that is indicative of amyloid protein changes<sup>50, 51, 52, 53</sup>. Samples were viewed on the Zeiss Axio Observer 7 fluorescent microscope with a Plan-Apochromat 63×/1.4 Oil DIC M27 objective (Carl Zeiss Microscopy, Munich, Germany), with the excitation wavelength set at 450-488nm and the emission wavelength set at 499-529nm. The % area of the fibrinoid microclot presence was determined by analysing micrographs using ImageJ 1.53e. After setting the scale, images are converted to 8-bit. The threshold, using the Huang setting, was then set by increasing the white background intensity to 255 and the black (fluorescence) signal intensity to 13-17. Next, we used the 'analyse particles' assessment and set particle size at 1-infinity. Three representative images were chosen per subject. The data generated were then analysed with GraphPad Prism 8.4.3.

### **Fibrinoid Microclot Analysis after addition of thrombin to create extensive fibrin clots**

ThT was used again to identify amyloid presence and load within clot networks. 49µL of PPP was incubated with ThT (again at a final exposure concentration of 5 µM) for 30 minutes at room temperature. 5µL of the sample was then transferred to a glass slide, followed by 2,5µL of thrombin (7 U.ml<sup>-1</sup>, South African National Blood Service). The sample was left to stand for 2 minutes in order for fibrin networks to form, after which a coverslip was placed on top of the clot. Samples were viewed on the Zeiss Axio Observer 7 fluorescent microscope with a Plan-Apochromat 63×/1.4 Oil DIC M27 objective (Carl Zeiss Microscopy, Munich, Germany), with ThT's excitation wavelength set at 450-488nm and the emission wavelength set at 499-529nm. Fluorescent intensity of fibrin clot micrographs was calculated using ImageJ 1.53e. 'Mean Gray Value' and 'area' were chosen as measurement settings.

### **Platelet Assessment Using Fluorescence Microscopy**

Two fluorescent markers, PAC-1 (FITC-conjugated) (340507, BD Biosciences, San Jose, CA, USA) and CD62P (PE-conjugated) (IM1759U, Beckman Coulter, Brea, CA, USA), were obtained to assess the state of platelets by using the Zeiss Axio Observer 7 fluorescent microscope with a Plan-Apochromat 63×/1.4 Oil DIC M27 objective (Carl Zeiss Microscopy, Munich, Germany). After centrifugation of the blood tubes and removal of the plasma, 20µL of haematocrit was slowly (due to its viscosity) pipetted and transferred to an Eppendorf microcentrifuge tube. After allowing the fluorescent markers to reach room temperature, 4µL of both PAC-1 and CD62P was added to the microcentrifuge tube containing 20µL of haematocrit. The incubation period lasted for 30 minutes, in a dark room, at room temperature. The excitation wavelength for PAC-1 was set at 450 to 488 nm and the emission wavelength at 499 to 529 nm; and the

excitation wavelength for CD62P was set at 540 to 570 nm and the emission wavelength at 577 nm to 607 nm. Platelet phenotype is assessed with the grading system we have recently implemented<sup>47</sup>, where platelet spreading and clumping scores were used and allocated a score of 1 to 4 for severity of spreading and clumping.

## Statistical Analysis

Statistics were completed on GraphPad Prism 9.3.1. Data were subjected normality tests (Sharpiro-Wilks). Parametric data were then subject to unpaired t-tests, and non-parametric data were analysed with the unpaired Mann-Whitney test. Data are represented as mean  $\pm$  standard deviation, or median [Q1-Q3]. Graphical data is represented as mean  $\pm$  SEM.

## Results

Table 1 shows the demographics and the disease scoring of the ME/CFS cohort using the International Consensus Criteria (ICC) questionnaire for ME/CFS patients<sup>48</sup>. Both healthy and ME/CFS population age data were normally distributed, and did not yield significant differences when analysed with an unpaired t-test ( $p=0.54$ ). Comorbidities are present within the ME/CFS population: 40% of subjects are afflicted with leaky gut/gut dysbiosis; 16% afflicted with POTS, psoriasis, fibromyalgia, gingivitis/periodontitis, hypercholesterolemia, and hypertension, 12% afflicted with rheumatoid arthritis, and cardiovascular disease; and 4% afflicted with orthostatic hypotension, mast cell activation syndrome, rosacea, and dysautonomia. The ICC questionnaire results indicate that this study's ME/CFS population predominantly experiences symptoms related to post-exertional neuroimmune exhaustion ( $7.8 \pm 1.6$ ), with subjects scoring the least in the 'immuno, gastrointestinal, and genitourinary impairments' section ( $5.9 \pm 2.7$ ). Scores that are registered as severe (i.e. with a score of 8-10) are also depicted. 60% of the ME/CFS subjects experience post-exertional neuroimmune exhaustion in a severe manner; 44% and 45% experience severe neurological and energy production/transportation symptoms, respectively; and 35% report enduring severe immuno, gastrointestinal, and genitourinary impairments. Our ME/CFS population therefore constitutes a sub-population experiencing predominantly post-exertion-related symptoms.

TEG® analysis of WB and PPP is shown in Table 2. Data for WB analysis from ME/CFS participants were assessed against a standard range of values for the TEG® analysis, as provided by TEG® guidelines. Data for PPP from ME/CFS participants were compared to those of the controls. With regards to the TEG® analysis of ME/CFS WB, no subjects fell outside of the normal range for TMRTG and TTG parameters, but our data suggests that WB from participants with ME/CFS fell outside the normal ranges for R, K,  $\alpha$  angle, MA and MRTG parameters (see Table 2). R and K angles reflect time-dependent properties, which indicate that our ME/CFS population clots at a rate higher than what is considered normal. ME/CFS participants exhibited larger and stronger clots (MA), which formed at a rate higher than controls (MRTG).

In PPP, significant differences between the control and ME/CFS groups were only observed within the  $\alpha$  angle (\*\*) and MRTG (\*\*\*) assessments, where the ME/CFS group exhibited higher clotting values, again pointing to a hypercoagulable state.

Figure 1A shows typical fluorescence micrographs of fibrinoid presence in PPP (without added thrombin) from a cohort of 25 participants with ME/CFS and from 15 healthy participants. Figure 1B shows a micrograph plate of a clot grading system that we developed<sup>47</sup>. PPP smears from the control group express little ThT signal, whereas the ME/CFS smears exhibit substantial fluorescence signals. Figure 3A represents the fluorescence signal of fibrinoid micrographs as mean % area of amyloid signal. A nonparametric Mann-Whitney test was performed, which indicated a significant difference ( $p < 0.0001$ ) between the control ( $0.10 \pm 0.54$ ) and ME/CFS ( $1.37 \pm 3.05$ ) mean % area amyloid. These qualitative and quantitative data suggests that fibrinoid burden is notably greater in this study's ME/CFS population when compared to the controls.

We also studied amyloid clot formation where thrombin was added to citrated PPP exposed to ThT, to form an extensive fibrin network. Figure 2 shows representative micrographs of healthy and ME/CFS fibrin networks. We also calculated the mean fluorescence intensity of the fluorescent signal (Figure 3B). Fibrin networks created from PPP of participants with ME/CFS exhibited a much increased fluorescence signal compared to that of healthy participants, with control and ME/CFS mean fluorescent intensity valued at  $0.11 \pm 0.19$  and  $1.69 \pm 1.69$  (\*\*\*\*), respectively.

Platelet morphology was also studied after adding two fluorescent platelet markers, namely PAC-1 (FITC-conjugated), which recognizes glycoprotein IIb/IIIa, and CD62P (PE-conjugated), which binds P-selectin (see Figure 4A). Platelet scoring was done as per our previously developed scoring system (see Figure 4B and C)<sup>47</sup>. The platelet populations from participants with ME/CFS exhibited a hyperactivated phenotype with significant spreading and granule release ( $2.72 \pm 1.24$ ). Comparably, control platelets are scored with a value of 1.00. 80% of ME/CFS haematocrit samples exhibited this phenotype, with 48% scoring 3 or 4 (severe end of platelet scoring range). Platelet clumping was observed ( $2.04 \pm 1.21$ ) in 52% of subjects, with only 32% scoring 3 or 4.

## Discussion

We have previously demonstrated that LongCovid/PASC platelet poor plasma (PPP) contains large anomalous fibrin/amyloid deposits (microclots) and hyperactivated platelets (Pretorius et al., 2021b). Due to the substantial overlap between LongCovid/PASC and ME/CFS symptoms and etiology we performed a series of experiments to determine if fibrinoid microclots are also present in ME/CFS plasma, and to what extent. We additionally characterized the constituent nature of identified ME/CFS fibrinoid microclots, as well as the viscoelastic properties of participant blood, which can determine the state of coagulability (hypocoagulable vs normocoagulable vs hypercoagulable)<sup>46,47</sup>.

TEG® analysis demonstrated that a high proportion of ME/CFS participants present with a hypercoagulable state (Table 2). In the WB analysis, several participants fell out of the healthy range, tipping towards the hypercoagulable side of the scale. This was noted in all parameters except TMRTG and TGG. In PPP, significant differences were identified in only the  $\alpha$  angle (\*\*) and MRTG (\*\*\*). However, mean ME/CFS PPP values for all TEG® parameters assessed leaned towards the hypercoagulable end.

Fluorescence microscopy identified fibrinaloid microclots within the haematological system of ME/CFS individuals with a burden significantly greater than that of controls (Figure 1 and Figure 3A). However, these fibrinaloid microclots are not as extensive as those we have previously documented in both acute COVID-19 and LongCovid/PASC<sup>25, 42, 51, 54</sup>. These microclots were probed for without the addition of thrombin, thereby indicating that these clot particles are likely circulating through the blood of afflicted individuals. Furthermore, these fibrinaloid microclots are amyloid in nature (as inferred by ThT staining), and as such have been shown to resist degradation via fibrinolytic means<sup>25, 42, 51, 54</sup>. These amyloid microclots may contribute to poor blood circulation and perfusion, possibly by blocking microcapillaries<sup>26</sup>, and impairing oxygen and nutrient delivery to various tissues. This provides a ready explanation for the fatigue and other symptoms experienced by individuals with ME/CFS. Furthermore, fibrinaloid microclots may contribute to further inflammation in the haematological system and at the endothelial linings of blood vessels in a feed-forward fashion.

Extensive fibrin clot networks (stained with ThT) were also induced where PPP were exposed to thrombin, and fibrin clots were assessed for the presence and load of amyloid fibrin(ogen). Note that this is different from fibrinaloid analysis as we are adding thrombin to form a clot network instead of probing for fibrinaloid microclots in the absence of exogenous thrombin – this gives us insight into the molecular profile of fibrin networks mimicking the terminal stages of the coagulation cascade. Amyloid protein changes were markedly increased in thrombin-induced fibrin clots from the ME/CFS group compared to controls.

Platelet morphology in the ME/CFS population varied across subjects, with 48% and 32% scoring as severe (3 or 4) for spreading and clumping parameters, respectively.

Together, our results indicate that clotting abnormalities are present in the haematological system of ME/CFS individuals. However, it must be noted that not all participants presented with hypercoagulable values when measured by the TEG®, and not all participants possessed platelets indicative of a hyperactivated phenotype. Further study is therefore required to determine what factors, including possible coagulation-promoting genetic variants or lifestyle/ environmental issues, may contribute to clotting/platelet activity in potential ME/CFS subsets. It is possible that identified clotting and platelet abnormalities may contribute to ME/CFS symptoms. Overzealous clotting leads to hypoperfusion of the vascular system, resulting in hypoxia. Additionally, associated inflammation damages the vasculature, further exacerbating circulatory issues. This might contribute to ME/CFS symptoms including fatigue, exercise intolerance, and cognitive impairment.



The presence of fibrinoid microclots and associated coagulation issues are present in ME/CFS, and point to a systemic vascular pathology and endothelial inflammation. Targeted therapies to address vascular and endothelial pathology may help and may be appropriate in certain individuals. Targeting vascular pathology might result in the amelioration of fatigue and other symptoms, which coincided with a decrease in fibrinoid microclot load<sup>26,47</sup>. However, it is important to note that the fibrinoid burden in ME/CFS seems to be less than that present in LongCovid/PASC<sup>25</sup>, thus caution is required when therapy in ME/CFS. Further research is required to determine if therapy targeted against vascular pathology could benefit individuals suffering from ME/CFS and presenting with hypercoagulability, significant fibrinoid microclot loads, and hyperactivated platelets.

## **Declarations**

### **Consent for publication**

All authors approved submission of the paper.

### **Availability of data and materials**

The datasets generated as well as figure micrographs analyzed during the current study are available on request.

### **Competing interests**

The authors have no competing interests to declare.

### **Funding**

Funding provided by PolyBio Research Foundation. DBK thanks the Novo Nordisk Foundation for funding (grant NNF10CC1016517). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### **Authors' contributions**

JMN: Sample and data analysis; wrote the paper; EP: Edited the paper, funding, co-corresponding author; study leader; AK: control participant identification, collection and screening; DBK: Edited the paper, funding, co-corresponding author. AP: Edit the paper.

### **Acknowledgements**

We would like to thank the patients and their families who participated in this study. We specifically like to thank Retha Viviers Founder of the South African ME/CFS foundation. Without her perseverance and support this study would not have been possible.

## **References**

1. Basted AC, Marshall LM. Review of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: an evidence-based approach to diagnosis and management by clinicians. *Rev Environ Health* **30**, 223–249 (2015).
2. Clayton EW. Beyond myalgic encephalomyelitis/chronic fatigue syndrome: an IOM report on redefining an illness. *JAMA* **313**, 1101–1102 (2015).
3. Johnston S, Brenu EW, Staines D, Marshall-Gradisnik S. The prevalence of chronic fatigue syndrome/myalgic encephalomyelitis: a meta-analysis. *Clin Epidemiol* **5**, 105–110 (2013).
4. Lim EJ, Ahn YC, Jang ES, Lee SW, Lee SH, Son CG. Systematic review and meta-analysis of the prevalence of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). *J Transl Med* **18**, 100 (2020).
5. Jason LA, Mirin AA. Updating the National Academy of Medicine ME/CFS prevalence and economic impact figures to account for population growth and inflation. *Fatigue: Biomedicine, Health & Behavior* **9**, 9–13 (2021).
6. Mirin AA, Dimmock ME, Jason LA. Updated ME/CFS prevalence estimates reflecting post-COVID increases and associated economic costs and funding implications. *Fatigue: Biomedicine, Health & Behavior*, 1–11 (2022).
7. Chia J, Chia A, Voeller M, Lee T, Chang R. Acute enterovirus infection followed by myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and viral persistence. *J Clin Pathol* **63**, 165–168 (2010).
8. Kerr J. Early Growth Response Gene Upregulation in Epstein-Barr Virus (EBV)-Associated Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). *Biomolecules* **10**, (2020).
9. Shikova E, *et al.* Cytomegalovirus, Epstein-Barr virus, and human herpesvirus-6 infections in patients with myalgic small ie, Cyrillicncephalomyelitis/chronic fatigue syndrome. *J Med Virol*, (2020).
10. Ariza ME. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: The Human Herpesviruses Are Back! *Biomolecules* **11**, (2021).
11. Rasa S, *et al.* Chronic viral infections in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *J Transl Med* **16**, 268 (2018).
12. Proal A, Marshall T. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome in the Era of the Human Microbiome: Persistent Pathogens Drive Chronic Symptoms by Interfering With Host Metabolism, Gene Expression, and Immunity. *Front Pediatr* **6**, 373 (2018).
13. Maes M, Twisk FN, Kubera M, Ringel K, Leunis JC, Geffard M. Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome. *J Affect Disord* **136**, 909–917 (2012).
14. Giloteaux L, Goodrich JK, Walters WA, Levine SM, Ley RE, Hanson MR. Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome* **4**, 30 (2016).
15. Hickie I, *et al.* Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ* **333**, 575 (2006).

16. Raijmakers RPH, *et al.* Multi-omics examination of Q fever fatigue syndrome identifies similarities with chronic fatigue syndrome. *J Transl Med* **18**, 448 (2020).
17. Keijmel SP, *et al.* A comparison of patients with Q fever fatigue syndrome and patients with chronic fatigue syndrome with a focus on inflammatory markers and possible fatigue perpetuating cognitions and behaviour. *J Psychosom Res* **79**, 295–302 (2015).
18. Al-Aly Z, Bowe B, Xie Y. Long COVID after breakthrough SARS-CoV-2 infection. *Nat Med*, (2022).
19. Wong TL, Weitzer DJ. Long COVID and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)-A Systemic Review and Comparison of Clinical Presentation and Symptomatology. *Medicina (Kaunas)* **57**, (2021).
20. Hunt J, Blease C, Geraghty KJ. Long Covid at the crossroads: Comparisons and lessons from the treatment of patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *J Health Psychol*, 13591053221084494 (2022).
21. Morrow AK, *et al.* Long-Term COVID 19 Sequelae in Adolescents: the Overlap with Orthostatic Intolerance and ME/CFS. *Curr Pediatr Rep*, 1–14 (2022).
22. Siberry VGR, Rowe PC. Pediatric Long COVID and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Overlaps and Opportunities. *Pediatr Infect Dis J* **41**, e139-e141 (2022).
23. Kedor C, *et al.* Chronic COVID-19 Syndrome and Chronic Fatigue Syndrome (ME/CFS) following the first pandemic wave in Germany – a first analysis of a prospective observational study. medRxiv, 2021.2002.2006.21249256 (2021).
24. Proal AD, VanElzakker MB. Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. *Front Microbiol* **12**, 698169 (2021).
25. Pretorius E, *et al.* 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 (2021).
26. Kell DB, Laubscher GJ, Pretorius E. A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications. *Biochem J* **479**, 537–559 (2022).
27. Kennedy G, Norris G, Spence V, McLaren M, Belch JJ. Is chronic fatigue syndrome associated with platelet activation? *Blood Coagul Fibrinolysis* **17**, 89–92 (2006).
28. Brenu EW, *et al.* Immune and hemorheological changes in chronic fatigue syndrome. *J Transl Med* **8**, 1 (2010).
29. Brewer JH, Berg D. Hypercoaguable State Associated with Active Human Herpesvirus-6 (HHV-6) Viremia in Patients with Chronic Fatigue Syndrome. *Journal of Chronic Fatigue Syndrome* **8**, 111–116 (2001).
30. Berg D, Berg LH, Couvaras J, Harrison H. Chronic fatigue syndrome and/or fibromyalgia as a variation of antiphospholipid antibody syndrome: an explanatory model and approach to laboratory diagnosis. *Blood Coagul Fibrinolysis* **10**, 435–438 (1999).
31. Bonilla H, *et al.* Comparative Analysis of Extracellular Vesicles in Patients with Severe and Mild Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Front Immunol* **13**, 841910 (2022).

32. Newton DJ, Kennedy G, Chan KK, Lang CC, Belch JJ, Khan F. Large and small artery endothelial dysfunction in chronic fatigue syndrome. *Int J Cardiol* **154**, 335–336 (2012).
33. Scherbakov N, *et al.* Peripheral endothelial dysfunction in myalgic encephalomyelitis/chronic fatigue syndrome. *ESC Heart Fail* **7**, 1064–1071 (2020).
34. Bertinat R, Villalobos-Labra R, Hofmann L, Blauensteiner J, Sepulveda N, Westermeier F. Decreased NO production in endothelial cells exposed to plasma from ME/CFS patients. *Vascul Pharmacol* **143**, 106953 (2022).
35. Lopes Pires ME, Clarke SR, Marcondes S, Gibbins JM. Lipopolysaccharide potentiates platelet responses via toll-like receptor 4-stimulated Akt-Erk-PLA2 signalling. *PLoS One* **12**, e0186981 (2017).
36. Li C, Li J, Ni H. Crosstalk Between Platelets and Microbial Pathogens. *Front Immunol* **11**, 1962 (2020).
37. Page MJ, Pretorius E. A Champion of Host Defense: A Generic Large-Scale Cause for Platelet Dysfunction and Depletion in Infection. *Semin Thromb Hemost* **46**, 302–319 (2020).
38. Antoniak S, Mackman N. Platelets and viruses. *Platelets* **32**, 325–330 (2021).
39. Branchford BR, Carpenter SL. The Role of Inflammation in Venous Thromboembolism. *Front Pediatr* **6**, 142 (2018).
40. Pretorius L, Thomson GJA, Adams RCM, Nell TA, Laubscher WA, Pretorius E. Platelet activity and hypercoagulation in type 2 diabetes. *Cardiovasc Diabetol* **17**, 141 (2018).
41. Aksu K, Donmez A, Keser G. Inflammation-induced thrombosis: mechanisms, disease associations and management. *Curr Pharm Des* **18**, 1478–1493 (2012).
42. Kell DB, Pretorius E. The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen). *Integr Biol (Camb)* **7**, 24–52 (2015).
43. Bester J, Soma P, Kell DB, Pretorius E. Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). *Oncotarget* **6**, 35284–35303 (2015).
44. Nunes JM, *et al.* Gingipain R1 and Lipopolysaccharide From *Porphyromonas gingivalis* Have Major Effects on Blood Clot Morphology and Mechanics. *Front Immunol* **11**, 1551 (2020).
45. Abou-Ismaïl MY, Diamond A, Kapoor S, Arafah Y, Nayak L. The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. *Thromb Res* **194**, 101–115 (2020).
46. Pretorius E, Swanepoel AC, DeVilliers S, Bester J. Blood clot parameters: Thromboelastography and scanning electron microscopy in research and clinical practice. *Thromb Res* **154**, 59–63 (2017).
47. Laubscher GJ, Lourens PJ, Venter C, Kell DB, Pretorius E. TEG((R)), Microclot and Platelet Mapping for Guiding Early Management of Severe COVID-19 Coagulopathy. *J Clin Med* **10**, (2021).
48. Carruthers BM, *et al.* Myalgic encephalomyelitis: International Consensus Criteria. *J Intern Med* **270**, 327–338 (2011).

49. Varin R, *et al.* Whole blood clots are more resistant to lysis than plasma clots—greater efficacy of rivaroxaban. *Thromb Res* **131**, e100-109 (2013).
50. Gade Malmos K, *et al.* ThT 101: a primer on the use of thioflavin T to investigate amyloid formation. *Amyloid* **24**, 1–16 (2017).
51. 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. *Prog Biophys Mol Biol* **123**, 16–41 (2017).
52. Pretorius E, Page MJ, Engelbrecht L, Ellis GC, Kell DB. Substantial fibrin amyloidogenesis in type 2 diabetes assessed using amyloid-selective fluorescent stains. *Cardiovasc Diabetol* **16**, 141 (2017).
53. Pretorius E, Mbotwe S, Bester J, Robinson CJ, Kell DB. Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. *J R Soc Interface* **13**, (2016).
54. Grobbelaar LM, *et al.* SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. *Biosci Rep* **41**, (2021).

## Tables 1-2

Tables 1-2 are available in the Supplementary Files section.

## Figures

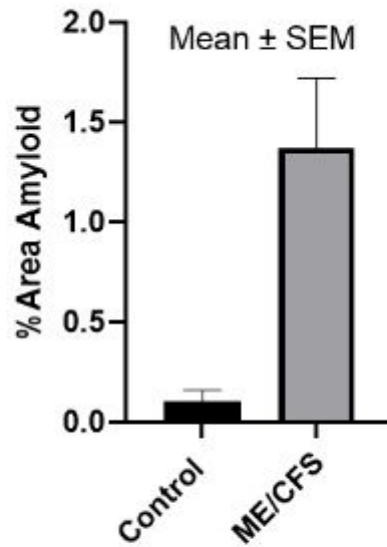
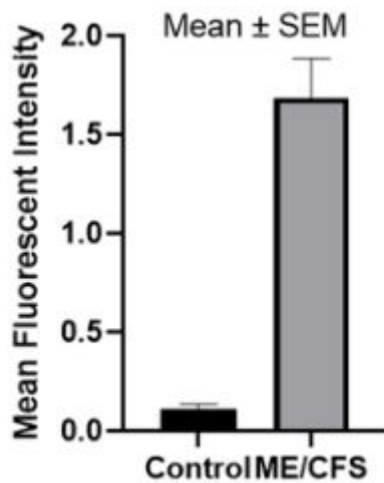
### Figure 1

**A:** Representative fluorescent micrographs of fibrinaloid microclot presence in platelet poor plasma from controls and individuals with ME/CFS. Images were taken at 63x machine magnification.

**B:** Fluorescence microscopy showing fibrinaloid microclots in platelet poor plasma (PPP) with representative examples of the different stages of different stages of fibrinaloid microclot formation. Stage 1 shows minimal microclot formation in healthy/control PPP which progresses to the presence of the severe microclotting Stage 4. Bottom row represents examples of stage 4 microclots using (A) bright-field microscopy, (B) fluorescence microscopy, and (C) an overlay of fluorescence and bright-field microscopy<sup>47</sup>.

### Figure 2

Representative micrographs showing thrombin-induced fibrin networks stained with ThT from healthy participants and participants with ME/CFS. Images were taken at 63x machine magnification.

**A****% Amyloid Area of ThT-Stained PPP****B****Mean Fluorescent Intensity of Thrombin-Induced PPP Clots Stained with ThT****Figure 3**

**A)** Mean % area of amyloid signal between control and ME/CFS groups represented as mean  $\pm$  SEM. A Mann-Whitney analysis yielded a significant difference ( $p < 0.0001$ ) with the ME/CFS group exhibiting a greater mean (1.37) than that of the controls (0.10). **B)** Difference in mean fluorescence signal between control ( $0.11 \pm 0.19$ ) and ME/CFS ( $1.69 \pm 1.69$ ) PPP fibrin amyloid networks induced by exogenous thrombin. A significant difference (\*\*\*\*) was determined by a Mann-Whitney test. Data is represented as mean  $\pm$  SEM.

**Figure 4**

**A:** Representative fluorescent micrographs of hematocrit samples from ME/CFS individuals stained with PAC-1 (green fluorescence); CD62P-PE (purple fluorescence); white areas represent overlap of the two markers. Images were taken at 63x magnification.

**B:** Fluorescence microscopy examples of the different stages of platelet activation and spreading, that was used to score the platelet activation in the Long COVID patients, with Stage 1, with minimally activated platelets, seen as small round platelets with a few pseudopodia, seen as healthy/control platelets that progresses to Stage 4, with egg-shaped platelets, indicative of spreading and the beginning of clumping <sup>47</sup>.

**C:** Fluorescence microscopy examples of the different stages of platelet clumping. With no clumping occurring in the healthy/control samples in Stage 1 (no figures shown), progressing to severe clumping of platelets as seen in Stage 4 <sup>47</sup>.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.png](#)
- [Table2.png](#)