Responses to Participants' Questions

The overarching goal of the R3 Seminars is to catalyze a shared understanding of the research being conducted by the scientific stakeholder community within the RECOVER Consortium. The R3 Seminars and the Q&As typically feature highly scientific material intended for researchers and clinicians. For other audiences interested in these topics, a link to the National Library of Medicine's MedlinePlus medical dictionary is provided at the end of the Q&As as a resource to help in understanding the scientific terminology.

This document provides responses* to questions raised by webinar participants related to the following presentations at the R3 Seminar ***Clinical Spectrum of PASC: Focus on Coagulapathies*** held on September 13, 2022:

- **Presentation 1: *Clotting and Platelet Pathology in Long COVID***

  Resia Pretorius, PhD

- **Presentation 2: *Long COVID-19: Role for Anticoagulants?***

  Jean Connors, MD

- **Presentation 3: *Why Should We Consider Antiplatelet Therapy in PASC?***

  Jeffrey Berger, MD, MS

- **Discussant: Shari Brosnahan, MD**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. For those with post-COVID commencing with the first wave in January 2020, are you (1) seeing higher rates of multisystem organ dysfunction, and (2) what testing is best for microclots for this patient group?

Responses:

Dr. Brosnahan: I think we're not necessarily seeing as much organ dysfunction as we were seeing in the first wave. This goes to Dr. Connor's point of how well vaccination has mitigated the inflammation. But I do think we're seeing some episodes of venous thromboembolism (VTE) come back in this current wave. I would say that I've seen ambulatory patients come with VTE, which had maybe been mitigated with the first wave of omicron. But as far as PASC goes and the microclots, I think that's a question for the panelists.

Dr. Pretorius: Currently, there are no methods except a microscope method to look for microclots. However, as I mentioned, the next step will be a flow cytometry method. Also, a microplate method has been developed with great success at the University of Manchester to find microclots more easily than with a fluorescent microscope. So, hopefully within the next months, we'll have various methods developed and tested and be able to compare symptoms with the presence of these microclots.

Dr. Berger: Personally, I'm fascinated with the microclots, and I think it's something that sounds very intriguing and interesting. I want to make a comment about the difference between all these potential biomarkers and excess risk. There are a lot of biomarkers that have been proposed. I'm intrigued by microclots. As it relates to treatment, there's a difference between showing an association between something and long-term outcomes, as compared with making a clinical decision for a particular therapy that has potential complications. That really needs to be the next set of studies, which is saying, "Okay, I see this. So, now let me see whether acting on this information will improve outcomes." I truly hope we get there, but I want to remind people that this is a very important missing step.

Dr. Connors: I'm glad you brought that up Dr. Berger because I think that's why I was trying to make my message so strong. We don't have good enough data to act on anticoagulating patients with therapeutic dose anticoagulation who have Long COVID, regardless of their biomarkers. And when I look at this, I'm fascinated as well by the microclots and what's driving them, and I think research absolutely needs to be directed toward the underlying pathophysiology. I'm currently on inpatient attending, and we have some thrombotic microangiopathy patients. We have a TTP (thrombotic thrombocytopenic purpura) and we have an atypical HUS (hemolytic-uremic syndrome); they all have microvascular thrombosis. We understand the mechanisms and the treatments do not involve anticoagulation. To Dr. Berger's point, we're not ready for prime time to act on these. I absolutely don't want my talk to be interpreted as "we shouldn't go down the road of looking at thrombosis"; however, we're not ready to give these Long COVID patients or PASC patients anticoagulation.

To add to your comment Dr. Pretorius, there were trials of tPA (tissue plasminogen activator) in patients with acute COVID and I reviewed them for the journals. A lot of them are small series and case reports. Intubated patients with bad respiratory compromise had transient improvements when given thrombolytic therapy. But when the minute therapy is stopped, they succumbed to their severe COVID. So, I think how we balance the proclotting and the anticlotting and the fibrinolytic phases are going to be other areas we need to investigate further.

Dr. Pretorius: I totally agree with both Drs. Berger and Connors. We need further investigations, further trials, and various combinations looking at various other products that we could combine before we can tell anyone to use these products, as it can be very dangerous. If you don't have a coagulation problem and you use anticoagulants and clot-busting molecules or products, it can be dangerous. So, we need further trials, and I believe three trials are starting in the UK and the US as well to look at a combination of antiplatelet and clot-preventing products to treat Long COVID. But before one can even go that route, one must wait for the outcomes. That is very important to note.

Dr. Connors: There was a great trial called ASPEN-COVID that looked at a compound called rNAPc2, an anticoagulant that inhibits tissue factor. It didn't have an effect compared with placebo. So again, we need a greater understanding of the mechanism. And I think to my complicated picture, we need to start at the inciting agents, because as I tell my inpatient team, all roads lead to thrombosis. So, keep doing that work and investigating those causes.

Q. There are peer-reviewed publications that show increased blood cell size and decreased deformability in COVID. Could this contribute to the capillary stress/inflammation and tissue hypoxia given that blood cells are known to pass single file through the capillaries? Can capillaries "stretch" to accommodate these larger and more rigid blood cells?

Responses:

Dr. Connors: As a hematologist, I'll start by saying there are a lot of reasons patients can have larger blood cells, including if they've had suppression and then they have reticulocytosis. But there can also be changes in the membrane components. Red blood cells scavenge nitric oxide, so that can be in our reservoirs. So, there can be a lot of imbalances that have to do with vascular tone. We're very familiar with these dynamics in patients who have sickle cell anemia where the cells become so rigid that they can't transverse the capillaries. So, I think this is an interesting concept. But, outside of sickling, even the larger that some cells get the more floppy and deformable they actually are. Again, this is another area that could be investigated.

Dr. Pretorius: I'd like to add that a group from one of the Max Planck institutes in Germany has developed a flow instrument where they looked at flow rate of red blood cells and deformability, and they did find changes in red blood cells and the deformability of all the immune cells. They also found microclots in trapping these highly deformable red blood cells. So, there definitely is a possibility that those red blood cells will not carry oxygen as they should and the perfusion over the endothelial layers carrying oxygen to the cells might be impaired carrying oxygen into tissues. So, that's an interesting phenomenon that should be studied. And that might be one of the reasons for the widespread organ damage that we note in Long COVID.

Dr. Berger: I'm not a hematologist, but I've been intrigued that in the past 5 years or so I've seen a lot of publications about a biomarker called RDW (red cell distribution width), and I wonder if any sort of pathological condition appears to be associated with it. I think it's fascinating. To be honest, I don't understand it well but I wonder if it's related to this. I know I've seen it in COVID as well. So, I wonder if that may shed any light on the discussion that everyone is talking about now.

Dr. Connors: I think it's hard to tease out because RDW is the distribution of sizes of the red cells and it's usually in a very narrow range of normal. Anything can perturb that. You have a GI bleed, you have an increased RDW because your reticulocytes are bigger. In an acute illness or acute infection, we have problems with erythropoiesis (the production of red blood cells), we have anemia of inflammation, and that changes the RDW. The MCV (mean corpuscular volume) goes down. You transfuse, you've got different MCV, you've got different RDW. So, I think it's hard to tease out. I think it's a fascinating area. We do know that red cells get trapped in clots all the time. And Dr. Berger, with your multiple associations, I'm always seeing surgeons talking about red clots and white clots. And, so, it's like, "Well yeah, okay, so red cells get trapped. We know."

But I think it's fascinating because there may be something more that COVID does. Why did SARS-CoV-2 wreak havoc across the world? No one has immunity to it. And I think people who get more severe disease or people that might have persistent disease may just not be able to handle the inflammation associated with it. They have an exaggerated response, or they can't shut it off. As we learn more about TMAs (thrombotic microangiopathies), we recognize that it may be a complementopathy; for example, how atypical HUS is actually a problem with complement regulatory genes. Getting back to the red cells, many things that affect erythropoiesis and they are a barometer or a marker for problems. But I'm not sure how much they themselves contribute, unlike platelets, monocytes, neutrophils, and extra cellular traps, for example.

Q. Does inflammation map to the microcloting observed by Dr. Pretorius if coagulation factors are not mapping the observed microclots, relative to Dr. Connor's observations?

Dr. Pretorius: We've shown that many of the inflammatory markers are in fact trapped inside microclots, and conventional blood pathology tests might be looking for them in the soluble part of the blood. We still need to find methods to study and determine the inflammatory molecule content of the microclots using easier methods than proteomics, which is the method we first used to find these molecules inside the microclots.

Q. Dr. Pretorius, are you seeing any change in the deformability of red blood cells in Long COVID patients? If so, what role does this play in the development of microclots? As vaccine-injured patients often have symptoms of Long COVID, what do you think is driving that response in this select group of patients?

Response:

Dr. Pretorius: Together with our collaborators from the Max Planck institute for Science of Light, we plan to study red blood cell deformability and interactions with platelets, microclots, and white blood cells. They've custom built a microfluidics instrument for my lab, and we hope to receive it soon. We've also received a South African Medical Research Council grant to study vaccine injury and we'll share the results as soon as we have them. Spike protein is probably an important factor.

Q. Have patients who are already on anticoagulants been looked at in relation to rates of onset of Long COVID?

Response:

Dr. Brosnahan: Studies are underway to investigate if the rate of anticoagulation affects the incidence of Long COVID.

Q. Is there a specific biomarker primary care physicians can use to screen for increased risk of clotting, such as D-dimer?

Response:

Dr. Brosnahan: There is no known biomarker to account for increased thrombotic risk in these patients. D-dimer elevation has been found in other cohorts to sometimes correlate with increased thrombotic risk, but it cannot be used as a screening tool for this, as some people are at increased risk but with normal D-dimer.

Q. What are the chances of resolution of antiphospholipid antibody syndrome developed post COVID?

Response:

Dr. Brosnahan: Once someone is diagnosed with antiphospholipid syndrome, there is no cure of this condition.

Q. Has there been an increased incidence of the diagnosis of coagulation disorders, including antiphospholipid syndrome?

Response:

Dr. Brosnahan: Despite early interest, the predominant mechanism of increased thrombotic risk on COVID does not appear to be antiphospholipid syndrome.

Q. Have there been increases in provoked and unprovoked deep vein thrombosis in patients with PASC?

Response:

Dr. Brosnahan: Studies have shown an increased thrombotic risk of both deep vein thrombosis and pulmonary embolism in a cohort of patients one year after an acute COVID Infection.

Q. What do Dr. Pretorius and the panel think about Bruce Patterson's work with statins, aspirin, and maraviroc to avoid hyperactivation of platelets and overall treatment for PASC patients?

Response:

Dr. Pretorius: Dr Patterson's work shows potential and I'm looking forward to seeing the published results from his group.

Webinar Slides

To request a copy of the R3 Webinar slides, please email RECOVER_ACC@rti.org

To Learn More

- Information about RECOVER research and to volunteer for studies: https://recovercovid.org/research
- Frequently Asked Questions about RECOVER and PASC: https://recovercovid.org/faqs
- CDC information: Information for the general public and for healthcare providers about post-COVID conditions: https://www.cdc.gov/coronavirus/2019-ncov/long-term-effects/
- For medical/scientific terminology: https://medlineplus.gov/healthtopics.html