Transcript

Claire Quiner

Good afternoon everyone and welcome to the RECOVER Research Review or R3 seminar. My name is Claire Quiner and I'm an epidemiologist with the RECOVER Administrative Coordinating Center. I'm the moderator of today's seminar, Clinical Spectrum of PASC: Focus on Coagulopathies. The goal of this seminar series is to catalyze the shared understanding of the research within the RECOVER Consortium. This is not intended to provide clinical guidance, rather the presentations and conversations today pertain to the research realm. Our presenters today will be unable to answer clinical or treatment questions, but will be prepared to discuss and answer questions pertaining to research in their field.

Before we move into introductions, I'd like to thank everyone who submitted questions in advance. Please submit any questions that arise during today's presentation using the Q&A feature in Zoom. During the presentation, we'll answer as many questions as possible. Some questions may also be answered within the Q&A feature on Zoom. After the seminar will post on recovercovid.org an FAQ with the recording of the seminar as well as answers for submitted questions relevant to today's presentation. Questions about other scientific topics will be addressed in future seminars and answers to broader questions about RECOVER will be available in the FAQs at recovercovid.org.

We have a great set of presenters today and I'd like to provide a brief bio of each of them, beginning with Resia Pretorius. She is a researcher, distinguished professor and chair of the Department of Physiological Sciences at Stellenbosch University in South Africa. Dr. Pretorius has published or has impressed 305 journal articles, she currently serves on the editorial board of two nature journals and is also a WHO panel member for expanding our understanding of post COVID-19 condition. She's filed numerous patents and she is the managing director of Bio Code Technology, a Stellenbosch University startup Company.

Moving on to Dr. Jean M. Connors. She is a hematology attending at Brigham and Women's Hospital and Dana Farber Cancer Institute. She's the medical director of the Anticoagulation Management Services and the Hemostatic Antithrombotic Stewardship Program and is also an associate professor of medicine at the Harvard Medical School. Dr. Connors serves as an associate professor for the Journal of Thrombosis and Hemostasis and the Journal of American College of Cardiology, Basic to Translational Science. She's participated in numerous clinical trials for patients with venous thrombosis in cancer and anticoagulation questions in general.

Moving on to Dr. Jeffrey Berger. He is an associate professor of medicine and surgery with tenure appointments in cardiology, hematology and vascular surgery and is the director of both cardiovascular thrombosis and the Center for the Prevention of Cardiovascular Disease at the NYU Langone Health. His laboratory studies cell biological processes relevant to atherosclerosis, thrombosis and platelet biology across different types of phenotypes of cardiovascular diseases.

Last but not least, we have a fabulous discussant today, Dr. Shari Broshnahan, who is an assistant professor of medicine and associate program director for the pulmonary and critical care fellowship at NYU School of Medicine. Her research is focused on venous thromboembolism, investigating factors which contribute to thrombotic risk as well as response to therapy. Today's speakers will share our current understanding in the gaps and in knowledge and how RECOVER will contribute to filling these gaps. With that, I'd like to hand it over to Dr. Shari.

Dr. Shari Brosnahan

Thank you guys so much for coming today. I'm very excited to start all this off. I think, we're going to first hear from Dr. Pretorius who will be talking to us about clotting, platelet pathology and long COVID. So, here we go.

Dr. Resia Pretorius

Hello everyone. Today I will be sharing with you our latest research on clotting pathologies and will spotlight our recent findings in COVID-19 and long COVID. I wish to acknowledge my research collaborators which include Professor Douglas B. Kell from the University of Liverpool, numerous clinicians, the hematologists and a team of biochemists. Postgraduate students as well as data scientists from the University and Mediclinic private hospital group approved sample collection at the facility and we received ethics approval for all the studies we are discussing here.

My lab and our collaborators from various labs over the world have been identifying inflammatory molecules in circulation that might be involved in and drive pathological clotting and we have also focused our research endeavors on studying the effects of increased circulating inflammatory molecules and how they interact with cells of the hematological system. We focus in particular on platelets and red blood cells, as well as on the clotting protein fibrinogen. We are also interested in identifying novel inflammatory molecules that might play a role in persistent symptoms of long COVID.

Over the years, we have published numerous papers that show the importance of inflammatory molecules in circulation and the role in abnormal blood clotting. I'm interested in platelet signaling and the role in abnormal clotting. Platelets in circulation play a critical role in healthy blood clotting. However, they can become overstimulated and can drive pathological blood clotting if there are inflammatory molecules in circulation. This can also happen in the presence of viral infections where platelets can act as important signaling entities. There's always also a complex relationship between the receptors on platelets and endothelial cells which circulating biomarkers and inflammatory molecules may bind to. As we now know, damaged endothelial cells and platelet hyperactivation are central in COVID pathologies. Our research group have also shown that these pathologies are in acute COVID and we've published it in various papers. In the context of COVID-19, platelets are therefore central

in immune activation and general coagulation pathology, and they can form various complexes and obviously also between cells. This is known as platelet clumping.

We are also interested in pathological blood clotting involving the main clotting protein fibrinogen, which is a soluble protein in circulation. If you focus your attention to the two cartoons shown in the figure there. On the left is a healthy protein structure with many alpha coils and few beta sheets. However, in the presence of inflammation, oxidative stress and circulating inflammatory molecules, this structure changes, where the alpha coils and twists into beta sheets as seen in A in the cartoon on the right. In plasma clots from healthy individuals, these clots have more alpha coils and have the ultra structure as shown in figure B. Various inflammatory conditions including cardiovascular disease and diabetes have a clot structure similar as shown in figure C. Please note thrombin was added to platelet pro plasma in B and C.

We have looked at blood clotting in acute COVID using scanning in electron microscopy. We found that platelets are damaged and clumped together and attached to red blood cells. The clotting protein that is supposed to be soluble spontaneous micro clots. In the micrographs on the far left you can see platelets that are hyperactivated. Note the yellow arrows. In the middle are micrographs where platelets are attached to red blood cells. Note the white arrows. On the far right you can see micrographs of spontaneously formed microclots that even glue together red blood cells. Note the blue arrows.

Now, with this ultra structural pathology in mind, we looked at platelet pro plasma and exposed it to a fluorescent marker called thioflavin T. It binds to open hydrophobic areas on damaged protein. This marker was first used to identify amyloid protein in the brains of Alzheimer's. In 2014 however, our research group discovered that thioflavin T also binds to misfolded fibrogen and fiber protein during pathological blood clotting. Here you can see the differences in platelet pro plasma protein structure in acute COVID-19 patients where we compare plasmas of acute COVID-19 to that of healthy and diabetic patient plasma samples. Here you can see microclot formation demarcated as the green signal from thioflavin T.

We found small areas of nearly undetectable abnormal clotlets that are present in plasma of healthy individuals as can be seen in the micrographs on the left. More abnormal micro clots are noted in plasma with of individuals with diabetes and also we found significant micro clot formation in acute COVID plasma seen in the far right. Also, note the scale of 10 micrometers at the bottom of the micrographs. As microscopy results are difficult to quantify, we suggested a micro clot and platelet grading system based on various stages of severity of microclot activation and based on various stages of platelet activation.

Here are examples of platelet grading system that we used. In our experiments, the microclot samples were exposed to two fluorescent platelet markers, CD62P, that is a pinkish signal and it's the marker for P-selectin that is either inside platelets on the membranes, or it can be found as shed from platelets as a soluble marker P-selectin. We also used PAC-1, which is the green signal that identifies platelets through the marking of the glycoprotein IIb/IIIa, which are on the platelet membranes. The micrographs plate on the left and the top row

shows examples of platelets from healthy control samples with minimally activated platelets. This is plate one. This plate shows increased platelets spreading and the beginning of clumping. The micrograph plate on the right shows platelet clumping. Here is our microclot grading system shown on the left. Stage one demarcates minimal microclot formation as found in healthy samples. We also suggest the various stages can be used as a numerical scoring system that might be used in both acute and long COVID.

Now with our knowledge of acute COVID-19, we also turned our attention to long COVID. We looked at blood samples from patients and soon realized that there are persistent microclots and widespread endothelial dysfunction and platelet dysfunction in these patients too. These pathologies may be central in causing the widespread symptoms in these patients and may lead to tissue hypoxia. We initiated a South African Long COVID registry in 2021. Here are some of the symptoms noted in long COVID patients and this is very similar to have to what has been found in the rest of the world. Brain fog, concentration issues, forgetfulness being a group of symptoms that were most prevalent in these individuals.

We also looked at comorbidities and we found high blood pressure, high cholesterol, rheumatoid arthritis and type 2 diabetes, the prevailing comorbidities in this patient group.

Here are some of the other results from participants with long COVID. On the left you can see the platelets from healthy participants and you can also see on the right, platelets from patients suffering from long COVID. Large platelet clumps can be seen in the long COVID population. We then, also planned an experiment where we looked at proteomics of healthy plasma versus type 2 diabetes, acute COVID and long COVID. We added diabetes samples as we know that people suffering from diabetes are more prone to severe COVID 19 symptoms. For proteomics analysis, we prepared platelet pro plasma and we followed it by our first trypsin digestion step. To our surprise, we found a visible deposit at the bottom of the Eppendorf tube in the acute COVID-19 and long COVID samples, but not in the diabetes and also not in control samples. Plasma proteins were therefore fully digested in controls in diabetes suggesting that trypsin could degrade all or then at least most of the plasma proteins in both the controls and the diabetes.

For proteomics, the sample was further filtered and we followed a second trypsin digestion step where we could solubilize the undigested pellet. We analyzed one microgram of protein of the supernatant and also the equivalent from the digested pellet deposit. We then viewed the unfiltered supernatant after the first trypsin step using fluorescent microscopy. We found that patients with type 2 diabetes and plasma from healthy samples digested fully and we couldn't find any signal through the micrographs in the top left. However, both microclots in were found in the groups of both acute COVID as well as long COVID.

Then we followed through with our digestion step and we looked at the content of these now digested clots. We detected various inflammatory molecules that were substantially increased inside the digested microclots. Of particular interest was a substantial increase in α2-antiplasmin and in fibrinogen alpha chains that retracts in-desolubilized fibrotic resistant microclots.

Here is the clotting cascade, just for those of you that are interested. Of particular interest to us was the fact that α2-antiplasmin prevents plot breakdown. We just completed a large proteomics study where we looked at 100 long COVID samples and 30 controls. Here are some of our most note really findings which include a down regulation of kallikrein, with an upregulation of Platelet Factor IV and Von Willebrand factor. And again, a marginal upregulation of α2-antiplasmin alpha. If you turn your attention to the clotting pathway diagram where these molecules may play a significant role, it again points to decrease fibrosis and eventually a fibrinolytic failure.

We also found a significant presence of antibodies trapped inside the microclots and pathologies noted in postviral syndromes, we therefore think have a definite golden thread running through them. Our findings suggest that hypercoagulation and vascular damage are key role players causing the various symptoms in these patients.

I wish to turn your attention to February 22 and the US government document where microclot presence, viral systems and auto antibodies together with organ damage were recognized as central pathologies to look into, in finding answers for long COVID.

I'm also very happy to report that our work was featured in 2020 June in Science and also, recently we were featured in Nature. Just on the way forward diagnosis and using our methodologies together with our team of international collaborators. And here I only show a few of them. Many, many more, the list was just too long to include it here. We will report back soon on more laboratory results from long COVID patients.

I'm happy to report that researches from Sheffield Hallam, as well as University of Manchester have confirmed microclots in non-COVID patients and could correlate the degree of microclot presence to disease severity. We desperately need clinical trials on treatments and we believe that a combination of preventing microclot formation as well as preventing platelet hyperactivation would do the trick. Treating only the one or the other will possibly perpetuate vascular damage.

Just a report from the WHO panel meeting today where I was a panel member, we are currently formulating the case definition for long COVID in children. This is desperately needed as children suffer significant symptoms when they contract acute COVID and have persistent symptoms going into long COVID. So, this is an exciting new endeavor for the WHO and I hope this will be available very soon.

I thank you for allowing me to share our insights and I'm looking forward to hear all your questions and see what the rest of the panelists talk about. Thank you.

Dr. Shari Brosnahan

Thank you so much. That was truly wonderful. I enjoyed that a lot and we will come back and focus a little bit more at the end. But now, we are hearing from Dr. Connors. Thank you.

Dr. Jean M. Connors

Hello and thank you for having me here. I think I need to swap my screens here. I'm going to shift gears a bit. I'm Gene Connors and I'm going to discuss long COVID-19 from a very clinical perspective. Is there a role for anticoagulants? And so, these are my conflict of interest disclosures. My agenda is quite simple. I'm going to review data for use of anticoagulants in acutely infected COVID-19 ambulatory patients and I'm going to review what current data we have on coagulation biomarker status in both convalescing and long COVID patients. Most of this data have just become available actually in the last few months.

I have used this slide since the spring of 2020 when we first became aware that COVID-19 was associated with a coagulopathy and thrombosis and we watched early reports come out of China when this now pandemic was localized and it was clear that increased D-dimer levels we're associated with disease severity and mortality. We came to appreciate that spring, that thrombo inflammation, the crosstalk between activation of inflammation and subsequent activation of coagulation was what was really driving this coagulation or this coagulopathy and that COVID-19 is a hypercoagulable state.

We are I think, all aware that both macrovascular thrombotic events occur particularly VTE and to a lesser extent arterial thrombosis, particularly in those most severely ill and in the ICU. But, we've also seen microvascular thrombosis and particularly pulmonary microvascular thrombosis, which is thought to contribute significantly to the hypoxemia and respiratory failure as well as demonstration of endothelialitis with disrupted anticoagulant function the abnormal vascular endothelial cells provide.

I show this micrograph from the Ackermann paper et al, for two reasons. One, is to show the multitude and high number of microthrombi in the alveolar capillaries scene here on this micrograph. But also, because one of the surgeons at our institution was involved Steven Menser and he and I were discussing this paper before it came out, discussing terminology. A comment that he said at that time has struck with me ever since. He has said he has seen this type of pathology in other types of pulmonary respiratory infections, this microvascular thrombosis, but not to the widespread extent that it is seen in COVID-19. And I think that speaks to the fact that we have a population that has never been exposed to this virus and have not developed any acquired immunity whatsoever.

So, based on these thrombotic findings and the recognition that this was a hypercoagulable state, multiple randomized control trials were launched and developed to address antithrombotics across all the patient spectrums. I'm going to focus on a few outpatient trials in those that are newly diagnosed and symptomatic and with the fundamental question that we all asked and that is, will early use of anticoagulants prevent macrovascular thrombosis and most importantly mitigate COVID-19 progression by decreasing microvascular thrombosis? As we are aware now, but when we designed this trial, we did not know the magnitude of extent of COVID in the population. But it appears that now, 90% of COVID patients are not admitted to the hospital and destined for outpatient treatment.

I'm going to start with the ACTIV-4B trial, the COVID-19 outpatient thrombosis prevention trial. Paul Ridker was the trial chair, I was the trial PI. Maria Brooks was our trial statistician and this was an NHLBI/NIH funded trial, as part of the active platform.

It's a very logistically challenging outpatient trial to conduct during a pandemic when infected patients had to quarantine at home. But the keys for the data that I'm going to show are that patients had to be within age 40 to 80 years, have been diagnosed with COVID within 14 days of randomization. They were then randomized to one of four groups, a placebo and antiplatelet agent aspirin, 81 milligrams or two different doses of Apixaban, the prophylactic and the therapeutic, in order to cover all the possible antithrombotic agents that we had at our disposal.

In the 657 randomized patients, the median time from COVID diagnosis was 7 days to randomization and the median time from randomization to initiation of study treatment was 3 days, important time parameters. What we found are demonstrated here in these graphs, both the primary endpoint and the bleeding outcomes.

The primary endpoint occurred in 3.6% of all randomized participants, regardless of whether or not they initiated treatment. This was much lower than we expected. Again, I think we were not aware of how many patients in the outpatient setting truly had COVID and our denominators may have been off a bit. But, what we also noted is that almost all of the participants who had an adjudicated positive primary endpoint, many of these events occurred before initiating treatment and all had progressive COVID-19 pneumonia. This happened very quickly in the time course of developing COVID-19. Subsequently, just published in June, 2022 were the ETHIC and the OVID randomized control trials. These trials were like ACTIV-4B. They took place in outpatients that were acutely infected. They were symptomatic and both trials compared enoxaparin versus no treatment. These trials, like ACTIV-4B also closed early due to lower than anticipated event rates.

The ETHIC trials randomized participants within 9 days of a positive test. Patients were assigned to 21 days of treatment with prophylactic dose of enoxaparin. They randomized 219 patients and in each group there were 12 events. Of those randomized to enoxaparin, 6 or half of them were admitted for COVID-19 pneumonia. Again, progression of what we presume is microvascular thrombosis, as well as the other inflammatory components of COVID-19 pneumonia. The OVID trial randomized participants within 5 days of a positive test and participants were assigned to either 14 days of enoxaparin again at the prophylactic dose of 40 milligrams versus no treatment and were assessed at 30 days. Of the 472 patients that were randomized, there were 8 events in each group. So, roughly the same event rate as we saw in active four B. All of these participants who experienced an endpoint were hospitalized for COVID related pneumonia and respiratory failure.

These were diverse populations. ACTIV-4B took place in the United States, whereas ETHIC enrolled participants from Belgium, Brazil, India, South Africa, Spain, and the United Kingdom and AVID, in Germany and Switzerland.

I need to point out that all 3 trials enrolled primarily unvaccinated populations of patients. Of these over 1300 participants across all three trials, we found remarkably similar results, different locations, different anticoagulant strategies. I have to say it was important that we have performed these trials because when we developed and designed these trials, there were no data to guide outpatient care and as we know, less than 10% of acutely infected patients are actually hospitalized. Again, events that did occur, occurred very early within 10 days of randomization across all three trials. And we had from 75 days to up to 90 days of follow up with no events really occurring outside that early window.

In all three trials, the majority of primary endpoints were hospitalization for COVID-19 pneumonia. So, the overwhelming majority of ambulatory patients with newly diagnosed symptomatic COVID-19, do not benefit from anticoagulant therapy. Both macrovascular thrombosis and progression of COVID-19 pneumonia occur very infrequently in these patients.

In a secondary analysis that we did of ACTIV-4B, looking at risk factors for primary endpoint, we can predict who is going to progress to severe COVID-19. And those factors for the most part include factors that we know are associated with more severe illness. Male sex, black race, Hispanic ethnicity. What's very interesting is the time from the SARS-CoV-2 positive test. So, for each day after you tested positive, the longer you went, each day had a 25% risk reduction in risk of developing more severe disease. CRP, much to Paul's delight and much to my chagrin fell out as a positive marker. Because, I was hoping we would see D-dimer there, but no CRP or marker of inflammation. A, the higher the C R P, the higher your risk for progression to severe disease.

And so, I'm showing the baseline characteristics last. These are what I consider most important and things that we need to keep in mind when we are moving forward and trying to figure out who is going to get long COVID. Because in our population of 657 randomized participants, the D-dimer level was in the normal range for 65% and was just between 1 and 2 times the upper limit of normal for another 25%. Only 10% of outpatients in our trial had a D-dimer that was greater than 2 times the upper limit of normal. CRP, similarly was elevated with the median of about 4. But yet, in an IQR they had, shows a bit of a range. But, these CRP levels pale in comparison to those that actually get admitted to the hospital.

And so, I'm going to discuss. I'm show this slide now and then I'm going to transition to what we have for data in the long COVID population and what we can make and surmise from that data. This data came from Ranucci's group in Italy, very early on when Italy was struck with COVID. Again, sort of before the rest of the world. They looked at IL-6 levels and fibrinogen concentration. This is where we first really began to appreciate that this was immuno thrombosis, thrombo inflammation and that inflammation was driving the coagulation abnormalities that we saw. As IL-6 levels rise, fibrinogen levels also rise. We all are aware that fibrinogen is an acute phase reactant. COVID-19 or SARS-CoV-2 does nothing different in terms of inflammation and elevation of pro-coagulant proteins.

Now, in this very busy slide, I'm going to look at some data that were published this May. In a UK post COVID clinic for patients who had impaired exercise capacity post COVID and looked at VWF-ADAMTS13 ratio, which I will describe.

They had 330 patients who had post COVID that are a median of 6 months after acute diagnosis and they assessed two functional parameters, the sit-to-stand test and the 6-minute walk and compare these with VWF parameters and D-dimer and coagulation levels. Now, I have to say these were one time measurements in these participants. There were no individual baselines. We do not know what these patients had for levels when they had acute COVID. But as you can see from this diagram here, this flow chart of the 330 participants exercise testing was performed in 84%. 80% of these people had normal exercise testing, and of those only 22% had an abnormal VWF-ADAMTS13 ratio, which I will discuss. Of the 20% that had abnormal functional exercise testing, it was almost 50/50. Normal ratio of VWF antigen to ADAMTS13 and elevated above 1.5 in about half of these, 20% of this whole population.

And so, what is VWF antigen ADAMTS13 ratio? VWF is secreted from vascular endothelial cells and ADAMTS13 is a protein that cleaves the ultra large VWF molecules and is not affected by inflammation. So, as VWF levels rise, this ratio is a marker for inflammation and endothelial cell disruption. You can see that in those that had a normal exercise test, we have the median right here and these are the 56 participants who had abnormal exercise tests. Many of these still had normal values. When we look at the median levels for Factor VIII, there was a slight increase in Factor VIII activity in those who had abnormal exercise tests, that was statistically significant. The same is true for the VWF antigen. It was elevated, but the DDI levels were exactly the same, in both those that had normal functional exercise tests and those that had abnormal tests. Amazingly similar.

And so, I think that this indicates that endothelial cell activation is definitely occurring or endothelial cell damage is persistent. But, we're not seeing activation of soluble coagulation factors. Another paper that I want to highlight that was just published in July of this year and the title is Sustained VWF-ADAMTS13 axis imbalance and endotheliopathy in long COVID syndrome is related to immune dysfunction.

This is the table one from this paper from the Irish group in which they assessed convalescent plasma samples from patients with COVID-19 and compared to acute COVID-19 in this table. And then, in the next set of experiments that I'll show on the next slide, they compare to 20 match controls. These convalescent samples were taken a median of 68 days after symptom resolution or hospital discharge. We don't know who in this group has long COVID. But again, I think the coagulation markers... And there're a lot of baseline characteristics in the rest of this table.

The coagulation markers show a clear difference between those that have acute COVID and those who have convalescent levels such that the fibrinogen level elevated in acute COVID is now normal in the convalescent plasma. Again, the DMR level, while we know it predicts for severity of disease and is associated with mortality, the

higher it is in acute COVID, returns to normal at this just 2-and-a-half months post COVID. CRP similarly low and IL-6 is low as well.

It's a very busy slide from this same paper. What I want you to focus on across these different panels is that the controls are in black, the convalescent patient results are in red and the acute COVID is in green.

While we look at again, the VWF-ADAMTS13 ratio here, IL-6 and 2 pro-angiogenic proteins that are from the Weibel-Palade bodies in vascular endothelial cells, angiopoietin-2 and osteoprotegrin and compare and look also at cellular activation. The convalescent plasma levels are starting to approach those of control compared to the severe elevations we see in those with acute COVID-19. And some of these values, for IL-6 for example, are very similar to controls as are these Weibel-Palade stored proteins of osteoprotegrin and angiopoietin two.

When we look at markers of inflammatory cell activation, we see that monocytes are still activated in these convalescent patients as well as activated CD-4 and CD-8 cells are there close to normal. Some of these patients have normal, some are elevated. Perhaps, these are the ones that will develop long COVID. I feel that these findings indicate sustained endothelial cell activation.

The last data slide I want to show in the last few minutes is that. That just came out about a month ago in August, was a little different, but it's actually looking at the outcome of macrovascular thrombosis in the UK BIOBANK and database of patients from the UK.

This was a retrospective propensity score matched cohort that compared 18,000 outpatients with COVID 19, not hospitalized when they were diagnosed and used propensity score matching to compare to 93,000 non-infected patients. And you can see a difference here between those that are not vaccinated and those that are fully vaccinated. Over 30 days, the cumulative incidents of VTE in these 18,000 patients who are infected is 0.6%. We never would've found a difference in ACTIV-4B, while with this low rate. But, these patients who had VTE had typical VTE risk factors that we know are associated in non COVID patients. Older age, male sex, obesity. There was an imbalance between number of patients who had a diagnosis of cancer seen in this curve as well as orthopedic fractures and whatnot. But, look what happens in the fully vaccinated participants. Those that had breakthrough infection compared to those not infected had a significantly lower event rate for macrovascular thrombosis.

In the last few slides, I want to just focus on the role of anticoagulation in acute COVID and discuss where we should go. From my perspective, the use of anticoagulation to prevent COVID-19 progression has been disappointing in acutely ill patients. The critically ill we've found no benefit for organ support or mortality. And I have to say, looking back, we've tried heparin to treat ARDS and it's never worked. In the moderately ill, again, my perspective, there's a 3% net clinical benefit, not the home run that we had hoped. There is a decreased need for organ support. There's a 1% difference in mortality before hospital discharge. There is a nonsignificant numeric decrease in VTE in these patients, but there's an increase in major bleeding.

The RAPID trial showed no difference in the primary endpoint up to 28 days using therapeutic dose anticoagulation, no difference in mortality before discharge, but fewer all cause deaths at 28 days. However, this

trial was really underpowered and had small numbers. It begs the question of whether long term effects of heparin have a role, but it's hard to justify the bleeding effect or the bleeding risk when the net clinical benefit at this stage of COVID is just not there. And so, therapeutic dose, I just have to say, does prevent VTE, especially in selected patients. But that's another topic for discussion.

So, where do we stand today with long COVID and coagulation biomarkers? We have a good start as you can appreciate from the data I just showed you. It's just come out in the last few months. The studies are limited by small numbers, one time sampling. Often these patients have no baseline values. The majority of patients with COVID are not hospitalized. But, we can predict for those in the outpatient setting who get acute infection, who will progress to severe acute COVID based on some baseline characteristics.

We cannot predict who will develop long COVID. This is currently not possible. Most long COVID patients have minimal disturbance of coagulation parameters, maybe 20% of those will. And so, it's difficult to justify use of anticoagulation in these patients. From my perspective, it's inflammation that's promoting activation of thrombosis and endothelial cell disruption in these long COVID patients. At least half of more of these long COVID patients, as I just discussed, appear to have normal coagulation profiles. And those that do have elevated coagulation biomarkers also have elevated markers of inflammation. And so, I think it is ongoing inflammation that's the culprit and not per se thrombosis.

This can be due to a variety of factors that have been discussed and will be discussed, whether it's persistent viremia, whether it's autoimmune phenomena that are triggered by acute infection. Or, whether what I think might be a culprit is genetic differences in ability to control infection, eradicate the virus and suppress inflammation including mutations or differences in complement regulatory genes, control of T-cell and B-cell response.

So, I think vaccination and strategies to control inflammation and not anticoagulants are likely to be the best options. I show you this cartoon as my parting words, because at the end result here, over on the right we have microvascular thrombosis. But, when you look at all of the factors that contribute to this microvascular thrombosis and you look at targeting X A or targeting II A, thrombin, the overwhelming majority of pathways really don't circle through this. And so again, I think for long COVID, anticoagulation is not the answer.

Dr. Shari Brosnahan

Thank you so much Dr. Connors. That was wonderful. I'd like to introduce our final speaker for today, Dr. Berger, We will be talking about antiplatelet therapy in PASC.

Dr. Jeffrey S. Berger

Thank you very much for the invitation. It's really great to be here and to talk about an area that I think is so important. It's actually humbling for me to follow Drs. Pretorius and Connors. I just learned a lot over the last 40

minutes and I think you'll hear a lot of the same themes. I'll spend a little bit more time talking about platelets and anti-platelet therapy, but I think we have learned a lot. It's pretty amazing how much we have learned, but I think there is a lot to still learn.

These are my disclosures for this talk. I think most pertinent is my involvement in the ACTIV-4A trial leadership looking at drugs that actually target immuno thrombosis.

You heard this earlier, this is just a different example. But, we learned early on that there was a lot of micro and macro thrombosis in COVID. There was a lot of platelets that was seen in pathology, much more than would have been expected. Working in Manhattan, in New York City, really at the onset of the pandemic, we were inundated with patients infected with COVID. Early on we saw an enormous amount of thrombosis. This was in all patients. This was in ICU patients as well as even in the non ICU setting, such that more than 10% of all patients who were not in the ICU had a macro thrombotic event. This was both arterial and venous. In fact, if someone had a thrombotic event, they were 80% more likely to die during the course of their hospitalization.

So, there was a lot of early work trying to figure out what was causing this. Some of the work we did here at NYU, sort of contributed to what you just heard about from Dr. Connors, which is this idea of thrombo inflammation. That it's more than just hypercoagulable mechanisms, it's more than just inflammatory mechanisms. It is the contribution of both. And in fact, when you look at sort of two crude biomarkers, and we chose these really because it was being tested clinically when you looked at patients and stratified them based on high and low D-dimer, high and low CRP, you see that each of them contributed to clinical events including death, critical illness, thrombotic events, even kidney injury. But, you saw this additive or this synergistic effect, that when you had elevation of both thrombotic and inflammatory biomarkers, your risk was even higher.

If you rewind very, very early on, there was a lot of thought of should we be targeting thrombosis? And I think it was stated really well back in the late summer, early fall in 2020 where Dr. Collins, the head of the NIH basically stated, there is currently no standard of care for anticoagulation in hospitalized COVID-19 patients. There's a desperate need for clinical evidence to guide practice. And this is really what led to the NIH leading with this ACTIV-4 initiative. You heard from Dr. Connors about ACTIV-4B, which is, once a patient gets infected, how do you treat them. Here at NYU and together with investigators at the University of Pittsburgh, we headed the ACTIV-4A trial, which was looking at hospitalized acute patients.

Now, fast forward. We published two papers early on, which was a really nice collaboration between our group ACTIV-4 together with REMAP-CAP as well as ATTACC. Really showcasing what you heard from Dr. Connors, which was that in critically ill patients, there was clearly no role for anticoagulation. There was no benefit in terms of organ support and there was an increased risk of major bleeding. It was a little bit different in the non critically ill patients where those patients appeared to have a clinical benefit in terms of organ support free days, approximately three or four days, but at the price of increasing the risk of major bleeding. And that gets to benefit

versus risk. But clearly, this has directed care not just here locally but around the globe in terms of taking care of hospitalized patients.

I wanted to talk a little bit about platelets. I think it's important to comment that despite best practices with anticoagulation, many patients are still experiencing thrombotic events. Many patients are still becoming critically ill and unfortunately dying. As someone who is interested in platelets, we noticed very, very early on that biomarkers of platelet activity were elevated in patients with, or in patients hospitalized with COVID-19.

In fact, when you looked at simple markers, thromboxane, even something as crude as the mean platelet volume, which is on your CBC, which actually looks at the size of your platelet, soluble CD40 ligand, soluble P-selectin. All of them after multi-variable adjustment were significantly associated with the endpoint of thrombosis or all caused mortality. In fact, when you looked at each individual endpoint, you saw nearly identical results.

So, we became very interested in terms of platelets and COVID-19. When you started looking at some of the early pathology results, this is in the bone marrow megakaryocytes. This is in the bone marrow of patients that unfortunately passed early on in the pandemic, you saw the virus in their bone marrow. And in fact when you looked at circulating platelets, you saw this same thing. So that the SARS-CoV-2 was actually getting into the megakaryocytes, was there by going into the platelet. And I think there has been a lot of data confirming this, not all of it. But I think it is fair to conclude that the virus is having an effect, both on the megakaryocyte as well as on the platelet.

When you look at platelet activity on the left, this is platelet aggregation. Patients that are infected with the virus have increased platelet aggregation. But what I think is very interesting, and we're not the only group to show this, but that the platelet transcriptome is dramatically changed in patients that have an infection with this virus. So clearly, the platelet architecture is changing. And in fact when you look at once again some of these crude measures, whether it's platelet size on the left or mean platelet volume, this is now in thousands of patients or with the immature platelet fraction, this is actually in a few hundred patients. You see that these crude measures of platelet activity are associated with in hospital critical illness as well as, or cause mortality.

Clearly, platelets appear to be associated. So I guess, our working hypothesis at the time was that the SARS-CoV-2 virus is getting into the megakaryocyte, getting into the platelet, causing increased platelet activity, changing the platelet transcriptome. Eventually causing critical illness, thrombotic events, as well as organ failure. The question is how and why? How is platelet activity contributing to COVID-19 pathogenesis?

And that led us to really, I would say, study the platelet, endothelial cell interaction. It's been well known that platelets directly influenced the endothelium. When you looked at all these autopsy samples, you saw tons of platelets in the micro and macrovascular lumen, adhering to the endothelium. So, the question was, we know that in this disease, patients were having critical illness, thrombotic events, organ failure. Our question was, are platelets inducing this via endothelial cell activation, Are they inducing this through vascular dysfunction?

Well this led to, probably a year's worth of work trying to sort of understand how this happened. We actually did show that platelets were able to induce endothelial cell activation platelets isolated from patients with COVID-19 compared to a whole host of controls. But, what was very interesting is that we found what we thought was the mechanism of how this was done. It was through the platelets releasing something called calprotectin, otherwise known as S 100A8/A9. The protein is called MRP 8/14. And very interestingly, collaborating with a colleague at Duke, Dr. Deepak Voora... He was doing another study looking just at the effect of antiplatelet therapy on the platelet transcriptome. Asking him to look at his data, looking at the outcome of these two genes, S 100A8 and A9, he found that aspirin had no effect. But a potent P2Y12 inhibitor was able to decrease this pro-inflammatory, this endothelial cell activation inducing protein with a P2Y12 inhibitor.

This was sort of exemplified and really showcased in this paper that we published now, I guess, a while back. But really, showcasing how platelets not just aggregating and causing platelet-platelet interactions. But really, how platelets are inducing this endotheliopathy that you just heard described by Dr. Connors, really showing that it actually had an effect on the endothelium directly.

So, there is some clinical data also to reinforce this, right? There're a lot of observational state studies. I will just show you one because it's not consistent with the randomized data. But this is one of the recent examples. This was a study done in Italy, looking at nearly 8,000 patients with COVID, many of whom were on antiplatelet therapy, either single or dual. And basically showing that overall it was really not a big difference in terms of antiplatelet therapy on some of the outcomes, when you compared to no antiplatelet therapy.

However, when you compared people who were on antiplatelet therapy to not being on anything. So, no anticoagulation or no antiplatelet therapy, those who were on antiplatelet therapy had a little more than a 20% lower risk of mortality, suggesting at least that antiplatelet therapy in the hospital period is effective in terms of outcomes. I'm always a little bit concerned showing observational data when you have randomized data. So, I think it's important to highlight there is randomized data. The RECOVERY trial, a very important trial. 7,351 patients were randomly allocated to aspirin, a very similar number to usual care alone. The primary outcome was 28 day mortality. There's no benefit of aspirin in hospitalization of patients with COVID.

We led the ACTIV-4A P2Y12 inhibitor component. This was on top of standard of care anticoagulation. Our primary endpoint was 21 day organ support-free days and we looked at thrombotic events or death. Very importantly, we stratified our population upfront to those that were critically ill as well as those that were not critically ill. In the non critically ill cohort, our trial was stopped a year ago now because futility was met. And looking at this bar graph, you see that the number of patients that died or had very few organ support free days was similar to those that received P2Y12 inhibitors, compared to those that did not. In fact, we had a 96% probability of futility for P2Y12 inhibitors in non critically ill patients.

A few months later, the REMAP-CAP data came out looking at antiplatelet therapy in critically ill group. Their primary endpoint, which was organ support-free days, was also not different, between the group that got

antiplatelet therapy compared to the group that did not. That's on the left. But what's really surprising, and I think what's very interesting and a big rationale for why we're talking about this today, is that they showed that there's perhaps a long term mortality benefit and the curves diverged over time. So in fact they found that 97% probability that antiplatelet therapy would improve survival to hospital discharge within 99.7% probability that it improved survival over 90 days. Really suggesting, maybe this is an appropriate strategy. In our data we have not replicated these, we are still looking at our data that will come out shortly. But, I will say that we have not seen such a dramatic finding, although we are going to combine our data with them.

So a lot more to come. But, I think pretty amazingly in a very short period of time, we learned a lot about different doses of heparin from ACTIV-4A. We learned about P2Y12 inhibitors. But, it's still ongoing. We are learning about P-selectin inhibitors and as you heard, the endothelium is a very important potential nidus and P-selectin inhibitor affects both the platelet as well as the endothelium. So, that's a very attractive target. And we are studying the SGLT2 inhibitors. Why do we care about thrombosis and platelets in PASC? I think, it's because there is persistent endothelial damage and dysfunction. As you heard from our first talk by Dr. Pretorius, that there is persistent clotting protein pathology in PASC. I think the limited data, at least to date, may suggest that there's some increased platelet activity over time. Although, I would highlight that it is limited to date.

I think we have learned a lot, we really have. We've learned a lot about micro and macro thrombosis and increased platelet activity and which therapies, and I'm not going to read this through. But, I think we still have a lot to learn. First of all, we have to learn about the mechanism and the pathogenesis of all the different variants. What works with one variant may be different from another. What works acutely maybe different from long term? We are still working on other drugs. But, I think it is amazing that if you look back in a relatively short period of time, which has been really devastating on many levels, the scientific community has gone together and I think a lot has been learned.

I wanted to thank so many people for this just because I get to be up here and give this talk. But really, this has work from many and thank you for your time.

Dr. Shari Brosh...: I just want to join, ask the three panelists to come back on and I'm going to have a little bit of discussion with everyone and synthesize. I think, I learned a lot from this. Always so amazing to hear from such great speakers. But, I had some specific questions for everyone.

I was very interested in Dr. Pretoria's work in terms of the actual formation of the thrombosis and these beta sheets that she was talking about. I was wondering if you wanted to touch at all on the shape of the thrombosis and maybe, the likelihood of having organ dysfunction related to that or if that had to do more with the thrombinolytic issues you were speaking about as well?

Dr. Resia Pretorius

Thank you for that question. Yes. I think, what we have been seeing is, from a pathological point of view. In any inflammatory molecule that maybe in circulation and that might also include spike protein can directly bind to the protein structure, the fibrinogen protein structure, changing it from a normal alpha coil, very few beta sheets into a more insoluble aggregate. And that is what we have been seeing. These insoluble aggregates entrap many of the inflammatory molecules that sometimes I hear patients as well as clinicians mention that very ill long COVID patients, when they are sent for regular blood tests to look for the soluble component in blood, the inflammatory markers that are usually looked for in a pathology test, that are looked for in the soluble component of blood. These tests come back within normal ranges. We actually found that these plated insoluble fiber micro clots entrap many of the inflammatory molecules that are actually... They're not necessarily available in the soluble component of the blood samples.

And furthermore, interestingly, we have also noted that D-dimer is also not increased in long COVID. And that might also be because clot breakdown does not take place as it should. So, these are failed fibrinolytic systems. And one of the molecules we found was α2-antiplasmin entrapped inside these microclots that prevent them from breakdown. So, the clot structure forming due to the presence of inflammatory molecules, we think, have got a lot to do with this micro thrombosis. And they may interact with the endothelial cells as well as platelets to form platelet-platelet interactions or complexes, or as Professor Berger noted, they also, these activated platelets then can also interact with these microclots and they may also interact with all of the other inflammatory cells in circulation. It just causes a pathological endothelial function, widespread systemic endothelial dysfunction in those images.

Dr. Shari Brosnahan

Interesting. It might be the pulmonologist in me, but I feel like it's almost like a blood sarcoidosis almost. Like you're making a little granuloma or something.

Dr. Connors, I was wondering what you were thinking about in terms of the timing of anticoagulation use. Because, a lot was made of that in the ACTIV-4A study, and the use of heparin being better in the acute level patients rather than the critically ill patients. But, that didn't seem to necessarily come through with the pre hospitalized patients. I was wondering what you think about that.

And then, I wanted you to touch also... You gave a little bit about how maybe X As are a little bit limited in their role in their pathway. And I wanted to maybe add on to the use of heparins versus DOACs there.

Dr. Jean M. Connors

Yeah. These are all excellent points and as Jeff said nicely that when this hit us right around the world, we didn't know what to do. The macrovascular and microvascular, the thrombosis just snowed us. I really do think that we all thought timing would matter and that by the time you reached end stage critical ICU level care, you had so much microvascular thrombosis that we couldn't prevent it. And so, the thought was, "Well, maybe we can prevent it in the outpatient setting and that will keep people from progressing." And I have to say, because of the constraints we had with the ACTIV-4B. One of the ways we might have been able to improve... Always looking how to improve... Is potentially get patients earlier, but we couldn't get them in. So, if you look at the ETHIC trial, patients had to be randomized within I think like nine days and they couldn't get the patients in. But, they had a much quicker turnaround time to starting and OVID the same thing.

And yet, even starting that early after diagnosis. They started within a median of two days post COVID positive test, no benefit to progression and all the endpoints were admission for progressive pulmonary disease. Now, is it just the alveolar proteinaceous gunk that's preventing airway? You could Shari tell us, better than me. Or, is it microvascular thrombosis that's contributing to the progression, is not clear. And again, so we have the moderately ill in the middle. And to Jeff's point about the long term effects, are we doing some... My view of some of what we're seeing is that we have microvascular clots that form early on and they may not contribute to severe disease and then, they're stuck as vascular scar and that may be contributing to poor profusion and brain fog or whatever.

But, we also have evidence that we have ongoing inflammation. And to that point, why does inflammation activate coagulation? It's so we can trap, like you said, Shari make those granulomas and trap those bacteria and those pathogens and kill them off. That may an abnormal pathologic response in the setting of inflammation.

And so, I think the timing for anticoagulation, for X A and inhibiting too is not helpful. And the studies in the moderately ill and ETHIC and OVID used enoxaparin. And there still was no obvious benefit even starting earlier than we did in ACTIV-4. So, I think it's not that it's just the multitude of targets and the anti-inflammatory properties of heparin. I think that the soluble coagulation factors are just overwhelmed by polyphosphate activation. But, the platelets may play a bigger role. The platelet vascular endothelial cell, monocyte, macrophage adhesion. Those are things I think we have to tease out.

I will say though, that we know vaccination decreases severity of disease and that as I showed you from, again, with all the limitations for propensity score matched program, even in the acutely ill, the rate of VTE, which is low in the ambulatory population, is decreased. That's where I think we have to head, right?

So, I think it's not the type of anticoagulant, it may not be the type. We could target all of them. We'd have people bleeding profusely, but we may not be mitigating that microclot process at any stage of the game. Sorry, if it didn't answer...

Dr. Shari Brosnahan

No, that was wonderful. I guess Dr. Berger, my question for you, it's so interesting to think about the virus actually causing different communications at the platelet level. Have you looked at the levels of viremia causing changes in these crosstalkings and have you seen episodic natures and how long have you been able to, from acute infection detect this difference?

Dr. Jeffrey S. Berger

Yes. Those are all really, really important questions. I think, early on when there was a big emphasis to sort of look at what is the acute effect of the virus on all these cell processes. I don't think we spent enough time on the questions that you're asking. I think we are doing that now, right? Which is, does it matter the amount of the virus circulating? Does it matter which variant of the virus? Do we know exactly how long these effects remain? These are all questions we are actively investigating. I think, early and upfront the intent was to understand why are we seeing what we are seeing clinically? And we tried to get to that as quickly as possible. You know from the literature, that we have an enormous amount of data that has shed light on a lot of the acute pathology we saw.

I think now, we're sort of getting into the nuances, which are so important. Especially when it comes to PASC, right? There is a specific proportion, a very important, but it's not the overwhelming majority of patients. But still, a lot of people have persistent problems. Understanding why and how, I think is our major goal right now. I think the NIH should be commended, right? Because they put in all this work. By the way, as well as many other agencies. This is not the sort of... Just in terms of all this work in the acute setting, we are now following patients longitudinally, in all of these patients who we have so much data, so that we can learn and understand what it is acutely that affects these long term complications. I think, we will get to answer a lot of those questions. I wish I can say we knew the answers now. I think we are on our way, but hopefully soon.

Dr. Shari Brosnahan

Of course. No. That's very thing helpful and I would say that's one of the reasons that we're having these seminars. This is part of the RECOVER initiative, which is to make a cohort of patients that will help us answer these questions that we're asking. And I think part of the reason to have these seminars is really to set up the next studies that we should be doing and what we should actually be looking at in these patients. So, that is all well said and I think that we have come so long from where we started, that it's just so amazing. I have time I think for one more question. I just wanted to bring it back to Dr. Pretorius. I was very interested to hear about that microclot grading system. I was wondering if you saw variability, on the lines of the last questions, variability in patients over

time or just intra patient variability in that?

Dr. Resia Pretorius

We have looked at quite a few long COVID patients and if they suffered from the symptoms that everyone has been reporting, then we did find in all of them platelet hyperactivation as well as microclot formation and from our grading system, the microscopic grading system between a two or three and a four. So depending on the severe the symptoms were the more severe the clotting pathologies we noted. We have also been working with various researchers in the UK that are also using a microclot grading system and quantifying clot numbers and they have been correlating symptom severity with presence of microclot content. They haven't focused yet on take pathology. That is the next step. So, definitely we have been finding that there's a correlation.

We have just been funded to develop a flow cytometry method for identifying microclots in circulation on a flow cytometer and I think that is a much more robust method than trying to do a morphology as a grading system on a microscope. So hopefully, we will have such data available within the next few months to actually quantify the number of microclots present in circulation and also then, perhaps find the amount of microclots, track them, the content of the micro clots in the flow cytometer and the inflammatory marker inside the micro clots. That might also be a nice additional method that could be used.

Dr. Shari Brosnahan

Wonderful, thank you so much. I think that brings me to the end of this little section. But, I think we're going to take questions from the audience.

Claire Quiner

Wonderful, thank you. Just addressing several questions from the audience, some of them submitted during and others submitted before the talk we'll get started off with, for those with post COVID commencing with the first wave in January of 2020, are you one, seeing... This is for the medical practitioners. One, seeing higher rates of multisystem organ dysfunction and two, what type of testing is best for microclots for this patient group?

Dr. Shari Brosnahan

I can take the organ dysfunction question. I think, we're not necessarily seeing as much organ dysfunction as we were seeing in this the first wave. This goes to Dr. Connor's point of how well vaccination has mitigated the inflammation. But, I do think we are seeing some episodes of VTE come back, in this current wave. I would say that I've seen ambulatory patients come with VTE which would, that maybe had been mitigated with the first omicron.

But as far as PASC goes and the micro clots, I think that's a question for the panelists.

Dr. Resia Pretorius

Perhaps I can answer that. Currently there are no methodology except a MI microscope method to look for micro clots. However, as I mentioned, the next step will be a flow cytometry method and then, also a micro plate method has been developed with great success at University of Manchester to actually find microclots in an easier method than trying to do it with a microscope, a fluorescent microscope. So hopefully, within the next months we will have various methods developed and tested and comparing symptoms with the presence of these microclots. So, watch the space.

Claire Quiner

Wonderful, thank you.

Dr. Jeffrey S. Berger

If I could just make one comment. Personally, I'm actually fascinated at the microclots, and I think it's something that just sounds very intriguing and interesting, I just want to make a comment about the difference between all these potential biomarkers and excess risk, which is that, there are a lot of biomarkers that have been proposed. I personally am intrigued by the micro clots. But, for us to make changes... Just because, I'm looking at some of for the questions, and should I treat based on this or not. I just want to remind I guess, the audience that there's a difference between showing an association between anything and long term outcomes, versus making a clinical decision of a particular therapy that has potential complications. Meaning that really has to be the next set of studies which is saying, "Okay, I see this. So, now let me see whether or not acting on this information will improve outcomes." I truly hope that we get there, but I just want to remind people that is a very important missing step.

Dr. Jean M. Connors

I'm glad you brought that up Jeff, because I think that's why I was trying to make my message so strong. We do not have good enough data to act on anticoagulating patients with therapeutic dose anticoagulation, who have long COVID no regardless of their biomarkers. And when I look at this I am fascinated as well by the micro clots and what's driving those and I think research absolutely has to be directed towards the underlying pathophysiology of developing these. But, I'm currently on inpatient attending, we've got some thrombotic microangiopathy patients hanging out here. We got a TTP, we got an atypical HUS, they all have microvascular thrombosis. We understand the mechanisms and the treatments do not involve anticoagulation. So I think, to just

solidify Jeff's point is that, we are not ready for prime time to act on these. Absolutely, I don't want my talk to be interpreted as we shouldn't go down the road of looking at thrombosis. But, we're not ready to give these long COVID patients or PASC patients or whatever acronym you want to give them anticoagulation.

I'm just going to add too Claire. I'm sorry because this goes to yours Resia. There were trials of TPA, in patients with acute COVID and I reviewed them for the journals. A lot of them are small series and case reports and patients. So thrombolysis, right? Thrombolytics. Patients had very well... Those intubated patients with bad respiratory compromise had transient improvements. But, the minute you stopped it succumbed to their severe COVID. And so, I think how we balance the pro clotting and the anti clotting and the fibrinolytic phases are going to be other areas that we need to investigate further.

Dr. Resia Pretorius

Yeah, I could just add to that. I totally agree with both Jeffrey and Jean. We need further investigations, further trials, combinations, 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 actually do not have a coagulation problem and you use anticoagulants and clot busting molecules or products, it can be dangerous. So definitely, we need further trials and there are I think three trials starting in the UK and the USA 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 definitely really important to note.

Dr. Jean M. Connors

There was a great trial called ASPEN-COVID, right? Looking at a compound called rNAPc2, which is an anticoagulant that inhibits tissue factor and it didn't have any effect compared to placebo. So again, we need more 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.

Claire Quiner

Wonderful, thank you. I think that answered several of the questions that we've received throughout this. I'm going to jump to a slightly different topic. There are peer reviewed publications which show increased blood cell size and decreased deformability in COVID. Could this contribute to the capillary stress/ inflammation and tissue hypoxia given blood cells are known to pass single file through the capillaries. Can capillaries stretch to accommodate these larger and more rigid blood cells?

Dr. Jean M. Connors

As the hematologist, okay, I'll start by saying there's 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. And so, there can be a lot of imbalances that have to do with vascular tone. We are very familiar with these dynamics in patients who have sickle cell and then the cells become so rigid that they can't transverse the capillaries. And so, I think this is an interesting concept. But, outside of sickling, even the larger some cells get, the more floppy and deformable they actually are. So I think again, another area that could be investigated.

Dr. Resia Pretorius

I can also perhaps just add, that a group from one of the Max Planck institutes in Germany have developed a flow instrument there, where they actually looked at flow rate of red blood cells and deformability and they did find changes in red blood cells as well as the deformability of all the immune cells. And they also found microclots in trapping these highly deformable red blood cells. So definitely, there 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 is absolutely a interesting phenomenon that should be studied as well. And that might be one of the reasons for the widespread organ damage is that we note in long COVID.

Dr. Jeffrey S. Berger

As a non hematologist I've been sort of intrigued that in the last, I don't know, five years or so, I've seen a lot of publications about a biomarker called RDW and I wonder any sort of pathological condition appears to be associated with it. I think, it's fascinating. To be honest with you. I don't understand it, but I wonder if it is related to this. I know I've seen it in COVID as well. So, I wonder if that is sort of shedding light on the discussion that you guys are all talking about now.

Dr. Jean M. Connors

Yeah, I think it's hard to tease out, right? Because, RDW red cell distribution with is the distribution of sizes of the red cells and it's usually in a very narrow window, a range of normal. Anything can perturb that. You have a GI bleed, you have an increased RDW because your reticulocytes are bigger. And so, in an acute illness, acute infection, we have problems with erythropoiesis, we have anemia of inflammation. That changes the RDW, right? The MCV goes down. You transfuse, you've got different mcv, you've got different RDW. So I think, it's really 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

Jeff, 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 are get trapped. We know."

But I think it's fascinating, because there may be something more that COVID does and I really think why did SARS-CoV-2 wreck havoc across the world? No one has immunity to it. And I think those that get more severe disease or those 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. And as we learn more about TMAs, we recognize that it actually may be a complimentopathy, right? Like atypical HUS is actually a problem with compliment regulatory genes. So I think, getting back to the red cell, there are many things that affect erythropoiesis and they are a barometer or a marker for badness going on. But, I'm not so sure how much they themselves contribute, unlike platelets, monocytes, neutrophils, and extra cellular traps and all that kind of stuff.

Claire Quiner

Great, thank you. And with that, I think we'll move into our closing remarks to be able to respect everyone's time here today.

Thank you so much again to our presenters and thank you to our audience for attending this seminar and engaging with the Q&A. As a reminder, a recording of today's seminar will be available on recovercovid.org within a few weeks and will be posting a Q&A document that has responses to the questions we received today, including those we did not have time to answer.

This slide lists the topics for future sessions, our three seminars are held on the second and fourth Tuesday of the month from 12 to 1:30 PM. We have some exciting topics come up and we hope to see you at those future sessions.

Finally, we have added three polling questions at the end of this seminar today to better understand the efficacy and interest in these seminars, as well as interest of the general group. We'd really appreciate it if you can take just a minute or two to provide feedback by answering these three questions.

Thank you everyone, and have a wonderful day.

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/