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**What Have We Learned About Inflammation and Thrombosis Since the COVID-19 outbreak?**

***Part 3*: Thromboinflammation Measurement and Treatment**

**Featuring:**

A person with a beard and mustache

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**Moderator**

**Mr. George Fritsma, MS, MLS**

**Laboratory Scientist**

**Guest**

**Brandon M. Henry,** **MD**

**Physician Scientist**

**Transcript of the Conversation – Part 3**

**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**: Well, thank you everybody for participating in our Part 1 and Part 2 of **What have we learned about Inflammation and Thrombosis since the COVID-19 outbreak.** Part three, our next discussion is:

**Thromboinflammation Measurement and Treatment**

**Mr. Fritsma**: Doctor Henry, what laboratory assays effectively measure the progression of COVID-19 coagulopathy?

**Dr. Henry**: Well, George, COVID is a very variable disease. It has a variable presentation ranging from asymptomatic to severe with a small but significant number of patients developing critical and fatal illness, though we have established risk factors that have proven to be quite true for severe disease or developments for disease, such as advanced age, obesity, cardiovascular disease, the variability in patient presentation and progression makes clinical monitoring in general a bit tricky, especially at the onset of the pandemic. We were able to identify the hallmarks of COVID-19 associated coagulopathy relatively early on in the pandemic and these have stood the test of time. However, we now know that SARS-CoV-2 impacts all facets of hemostasis as we talked about in previous conversations, primary, secondary as well as fibrinolysis. And this makes monitoring progression of coagulopathy a bit challenging. But given that, I think sticking to the basics is best. Let's look at a few slides with a little bit more info.

Now what I like to call the early laboratory hallmarks of COVID-19 associated coagulopathy are really four things: early on, we’ve seen elevated D-dimer—It's not necessarily going to stay elevated over the disease course, but early on we do see an elevated D-dimer, and that's really because of activation of fibrinolysis within the Long COVID production of pulmonary micro clots, we have high fibrinogen that's driven by inflammation. We have mildly prolonged PT as well as sometimes prolonged APTT and mild thrombocytopenia. Thrombocytosis can also occur, but I think I see a bit of a mild thrombocytopenia a bit more frequently. I would say these are the really the four key hallmarks of the earliest signals of COVID-19 associated coagulopathy, and all four of these, George, we found to be independently associated with poor disease progression or outcomes.

Now we also can look at what I call microangiopathy. We talked a little bit earlier about that. I think that one of the main drivers is sort of this secondary thrombotic microangiopathy phenomena, and we can look over, in a patient to see whether or not some of these factors, some of these variables are starting to become evident. We can look at some laboratory signs such as increasing LDH [lactate dehydrogenase], decreasing hemoglobin or haptoglobin, elevations in serum creatine of course indicating AKI [acute kidney injury], elevation of bilirubin and D-dimer. Respecting D-dimer, it may be normal, especially after the initial phase. Because we talked about that early on we do have fibrinolysis. But overtime that PAI-1 becomes really high, and TPA becomes worn out and start to get that sort of exhaustion of fibrinolysis.

So all this might point to a progression, especially seeing these changing rapidly of coagulopathy and really progressing to that sort of secondary TMA. And even looking at each one of those factors like I mentioned earlier, we've seen it. Independently, they're associated with disease progression, severity, and even mortality. Looking at LDH, LDH is a very commonly measured inflammatory marker but also measures cell injury. Also a major indicator that we use for, evaluating TMA. And we can see those elevations in LDH, we found in our own analysis, be associated with, significantly increased odds 6-fold of developing severe disease and 16-fold increase odds if you have elevated LDH of dying from COVID-19. So overall there are some biomarkers that we can use, but I think, overall we want to stick to the basics just because of the complex mechanisms that we see, it's very hard, to say that there are definitive things that we should really be using from monitoring the progression of coagulopathy.

**Mr. Fritsma:** Thank you, although we're to stick to the basics, we did previously discuss thromboinflammation and its role in driving the pathology. Are there any specific biomarkers for thromboinflammation?

**Dr. Henry**: Yes, good question, George. One biomarker that I've really come to appreciate since the onset of the pandemic is the von Willebrand factor antigen to ADAMTS13 activity ratio. You can use it backwards. I like ADAMTS13 to VWF ratio, but both are used. Now, Von Willebrand factor is a large adhesive multimeric protein involved in hemostasis—specifically platelet adhesion via GP1b—as well as binding collagen, factor VIII as well as number of other functions. The larger the size or number of VWF multimers, the greater functionality of the protein.

Now a deficiency or defect in von Willebrand factor can lead to something we call von Willebrand disease that can cause bleeding while an increase in von Willebrand factor may cause thrombosis. So low levels we get bleeding, high levels we get thrombosis. And thrombosis when we have the high molecular weight form, the more active protein. Today, however, we have another protein, ADAMTS13 and that stands for A Disintegrin And Metalloproteinase with Thrombospondin motifs type 13, a very long name. So we'll just call it ADAMTS13, and that's primarily responsible for controlling the size of von Willebrand factor in plasma. It binds and basically proteolytically cleaves VWF multimers, and that's sometimes called VWF factor cleaving protease. The relationship between von Willebrand factor and ADAMTS13 we’ll call the von Willebrand factor ADAMTS13 axis. Now, the relative levels of both VWF and ADAMTS13 in the normal state can be described as a percentage of normal which we would basically average to be approximately 100%. So, they coexist in this balance that maintains normal primary hemostasis.

Now going back to one of our earlier Coag Conversations, we talked about secondary microthrombotic angiopathy as the driver of COVID-19 associated coagulopathy. And I briefly showed our research on von Willebrand factor and ADAMTS13 in relation to COVID-19 acute kidney injury. What we see in COVID-19 is a major increase in the amount of von Willebrand factor circulating in blood. And this is due to the proinflammatory state, specifically, high levels of IL-6 which stimulates the production and release of acute phase reactants, including von Willebrand factor as well as other proteins which we commonly associate with inflammation, CRP, fibrinogen, ferritin, but also regulation components like factor VIII and prothrombin. So, we get this pro inflammation that's really causing this massive release of von Willebrand factor and then von Willebrand factor starts to consume the available ADAMTS13 that's around to cleave it and that results in a decrease in ADAMTS13 activity level. Now it's important to note that this decrease in ADAMTS13 is relative. When we measure it in the lab, we see a decrease, but it's relatively mild. Nowhere near the levels we would see, for example, in patients with thrombotic thrombocytopenic purpura, or TTP, which is a deficiency of ADAMTS13. It's a relative relationship. In other words, in the presence of this massive surge of von Willebrand factor, the level of ADAMTS13 available is inadequate to maintain normal hemostasis, resulting in a prothrombotic state.

**Mr. Fritsma:** You know, that is interesting. We've talked about ADAMTS13 before and had some discussion last year with **Dr. Long Zheng** from University of Kansas about that. Are there other thrombotic conditions that the VWF-ADAMTS13 axis looks similar to COVID?

**Dr. Henry:** Measurement of the VWF-ADAMTS13 axis is probably a bit underutilized and in my opinion probably has significant diagnostic utility outside of COVID-19. I really believe it's key to look at the balance between these two proteins, when going through differential diagnosis and they give insight into whether a patient may be more prone to thrombosis risk or bleeding risk and we have published a few really great papers on this. Let's look at a few slides and we can look how it looks in different conditions.

So here we sort of have an order, I just wanted to point out how the system interplays. We can see we have platelets. We have the GP1b and we have our von Willebrand factor right here these large multimers of von Willebrand factor. What happens is, in normal hemostasis, ADAMTS13 comes along and cleaves these large multimers of von Willebrand factor and that enables normal hemostasis. However, when we have a deficit of ADAMTS13 for whatever reason, we don't have that cleaving of von Willebrand factor. We just get these large multimers that bind a bunch of platelets and this results in thrombosis.

So, we've published a couple great papers. I put this up here as referenced to the listeners to kind of go back to if they're more interest in this topic. I'm not going to have time to get this in detail, but I'm going to go over the highlights of what we think about when you look at the VWF-ADAMTS13 axis. So, I put what we expect, both in terms of laboratory values as well, a visual representation of the balance between the axis. In normal individuals we have a relatively appropriate balance between VWF and ADAMTS13 and that maintains proper normal hemostasis.

Let's look at a few disease states. Let's start with thrombotic thrombocytopenic purpura. This is a disease where we have some type of deficit in ADAMTS13. Due to an antibody or some type of genetic condition that results in us not having ADAMTS13 available to cleave von Willebrand factor. What happens is the balance becomes shifted, so we have too much of VWF and that leads to an imbalance. There's not enough ADAMTS13 available to cleave the VWF and we get a state on which the ratio is greater than 15, up to infinity and we get a high thrombotic risk.

We think about COVID-19. We're going to look at COVID-19. We talked again about the imbalance that we have. We have inflammation driven release of von Willebrand factor. And though we have normal levels of ADAMTS13, it's just inadequate in order to be able to balance the amount of von Willebrand factor that's circulating. We don't quite see the levels of ADAMTS13 that we would see in TTP, very low levels. We see a minor drop in activity as it's just used up, but the relative balance between the two does result in a significant thrombotic risk, and usually the ratio between that we see instead of being appropriate what we see here between the .5 to 2 range; it's very close to a one to one ratio between them. We start to see a ratio that's two to 20 in favor of von Willebrand factor to ADAMTS13 that results in this sort of thrombotic risk.

We can look a little bit at von Willebrand disease. We look at Type 3. Type 3 is when we as a quantitative, so we do not have that von Willebrand factor at all. And the ratio then becomes very low because we do not have any von Willebrand factor. We have lots of ADAMTS13. And then just eating up whatever von Willebrand factor is around and that leaves a significant bleeding risk. We can't maintain proper hemostasis. We can't maintain proper clotting because there's no von Willebrand factor. We look at most other forms about von Willebrand factor. These are more qualitative as opposed to quantitative.

Again, a similar situation. [Referring to a slide being shown] Not as severe, but again because ADAMTS13 is predominant, at least in relation to VWF appropriate balance, we get bleeding risk. So overall looking at the balance between these two, especially when we're not sure about what a patient condition would be, we're considering about TMAs and stuff. It may prove very useful to consider the balance of these two and measuring them together may be quite useful.

**Mr. Fritsma:** This is very interesting to me. Let's move on. Let's talk about therapy. Are there therapeutic avenues explored for targeting the secondary TMA in COVID-19?

**Dr. Henry**: Treating secondary thrombotic microangiopathy requires treating the driving force. In this case it's the thromboinflammation of COVID-19. Evidence to date paves the way to evaluating specific therapies already in use for other secondary microangiopathies such as complement inhibitors like eculizumab although the results for this and similar agents including ravulizumab have not been, I'd say, overly successful. In trials, preliminary data do suggest that the therapeutic approach may complement other therapeutic strategies to improve survival and reduce hypoxia, especially in critical illness. So, they help. I don’t know if they're a miracle drug, but they definitely can play a key role in the illness process, by reducing the illness. But, as we pointed out, the coagulopathy picture is very complicated.

It's very intricate, but at least we can see that targeting complement as a component does seem to reduce the amount of the severity of the illness and points to TMA being a driving factor. We can consider the use of recombinant ADAMTS13. This is another possible option to counteract the abnormally up regulated von Willebrand factor in the plasma. However, this still needs to be really tested in future trials.

I think another consideration is plasma exchange, which is currently being investigated. There was one small study done by Arulkumaran and colleagues. They utilized plasma exchange in seven critically ill patients, I believe with COVID-19 ARDS [acute respiratory distress syndrome] and they found that plasma exchange, compared to the controls, five out of seven controls developed AKI whereas none of the patients who received plasma exchange developed AKI. So, using a therapeutic approach that we use for TTP may also be useful in the management of COVID-19. So, I think about those approaches the using the complement inhibitors. It's talked about complement as an important component to the team of ADAMTS13, plasma exchange or potential therapeutic avenues that are currently being explored for treating the TMA that we see associated with COVID-19.

**Mr. Fritsma**: Thank you. And one more question I have for you. What do we know about the laboratory values in Long COVID and are there recommendations now for monitoring patients after the SARS CoV-2 infection?

**Dr. Henry:** So as of now we have relatively limited data on laboratory values in Long COVID. A challenge certainly is that as we talked about previously that Long COVID is extremely heterogeneous with a nearly infinite list of clinical symptoms and variable clinical presentations. And as I mentioned, we talked about it probably represents several different diseases. Now a few studies have looked at levels of D-dimer after COVID-19. Finding somewhere between 15 and 25% of patients have elevated D dimer after several months after infection, which matches the incidence of Long COVID, but moreover, it has been demonstrated that this increase in D-dimer is actually independent of sort of the inflammatory picture.

Now, looking specifically at Long COVID, there was a really great preliminary study by Pasini and colleagues looking at 75 patients with Long COVID at two months post discharge. They found that all patients with Long COVID had very high levels of very high serum concentrations of ferritin and D-dimer. Eighty-seven percent had low levels of hemoglobin, 73% had elevations in ESR and CRP and 27% had elevations in LDH. This certain picture of low hemoglobin, elevated LDH sort of hints at a picture of ongoing thrombotic microangiopathy. But the fact that 100% of patients had elevated D-dimer suggests that these patients are at increased risk of thrombotic events, and this supports the clinical data that we talked about previously.

Looking at the elevated risk of MI, the elevated risk of stroke, elevated risk of DVT, PE that we see in patients we have to be very mindful of this and that kind of leads to the question—what should we be monitoring in patients after COVID? I think at this point there's limited data to draw full recommendations upon, however, I think it's reasonable to monitor D-dimer levels in patients with Long COVID symptoms or any patient who may be really at elevated risk at baseline; elevated risk of these types of events who develop COVID, even if it's mild COVID. Maybe we should be monitoring those patients a little more carefully; monitoring their D-dimer because of what we see from some of these other studies about the long-term cardiovascular outcomes. I think that we need to evaluate Long COVID on a case-by-case basis and consider what testing we should do based on that patient’s particular risk profile.

So, I think patients who, as I mentioned already, have baseline increased risk of thromboembolic events. We should be monitoring those patients more aggressively, especially Long COVID and we should consider possibly consider early anticoagulation during their course of COVID-19 that we kind of consider we can we continue. At least throughout this risk period I'm seeing is at least one year, increased risk of major thrombotic events.

So, George, I have to say we urgently need more studies on the laboratory values in Long COVID and our group will be making this a priority in the coming months.

**Mr. Fritsma:** Thank you, I'm going to just follow up with that D-dimer question again, because the conventional wisdom about D-dimer is that elevated D-dimer is a promiscuous result, related mostly just to generalized chronic inflammation. And it sounds like what you're saying is using D-dimer and showing that maybe there's some ongoing thrombotic activity would be an indication for treatment. Am I saying that correctly?

**Dr. Henry:** Yes, I think you are. And, of course, we don't have definitive evidence. Either way and it's hard to make these generalizations. But yes, I think you're 100% on point. If we're seeing patients who have these elevated D-dimer after COVID, we should be taking that very seriously. We shouldn't be disregarding that remnant of COVID; that it's not harmful.

I think we should take it as an ongoing, potential risk of something very serious and address that appropriately. I think the clinical data that we've seen in the past few months, especially these long-term cardiovascular outcomes, really provide the clinical evidence to back up exactly what you just said.

**Mr. Fritsma:** Well, that's very interesting to me and that really is some new information. Thank you.

Well, this concludes today's conversation. Please access all three of our COAG conversations on the subject: **What we learned about inflammation and thrombosis since the COVID-19 outbreak**.

As you look at the website, you'll see that we'll also have a transcription of these talks, and you're invited to add your comments, as well, at the end. Doctor Henry thank you for your expertise and thank you to the audience for participation in this very fascinating topic. Thank you very much.

**Questions or Comments: Webinars@BioMedicaDiagnostics.com**