EXPLORING NEUTROPHIL EXTRACELLULAR TRAPS

Researchers are taking what they have learned about blood clotting in antiphospholipid syndrome and applying that knowledge to COVID-19

Jason Knight, MD, PhD, associate professor, Division of Rheumatology, and research collaborators across Michigan Medicine have spent the better part of the past 10 years focused on the study of antiphospholipid syndrome (APS), an unpredictable, chronic autoimmune disease that can cause dangerous clots to form in the body’s blood vessels. “These clots can lead to serious and sometimes life-threatening complications, including stroke, heart attack and late-term pregnancy losses, which are all very devastating,” says Knight. “We are working to advance the understanding of the disease process and identify new, sophisticated approaches to the treatment of APS that are both personalized and proactive.”

In addition to his primary role as a scientist, Knight is also a practicing rheumatologist. “In this role, I round in University Hospital once a year, typically during the first two weeks in March. In 2020, this happened to coincide with the onset of the first surge of COVID-19,” he says. “Things were still pretty normal during that first week. But, in the second week, everything changed with all the COVID-19 admissions to the hospital.”

The second positive COVID-19 test at Michigan Medicine was a lupus patient that Knight and colleagues evaluated on their consult service. “We were called on to determine whether this patient was having a lupus flare,” says Knight. “Our thought was that it actually looked like something else, and we feared it could be the COVID-19 we’d been hearing about.”

When Knight finished rounding, he started a deeper dive into the COVID-19 literature. “At the time, it was mainly just papers coming out of China. Some of the papers were preprints and not yet peer-reviewed, and some had been rapidly peer-reviewed,” he explains. “One of the things we noticed, and this is not so common for most viral infections, was that patients with COVID-19 that were having a severe disease course tended to have very high levels of neutrophils in their blood.”

Neutrophil Extracellular Traps

Knight explains that one of his lab’s first discoveries in APS centered around structures called neutrophil extracellular traps, or NETs. “It’s not that we discovered NETs, but we did discover their relevance in APS,” he says. “NETs come from a type of white blood cell called the neutrophil. They are the cells designed to be the first line of defense, and they come pre-armed with all of the tools they need to turn back an invader like a bacteria or a virus. But when the brakes don’t work well, or they’re overstimulated, neutrophils can cause a host of problems because they have all of this potentially toxic stuff inside of them.”

He notes that one of the ways in which neutrophils combat bacteria is to shoot out spiderweb-like NETs that are built out of DNA. “These NETs become much larger than the original neutrophil itself by taking all this condensed DNA from the nucleus and spilling it out into a web. What we found is that, when this happens in the bloodstream, it is a significant cause of blood clotting.”

Early on in the pandemic, research was rarely focused on the connection between COVID-19 and blood clotting. “That changed pretty quickly as March went into April, and as more reports started to surface that when people get very sick
from COVID-19, blood clotting is a big part of that,” says Knight. “It’s not only blood clotting in the big vessels, but also in the microscopic vessels, including in the lungs. In the very first COVID-19 autopsy series that was released, the lung damage looked more like it was coming from blood clotting, versus what we’re used to seeing in patients in the ICU with pneumonia. And actually, in some ways, it looked like the type of pathology we would see in APS. So we thought maybe it would be worth looking for NETs in COVID-19 patients.”

Collaboration
In the meantime, Knight had initiated a collaboration with Ray Zuo, MD, clinical assistant professor, Division of Rheumatology, who was working on APS in Knight’s lab, and Yogendra Kanthi, MD, assistant professor, Division of Cardiovascular Medicine, and a Lasker Investigator at the National Institutes of Health’s National Heart, Lung, and Blood Institute. “The three of us together thought we would do a study, and we initially tried to write a protocol where we would consent patients in the hospital to give us a blood sample,” he explains. “All we really needed was a sample to do the early studies, but this was a chaotic time and PPE was in short supply. There were no protocols for whether researchers would be allowed to approach COVID-19 patients for research purposes.”

After spinning their wheels for the better part of a week, Knight, Zuo and Kanthi moved to a different train of thought. “We would work with the clinical labs that, once they had done the regular testing ordered by the clinician, had leftover blood samples that got thrown away,” says Knight. “We submitted an application to the U-M Institutional Review Board (IRB) to address the issue of whether patients needed to consent for us to take samples. The IRB agreed that, given the urgency of the situation, the difficulties with consenting in COVID-19, and the fact that these samples were going to be thrown away anyway, we were approved to study the samples without signed consent.”

Soon thereafter, Zuo traveled all over the hospital and managed to get his hands on several COVID-19 blood samples. “Initially, we had 50 patient samples from COVID-19 patients,” he says. “Eventually the blood sample collection process was nicely organized by Dr. Anna Lok’s team and the U-M COVID-19 Biorepository. They ultimately started doing this in a larger-scale fashion than what we had done initially. And now they have a lot of these discarded samples in their freezer that researchers can use.”

Labs Closing
Also happening universitywide in the early days of the pandemic was that laboratories were being shut down, including Knight’s research lab. “Although we had figured out a way to get blood samples, we did not have an open lab with which to continue the process,” says Knight. “So we wrote to Steven Kunkel, PhD, Peter A. Ward Distinguished University Professor; executive vice dean for research, Medical School; chief scientific officer, Michigan Medicine; and endowed professor of Pathology Research, to appeal that our lab be reopened.”

NETs in Covid-19
Knight and colleagues started the pilot on April 1, 2020, and, in fact, found exactly what they had predicted: that there were high levels of NETs in the blood of COVID-19 patients. “The important thing we could tell, even only testing 50 patients, is that higher levels predicted more severe disease. People on the ventilator had much higher levels than people who were less sick and who were still breathing.”

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normally, or weren’t needing oxygen support,” says Knight.

To highlight the advances of their work, Knight and the team wrote a paper titled, “Neutrophil extracellular traps in COVID-19,” published in JCI Insight on April 24, 2020. To date, the article has been cited more than 500 times, which is considered a sizable accomplishment for basic science research in just a year’s period.”

“I think it has so many citations because a lot of people have now done something similar and are citing back to our original work. It would be fair to say the work has been replicated on every continent. Well, except Antarctica,” he says.

Expanding Concepts

Knight reports that their work has informed a number of clinical trial concepts for the treatment of COVID-19, including a randomized clinical trial on a drug called Dipyridamole, an FDA-approved anti-clotting agent. Written by Kanthi, the trials launched in May. “Recruitment was very slow in the summer, as this was an inpatient trial and hospitalizations for COVID-19 were quite low then. But eventually the second surge happened, and we recruited 100 people by January 2021,” says Knight. “Right now we’re analyzing those data to see if this drug made a difference or not in COVID-19 patients when they were admitted to the hospital.”

Antiphospholipid Autoantibodies

Around the time Knight and colleagues were discovering NETs in COVID-19, a Chinese research group published a paper in the New England Journal of Medicine, reporting three patients who had COVID-19 and blood clots, and in whom antiphospholipid antibodies were detected. “These are the autoantibodies that occur in APS that we studied pre-COVID-19. And these autoantibodies can sometimes be associated with infections,” he explains. “When the body is under stress, it can be a trigger for these autoantibodies. Although this was known, no one had ever really dug in deeply to understand if they might be causing problems.”

Knight and colleagues wanted to know whether these autoantibodies, if detected transiently during an infection, could signal trouble. “We used the same types of samples that we had used for the NETs study, and we measured a whole panel of these autoantibodies. We eventually had 172 subjects,” says Knight. “And we found that a little more than half of the subjects had at least some level of these autoantibodies in the blood. These were all people in the hospital with COVID-19. And so we thought that was pretty interesting, but this still didn’t prove that they were causing any trouble.”

So Knight and colleagues purified the autoantibodies, which is something they were well versed in, given their prior research in APS. “We got approval from the animal regulatory body, which involved a lot of email exchanges, to inject these autoantibodies into mice. This is something we had done pre-pandemic with APS research to see if they caused blood clotting or not,” he says. “And that is what we found. So if we take these autoantibodies from COVID-19 patients and transfer them into mice, the mice show higher levels of NETs, and they also develop really large blood clots.”

What followed was a paper co-authored by Knight, titled “Prothrombotic autoantibodies in...
MOVING FORWARD

Jason Knight, MD, PhD, associate professor, Division of Rheumatology, says that he and his colleagues plan to go deeper, and have submitted grants to look more generally at autoantibodies in serious infections. “It isn’t that no one has ever thought about infection-induced autoimmunity. But we wonder if that is perhaps more prevalent than we realized,” says Knight. “It is interesting that while we were discovering these autoantibodies in COVID-19 causing blood clots, other types of potentially problematic autoantibodies were being described by others. One example is autoantibodies that turn down the volume on antiviral cytokines.”

Knight says it’s possible that this whole new field of functional autoantibodies might have its origins in evolution. “There was surely an era in human history when bleeding to death on the cave floor was a much more important concern than getting a blood clot in your leg when you’re on an airplane,” he reflects. “So perhaps some of these autoantibodies were designed as kind of an emergency last gasp response. I tend to think that all of us have this autoimmune potential living inside us. And COVID-19 seems to be really good at bringing it out.”

Applying Knowledge

Knight’s lab remains one of the major research centers in the United States studying APS. “As a disease, APS is relatively understudied compared with the impact it actually has,” he says. “To say that we have been toiling in obscurity would be a tad histrionic, but I think it is fair to say that our work with APS was not getting too much attention. But it seems our prior work put us in a good position to make some discoveries related to COVID-19. This is a reminder about the importance of researching relatively rare diseases, because you may not know at the beginning of that research where it could take you. Before 2020, there was no such thing as COVID-19. But now there is. So we are taking what we have learned about blood clotting in APS and applying that knowledge to the study of COVID-19. We really hope it makes a difference.”