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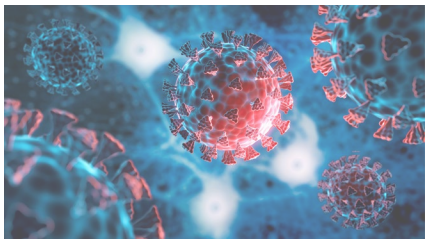

What Have We Learned About Inflammation and Thrombosis Since the COVID-19 outbreak?
***Part 1*: Relationship of Viral Infection and Inflammation—the Foundation for Thrombosis**

Featuring:

Moderator
Mr. George Fritsma, MS MLS
Laboratory Scientist

Guest
Brandon M. Henry, MD.
Physician Scientist

Transcript of the Conversation – Part 1:
Mr. Fritsma: Welcome to Coag Conversations, an educational series sponsored by BioMedica Diagnostics of Windsor, Nova Scotia, Canada. I'm George Fritsma, faculty for the University of Alabama at Birmingham School of Medicine, Division of Laboratory Medicine, and proprietor of the Fritsma Factor, your Interactive Hemostasis Resource where we exchange current coagulation information.

We welcome you to our three-part series: **What have we learned about Inflammation and Thrombosis since the COVID-19 outbreak?**

Our guest is Doctor Brandon Henry, a Physician Scientist and Investigative Pathologist affiliated with the Cincinnati Children's Hospital Medical Center, in Cincinnati, OH, USA.

To date, he has published over 300 articles, including 150 plus articles on SARS-CoV-2 infection, which have already been cited over 10,000 times. Doctor Henry is America's most published COVID-19 scientist and according to a recent analysis of Scopus, he is ranked among the most published and most impactful researchers in the world of COVID-19. Doctor Henry is the leader of multiple international studies investigating COVID-19. His research focuses on the intersection of virology, immunology and hemostasis. Over the last two years he worked to unravel COVID-19 pathophysiology, elucidating the mechanisms driving COVID-19 associated coagulopathy, immunopathology and multi-organ injury.

Doctor Henry serves as an international advisor on COVID-19 response to multiple regional and national public health agencies and is the Chair of the International Federation of Clinical Chemistry and Laboratory Medicine SARS-CoV-2 Variants Working Group. Doctor Henry, do you have a few comments?

Dr. Henry: Hi George, I'm excited to be here today. The significant progress that we have made in studying COVID-19 associated coagulopathy over the last couple years will pay many dividends in the years to come for

many other thrombotic diseases. Indeed, it has become very clear to physicians and scientists that thrombosis and inflammation are inherently linked and that we can't study one in isolation without consideration of the other. So, the major scientific advances in understanding thromboinflammation seen in COVID-19, I believe, will cause a paradigm shift in our research approach to many other conditions such as myocardial infarction and stroke. This will not only advance our scientific knowledge, but really enable new therapeutic approaches, even novel diagnostics. So, with that, I'm excited. Let's dive into our talk.

Mr. Fritsma: Thank you, that's very interesting. As I said, our overall title is ***'What have we learned about inflammation and thrombosis since the COVID-19 outbreak?'***

We will have 3 brief presentations, and beginning with Part One—***Relationship of Viral Infection and Inflammation—the Foundation for Thrombosis***

Let me start, Doctor Henry, how can we describe the inflammatory pattern observed in SARS-CoV-2 and how does it compare to other infections and critical illnesses ?

Dr. Henry: The pattern of inflammatory biomarkers that we see in COVID-19 really reflects a state of thromboinflammation. We see a widespread dysregulation of the immune system with both strong pro and strong anti-inflammatory factors, though we often become a bit fixated on the pro-inflammatory side and we forget about the anti-inflammatory side. Because of that, I'm a bit hesitant to describe what we see in COVID-19 as a cytokine storm, it's a term commonly used, but we'll jump back to that in a few minutes. We also see a biomarker pattern indicative of the arrangement of hemostasis even in relatively mild cases of COVID-19. We still see some of these signs that, hemostasis, there's something going on there and that may explain some of the long-term consequences of COVID-19, such as increased risk of myocardial infarction and stroke, and in patients who only had mild cases, even asymptomatic cases.

Let's jump to a few slides to discuss this a bit further, including how we got to where we are now, and we'll make a comparison to a few other infections and critical illnesses.

Dr. Henry: So George, this is a paper that we did really early on in the pandemic. We were able to highlight what biomarkers really indicated a progression to a severe or even fatal course of COVID-19, and it's quite broad and it really indicates that we see widespread dysregulation of nearly every system in the body. We have multiple hematological changes. One, I'd like to point out is that we have a declining lymphocyte count, which usually kind of is an indicative marker of an immunosuppressive state, but at the same time you see elevated neutrophil counts. We've seen declining hemoglobin and we'll talk about that a little bit later. We see liver biomarkers. We see kidney biomarkers. We see high LDH [lactate dehydrogenase], indicative of cell injury. Myoglobin and cardiac troponin, cardiac biomarkers and of course the coagulation on that side. The PT and the D-dimer at least very early on in disease course, and we have a whole array of inflammatory biomarkers that that are elevated.

Note, we did a lot of detailed analysis in our own studies. One of the interesting things that we found was that the anti-inflammatory cytokine response actually was a stronger predictor of disease outcome and disease progression than the pro-inflammatory response. So the anti-inflammatory markers had a greater predictive

power than the pro-inflammatory markers. There was an interesting study that was done when we talked about comparing how does COVID-19 look at sepsis.

Or how does COVID-19 compare with ARDS [acute respiratory distress syndrome] or trauma compared to other critical illnesses? This was a really great study done by Matthijs Kox [Radboud Univ Med Ctr, Netherlands] and what they did was they compared COVID-19 with ARDS to sepsis with ARDS and with no ARDS. As well as to trauma and a couple other conditions. What they found was that levels of all three cytokines that they measured, tumor necrosis factor [TNF] interleukin 6 [IL-6] and interleukin 8 [IL-8] were significantly lower in patients with COVID-19 as compared to those with septic shock with ARDS. So, as I think, a better way to say it is that we see an immune dysregulated state but not necessarily a true cytokine storm. It's just a complete dysregulated immune response. So as my friends Giuseppe Lippi and Mario Plebani like to call it, “It's a cytokine breeze more than a cytokine storm.”

Mr. Fritsma: This is really interesting. It's a little bit of a different look than what most of us have been seeing for the last two years. And another question; I take a lot of interest in the coagulation mechanism. Does the coagulation mechanism itself contribute to COVID-19 pathophysiology?

Dr. Henry: No, early on in the pandemic my Italian colleagues and I were amongst really the first to begin to observe and publish that it appeared that COVID-19 outcomes were strongly associated with admission biomarkers indicative of some derangements in hemostasis. For example, we published an analysis showing that even mild thrombocytopenia at admission was associated with increased severity and mortality. As SARS CoV-2 spread around the world, this was followed by other clinical observations of numerous major thrombotic events in patients with COVID-19. Even in those with no pre-existing history or baseline risk factors that would suggest that they would be subjected to these types of events. Now over two years later, it's quite clear that COVID-19 is as much a pulmonary disease as it is a vascular disease. And I think it's both, it's a pulmonary and vascular disease. Well, at first that coagulopathy is mostly limited to the pulmonary microvasculature. As the disease progresses, it becomes systemic, impacting the microvasculature of multiple organs, leading to multi organ injury, AKI [acute kidney injury] cardiac injury, liver injury, etc. And eventually leading to pulmonary and systemic macrothrombosis and thromboembolic events. These are very strong drivers of patient prognosis and overall outcome. But the mechanistic picture is extremely complex and despite two years of research we are still working to unravel it all. I have a slide here I want to show you that shows a few of these mechanisms.

This is a paper that we published in Mayo Clinic Proceedings where we really dived into the meat of what COVID-19 associated coagulopathy is and we can highlight what the most frequent thrombotic events and complications we see in patients with COVID-19. We have our venous thrombosis emboli. The in situ pulmonary thrombosis vs arterial thromboemboli like MI, stroke in the Long COVID and many other systemic thromboemboli with DIC and systemic arterial events. And what we have, this overview of the mechanistic picture of what we think at least based on the data that we have now, is really driving the pathogenesis of this COVID-19 associated coagulopathy. And I have to say, George, this is mostly an oversimplification because the more it comes out, the more and more complex this picture gets, and we see how intricate that everything is.

But I think if I had to point out a few things that are really driving it, we have to go to endothelial injury. Endothelial injury is really key and endothelial injury may be driven by the fact that the virus itself is damaging the endothelium. It may be in inflammatory factors that are driving the endothelial injury and the endothelial injury is really the mechanistic heart of what's driving COVID-19 associated coagulopathy. But it's not the only factor, but endothelial injury results in increased release of von Willebrand factor. And inefficient cleavage of the ultra large von Willebrand factor molecules that we talked about a little bit, but hopefully later in the talks we will have time. We have many other factors, a few which I'd like to point out, for example, is release of plasminogen activator inhibitor one [PAI-1] and this will lead to hyperfibrinolysis and then we have a neutrophil activation. This is becoming a new area of real focus and I think it'll have major implications for almost every thrombotic disease, and we get the release of these what we call neutrophil extracellular traps, and these will lead to activation of blood coagulation.

And then on top of it, we have patients who are prolonged and have prolonged immobilization. So, it really becomes a perfect storm. We have components of primary hemostasis being dysregulated. We have massive activation of platelets. We have activation of a secondary hemostasis. We have inhibition of fibrinolysis, at least a transient state of suppression of fibrinolysis. And then, of course, we have the inflammation factors which are just feeding into this whole system, so it really becomes a messy, complicated picture that we see in COVID-19. Over time we will continue to unravel this, but I think a lot of the fundamental mechanisms and the relationships between these different systems will play a key role in helping us understand thromboinflammation before any other disease states.

Mr. Fritsma: Thank you. I think a lot of us are trying to put this into one category or another, so let me just ask you this. If you could categorize the coagulopathy, would you call it a septic intravascular coagulation, which is labeled as SIC? Early on, people were saying, oh, it was DIC. I think most of the early publications out of China said DIC, or would you think it's a TMA, thrombotic microangiopathy? Can we categorize it that way?

Dr. Henry: This is a great question and it's a very challenging question to answer. Defining COVID-19 associated coagulopathy, or at least categorizing it according to our pre pandemic clinical definitions of coagulopathies, is very difficult. First SARS-CoV-2 is unique in that it seems to directly impact all facets of hemostasis, like I showed in the previous image. Primary hemostasis, secondary hemostasis and fibrinolysis are all deranged. All of them. Second, I think we see components of DIC, SIC and TMA in patients with severe COVID-19. All of these we see components of them.

Though, overall, the thromboinflammation that we see in COVID-19 to me, and at least my opinion, best reflects the state of secondary thrombotic microangiopathy. Now that's not to say that we do not see DIC in COVID-19, because we absolutely do see patients in the ICU with COVID-19 related DIC. But I do not think that is the predominant form or the predominant driver of COVID-19 associated coagulopathy. Although there are some characteristics of COVID-19 associated coagulopathy that are similar to DIC, such as increased D-dimer or a trend towards lower platelet counts, there are a number of distinctions.

First, fibrinogen is increased in COVID-19 associated coagulopathy which contradicts the presence of a consumption coagulopathy like DIC. Fibrinogen, like von Willebrand factor and a number of proteins, is an acute

phase reactant so those are increased in the presence of inflammation. Moreover, the thrombocytopenia and the increase in D-dimer does not reach the severity observed in DIC, such as in patients with sepsis. So based on the reported data to date, the D-dimer is really interesting. We believe that the elevated D-dimer that we see early in the disease course is reflective of pulmonary inflammation with local activation of blood coagulation, really within the lung. Initially there's sufficient balance of TPA, tissue plasminogen activator, in relation to what I mentioned earlier, plasminogen activator inhibitor [PAI-1], which inhibits fibrinolysis. So, we have more than enough TPA which allows for adequate activation of fibrinolysis. However, as the disease progresses, there is consumption of plasminogen as observed by the low plasma values in severe COVID-19 that we and other groups published along with inflammation driven elevation of PAI-1 and depletion of TPA which leads to a state of hypofibrinolysis allowing sort of that perpetration of pulmonary and then systemic thrombi.

Let's jump to a few slides so I can highlight why I think we see a secondary TMA type phenomenon. I want to point out a few interesting studies that we've done that really highlight why I think we see a secondary TMA. This is one study we did, but we looked at the balance between ADAMTS13 and von Willebrand factor and what we've seen in our research, and in our studies, we've been using AKI, George, as our primary outcome measure. The reason for that is that the hallmark organ injury of TMA is, classically, acute kidney injury. And what we looked at was in patients with COVID-19, did the balance between ADAMTS13 and von Willebrand factor suggest the state of secondary thrombotic microangiopathy? What we found was yes, and matter of fact, as we can see, severe AKI was associated with low levels of ADAMTS13 activity and high von Willebrand factor antigen levels. Similarly, we've often also done multiple studies looking at complement, another really key player. Because TMA is really a thromboinflammatory disease with many components that are in complement play a major role we target complement for treating different forms of TMA. And we also saw similar things that complement with reflective of severity and in a multi-center study we did with my friend Zoltan Prohaszka, MD in Hungary, we found that complement levels at admission did reflect our ability to predict a severe acute kidney injury in coronavirus disease 2019. And finally, another interesting point; we looked directly for signs of endothelial injury and the relationship between COVID-19 associated AKI. We looked at angiopoietin 2. Angiopoietin 2 enhances endothelial inflammation and hyperpermeability and we also found a similar picture that levels of angiopoietin 2 were associated with severe AKI and need for renal replacement therapy.

Dr. Henry: So to me now, this sort of starts to put together a picture both from the clinical side and laboratory side that we sort of see as the main driver, maybe a secondary microangiopathy.

Mr. Fritsma: Thank you, that's very interesting. The one thing we still wanted to talk about is some of the novel variants that have come along such as Omicron. With respect to the pathophysiology and thrombosis, what does the future hold in this regard?

Dr. Henry: I think that this is a really important question and it's a question that public is asking, scientists are asking, physicians are asking. At this point, we don't truly, totally understand how the various combinations of mutations combined in the different variants impact pathophysiology, nor the prothrombotic state associated with COVID-19. There are a few important points to consider when trying to evaluate this. First, the virus has continued to diversify at an extremely rapid rate. I think as we all know. And looking at just within Omicron, we now have numerous sub lineages that we struggle to keep up with. Now, it might be tempting to say well, all

those sub lineages, minor differences, and they're likely insignificant to the pathophysiology. But just looking at two of the first sub lineages of Omicron identified, BA.1, which was responsible for the first wave has now mostly run its course, versus BA.2, the so-called stealth variant not really stealth, but was so called that early on, that's now dominating world. There are more different mutations. They're more genetically different, BA.1 versus BA.2., than the original Wuhan strain, Delta or Alpha versus Delta. In other words, BA.1 and BA.2 are more different from each other than Alpha, Beta or Delta were from each other. So, BA.1 and BA.2 are very distinct viruses, which makes interpreting the clinical data very challenging.

As well, studying them in the lab, as they require different conditions to study. Second, while BA.1 seemed to show a reduction in intrinsic severity, some new data just came out that suggest that BA.2; BA.2.2 which was predominant at Hong Kong, retains the pathogenicity and mortality rates similar to that of the original virus in vaccinated persons. So, in other words, doesn't really have that mild aspect that we saw in BA.1. And I'm going back to the pathology side, for BA.1, evidence suggests that the virus actually prefers a different cell entry mechanism than that of the original Wuhan strain, preferring an endocytosis route over the TMPRSS 2 mediated cell entry pathways. What this really means, coming back to understanding of pathophysiology, was that BA.1 has different tropism for tissues. Tropism is the capability of a virus to infect a distinct group of cells within the host, so we consider neuro tropism or cardiac tropism or vascular tropism. We don't totally understand what it is, but we know that the virus has different tropism just within the lung. Omicron BA.1 prefers to replicate in the upper respiratory tract, one of the factors that makes it less pathogenic as opposed to the lower respiratory tract preference that we saw with the Delta variant.

This likely significantly impacts technology, but we don't know how so yet in the context of the entire tissues of the human organism. Moreover, we don't understand this yet for the other sub lineages, we're now studying such as BA.2 or BA.4 and BA.5 which were recently added to The WHO [World Health Organization] watchlist. So hopefully we'll understand this better in time, but it's very challenging and we certainly need to see how it plays out in pathophysiology and then, in turn, the COVID-19 associated coagulopathy.

Mr. Fritsma: So it looks like we've got a long way to go in in characterizing all of these varieties.

Dr. Henry: Yes, I think we've still got a ways to go but scientists are working on it day and night. And in time we will have better understanding of how this virus behaves.

Mr. Fritsma: Very good. Well, this concludes today's conversation. Please join us again next month as we continue our discussion about what have we learned about inflammation and thrombosis since COVID-19 outbreak. Doctor Henry, thank you for your expertise and thank you to our audience for your participation.