Coag issues occupy COVID's central stage

Karen Titus

July 2021—The pandemic's reach has often been portrayed in shades of red, signaling surging COVID-19 cases across states and countries. Vaccination maps, on the other hand, tend to render progress in more soothing tones, typically in the green family.

But in coagulation laboratories, one small portent is colored blue—specifically, blue-top sodium citrate tubes.

In recent months, laboratories began voicing concerns about tightening supplies. They've spoken with their vendors; some have reached out to new ones. And though no one wants to think about limiting testing if supplies truly become scarce, it wouldn't be the first time labs have had to steer through these waters.

The tubes are a functional symbol of the continued complexities of COVID-19-related coagulopathy, as physicians try to understand and respond to the pathophysiology of infection that leads to a thrombotic event.

As the pandemic has churned on, much has started coming into sharper focus. Prepublication persists, but physicians have begun to sort through the past 18 months and, as many have put it, to "do the science." Time and experience have helped physicians see what it entails, including what it looks like and how to test for it.

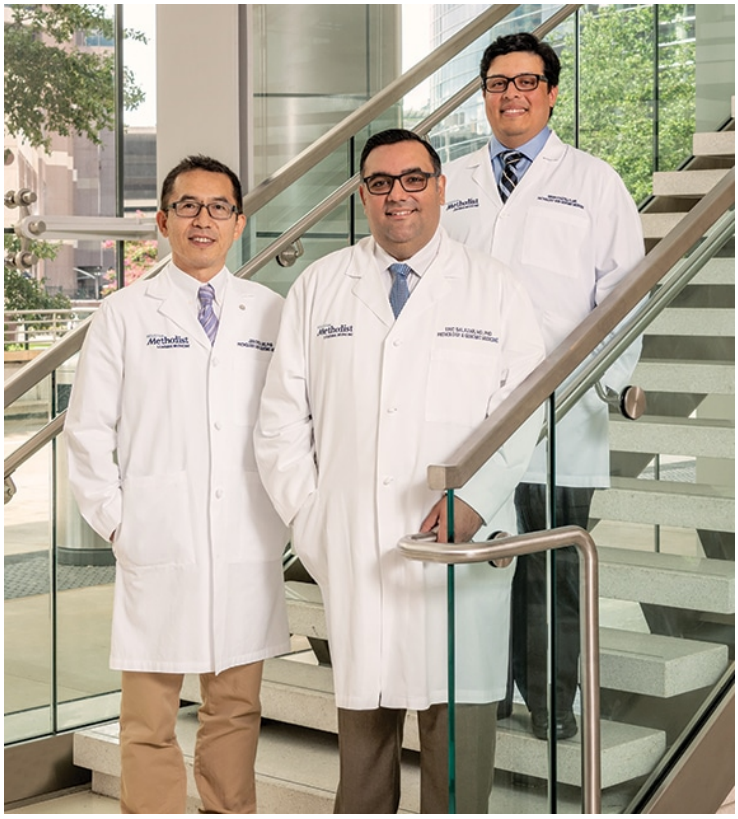
Nevertheless, "COVID-19-related coagulopathy is a relatively new topic," says Eric Salazar, MD, PhD, medical director of the coagulation laboratories and assistant professor, Department of Pathology and Genomic Medicine, Houston Methodist Hospital. "It's still an evolving field. So some of the concepts aren't as solidified just yet as they may be for other entities that cause coagulopathy."

In other words, he says, "We're still learning about its pathophysiology."

In typical pandemic fashion, that includes learning on the fly. As the vaccines rolled out more widely, labs have had to address concerns about thrombosis and thrombocytopenia related to two vaccines. The worry was great enough to put a temporary hold on the Johnson & Johnson/Janssen vaccine in the United States, and has sent labs scrambling, including after those blue-top tubes.

From the earliest days, coagulopathy has clung to COVID-19. "There was a lot of urgency around this topic," says Kristi J. Smock, MD, medical director of the hemostasis/thrombosis laboratory, ARUP Laboratories, and professor of pathology, University of Utah School of Medicine, as physicians worked to figure out how to treat patients, identify those who would develop complications, and which coagulation tests would help with both.

Those embryonic experiences have given way to somewhat more solid footing. "I think people are fairly set on the idea that COVID-19 patients, especially the ones who are more critically ill, have coagulopathies," says Dr. Smock, who is vice chair of the CAP Hemostasis and Thrombosis Committee. "They're at high risk for having thrombotic events. And there's a laboratory profile that suggests a worse prognosis, because it's identifying the sicker, more advanced patients."



Dr. Eric Salazar (center) at Houston Methodist Hospital with Jian Chen, MD, PhD (left), and Brian Castillo, MD, all of the Department of Pathology and Genomic Medicine. Together they work on the challenges that COVID-19-related coagulopathy presents, including those of vaccine-induced immune thrombotic thrombocytopenia. [Photo by Hall Puckett]

Work to identify the best tests is—like the pandemic—ongoing. “We have landed on standard recommendations for the ‘typical’ COVID-19 patient,” says Dr. Salazar, including PT, aPTT, fibrinogen, and D-dimer. “And certainly a complete blood count can be helpful.” More esoteric tests are available, but their clinical utility is unclear, says Dr. Salazar, who is a member of the Hemostasis and Thrombosis Committee.

The initial urgency may be gone, but the need for testing is not.

Even in places that have been relatively unscathed by the pandemic, such as Vermont, the lab has been drawn into related coagulation testing, says Andrew J. Goodwin, MD, professor of pathology and laboratory medicine at the University of Vermont Larner College of Medicine, vice chair for quality and clinical affairs at the University of Vermont Medical Center, and chair of the CAP committee. UVMMC is the state’s largest tertiary hospital, and while the numbers were relatively small compared with those in other parts of the U.S., “we had patients in the ICU where COVID coagulopathy was a concern. We certainly saw an uptick in our D-dimer requests,” along with other related lab tests.

Larger centers were and are even busier. “We’re doing *a lot* of testing,” says Geoffrey Wool, MD, PhD, medical director of the coagulation laboratory and associate professor of pathology at the University of Chicago and a CAP committee member as well. “Our D-dimer volumes have more than doubled. Our fibrinogen testing has gone up; our TEG [thromboelastography] testing has been high and continues to be high.”

He adds, “We’re all seeing COVID coagulopathy,” a well-described mix of very high D-dimer levels, normal to very

mildly reduced platelet count, high fibrinogen, and normal PT and aPTT. High von Willebrand factor levels and generally reduced ADAMTS13 levels are part of the picture as well, he says.

At Houston Methodist, Dr. Salazar says, “We have an eight-hospital systemwide COVID algorithm that guides clinicians about which laboratory tests to order” and which treatments to consider. The inflammatory bundle for COVID-19 patients—particularly those who are admitted—includes D-dimer, fibrinogen, PT and aPTT, as well as LDH, CRP, ferritin, IL-6, and IgG.

Dr. Smock points to guidance released by the International Society on Thrombosis and Haemostasis, including an interim document she still considers valid (Thachil J, et al. *J Thromb Haemost.* 2020;18[5]:1023–1026). It says a coagulation profile is reasonable in patients who are sick enough to be admitted to the hospital—including D-dimer, PT, platelet counts, and fibrinogen—to get an understanding of the level of activation in the patient’s coagulation system, which tends to be correlated with disease severity and prognosis.

These tests might also be useful as a patient stabilizes. It might be reassuring to do trending in some of those lab values, says Dr. Smock, to see if they’re improving in parallel with patient status.

As labs sort through all this, questions endure about the nature of COVID-19-related coagulopathy. Is it different from other types of disease-related coagulopathy, such as disseminated intravascular coagulation or sepsis-induced coagulopathy?

“It does appear to be distinct in some respects,” Dr. Salazar says. It’s rare, for example, for patients with COVID-19 to progress to the coagulopathy-meeting criteria for overt DIC. Usually they have a mild thrombocytopenia, he explains, in contrast to the more severe thrombocytopenias seen in other cases. Usually the fibrinogen level is quite high; ditto for D-dimer levels. The PT and aPTT are only mildly prolonged. This will be accompanied by a parallel rise in inflammatory markers, like C-reactive protein, IL-6, and others. And in contrast to other types of disease-related coagulopathies, where patients tend toward bleeding diathesis, COVID-19 patients “very clearly have much more of a thrombotic tendency.”

COVID-19-related coagulopathy strongly resembles sepsis-related coagulopathy, Dr. Smock says. “Another term you’ll hear is immunothrombosis.” The implication is that strong immunologic responses and reactions in COVID-19 patients can be associated with activation of the coagulation system.

Both observations “are pretty well settled,” she says.

But COVID-19-related coagulopathy is not immediately self-evident. Not only do physicians not always know it when they see it, but often they don’t know *what* they’re looking at.

Early on, the discussions focused on the meaning of the lab test abnormalities in patients with COVID-19—such as elevated D-dimers, prolonged PT, and decreased platelet counts—that resembled DIC. “People were discussing, *Is this a profile of DIC, or is this something else?*” she says.

Dr. Goodwin and his colleagues were among those asking that question. Noting that fibrinogen levels dropped off quickly in patients who did not survive, “Our question was, ‘Is this coagulopathy, and ultimately what we believed to be disseminated intravascular coagulation, slightly different in COVID patients versus non-COVID patients?’ That’s what a lot of people were trying to figure out—is this DIC?

Most patients don’t actually fit with DIC, Dr. Smock says, although some severely ill patients—“at the most extreme end of the spectrum”—may look like they have full-blown DIC, with extremely high D-dimers and consumptive coagulopathies.

Dr. Wool agrees that only the sickest patients—those with a COVID-19-related pneumonia and in need of ECMO support, or who get a secondary bacterial pneumonia and become septic—go on to develop “what looks like a real DIC. Everyone else has this interesting constellation of very high D-dimer levels, but other things sort of stay maintained or are not particularly perturbed.”

Dr. Wool

There's also been debate over whether COVID-19 differs from the first SARS epidemic, as well as whether it's different from the severe annual influenzas that can land patients in the ICU. It seems to be different in some ways, Dr. Wool says, but as researchers do rigorous look-backs at earlier outbreaks, those cases appear to have many similarities to COVID-19. "Maybe we're just learning more about the coagulation perturbations in really proinflammatory viral infections."

The rate of venous thromboembolism is increased in COVID-19 patients, for example—but, Dr. Wool asks, is it increased compared to another patient with the same comorbidity and age who's in the ICU for severe influenza and requires intubation? "I'm not sure," he says.

Then again, there's been reported increased risk of arterial stroke for COVID-19 patients, Dr. Wool continues. Moreover, the high D-dimer-associated morbidity and mortality may not be explained only by thrombosis. "I think the D-dimers are reflecting a degree of inflammation and infection."

If patients also have hemolysis or icterus, Dr. Wool says, the challenges mount, particularly for the patient on ECMO or who have liver dysfunction. In these sickest of patients, the aPTT is shortened more than might be expected for the degree of heparinization due to the high factor VIII level. Anti-Xa-driven heparin monitoring is recommended for these patients.

Prophylactic anticoagulation has become standard of care, though this doesn't generally require laboratory monitoring.

Therapeutic dosing, generally involving unfractionated heparin drips, is another matter. Because COVID-19 is such a proinflammatory disorder with a robust acute-phase response, factor VIII levels can be quite high, Dr. Wool says. "So these end up being patients where the PTT-to-anti-Xa heparin level correlation is never perfect." A sort of tug of war ensues: The heparin prolongs the aPTT, but the high factor VIII level speeds up coagulation and shortens the aPTT. "So the PTT isn't quite as long as you'd expect, based on that heparin level. And when we look at our dedicated heparin level, it's higher than you'd expect, based on the PTT," because of the factor VIII levels.

"Some of our ICUs have found these patients harder to anticoagulate," says Dr. Wool, who adds he's frequently called on to explain the discrepancy to his colleagues. These patients may look more heparin refractory, given that the aPTT doesn't increase as expected by increasing the heparin drip rate, whereas turning to an anti-Xa might appear to be more therapeutic.

That points Dr. Smock to her wish list of questions she'd like to see answered. "Even though it resembles the coagulopathies that you see in sepsis, which is a much broader type of category, there are still questions about whether there are any unique features to this that are specifically COVID related. And, what are all the pieces of coagulation that become deranged in the most severely ill patients?"

Also on that we-need-to-know list: a better understanding of the reasons behind thrombotic events such as PE and DVT, which in patients with COVID-19 may not be easily clinically apparent. "People have tried to understand the reasons for that risk of those types of thrombotic events," Dr. Smock says. The literature looking at the mechanistic factors points to robustly acute-phased coagulation factor proteins that can increase in people who have strong inflammation and stress: increases in factor VIII and fibrinogen, and possibly immune complex activation of platelets, as well as consumption of anticoagulant proteins. "Just the things the body does in times of stress, to improve hemostasis to protect against bleeding, appear to be at play in some of these thrombotic events."

Understanding the underlying mechanisms is more than just an intellectual exercise, Dr. Salazar notes, especially if it helps labs identify patients who might be at risk for thrombotic outcomes. Determining the precise underlying mechanisms may also lead to more efficacious targeted therapy.

Dr. Wool lauds the number of well-done autopsy studies that have appeared, but says he'd like to see additional functional studies that focus on lung, to determine whether that is indeed the site of fibrinolysis. The presumption is that the plethora of high D-dimers seen in COVID-19 come from lysing the abundant fibrin deposited in the lung microvasculature—"because we're not seeing fibrinolysis in the bloodstream ex vivo when we're doing testing"—but it hasn't been proven.

The thirst for knowledge extends to questions about the tests themselves.

Dr. Wool, for one, has his eye on the technicalities of D-dimer testing. In cases where offline dilutions are required, "What is the best diluent to maintain the matrix? Are we doing this the best way?"

That's coupled with clinical questions that need to be answered: What does a D-dimer of greater than 20 ng/mL mean? How different is a D-dimer of greater than 100 ng/mL from one that's greater than 200 ng/mL? "I think we would all suppose that if the levels are that much higher, the patient is at even higher risk of clotting and COVID-19-related mortality, but that hasn't been proven either," Dr. Wool says.

Finally, what clinical outcomes might be linked to platelet function testing? "A lot of groups see platelets coming out partially activated, but it's interesting that it seems to depend on the methodology."

Dr. Wool says his lab is using flow cytometry-based platelet function testing in research settings, trying to nudge it toward clinical use.

"We see some interesting things in COVID-19," he says, including subsets of activated platelets that tend to be unresponsive to platelet-activating substances. "It's like they've already been stimulated."

He suspects that's related to the body's prothrombotic state. "When we look at them, they already have some markers of activation." Other groups have reported similar findings, he says. "We're working on ways to standardize the identification of this."

Platelets circulating in COVID-19 patients, Dr. Wool says, tend to be bigger and less mature, implying increased platelet turnover. The fresher platelets are more commonly identified in the peripheral blood, a marker, he suggests, of a compensated platelet consumptive state.

One could consider the many complexities the good old days of COVID-19 coagulopathy testing. More recently, questions about vaccine-related complications have filled laboratories' dockets. Though the complications are rare, "they're causing quite a hubbub," says Dr. Smock.

It's generally referred to as VITT (vaccine-induced immune thrombotic thrombocytopenia), although, like the names of people who arrived at Ellis Island, it may be subject to change. When Dr. Smock and her colleagues worked with the CDC to investigate a case at UHealth this spring, she noted the CDC used TTS (thrombosis and thrombocytopenia syndrome), a tag notable for the absence of the word "vaccine."

COVID-19 itself was targeted by a geographic slur at the start of the pandemic; likewise, the variants have moved through a series of sobriquets (numbers, countries of apparent origin) that now seem to have settled on Greek letters.

Given the festering misinformation around the vaccines, Dr. Smock wonders if TTS seems less polarizing. ("There's always some weird, new horrible aspect to the pandemic," she says.)

Whatever the reason, or the final name, it's worth noting that both VITT and TTS appear to be in use right now, Dr. Smock says, since "It can make it hard to find things in the literature."

Thinking about these rare complications, she says, “To me, the biggest questions are, why does this happen in some patients and not to others? That would be useful to know. Why have these two adenovirus vector vaccine constructs been implicated in this, while other vaccines haven’t? What’s that actual mechanism? Is it something about the vaccine, like the viral capsid?” And while the two vaccines are similar, she says, they’re not identical, which raises further questions.

Vaccination is sort of the thumb on the scales with COVID-19. Says Dr. Salazar, “At this point, we’re not getting as many questions for the typical COVID-19 patient.” Early questions about the utility of viscoelastic testing, as well as lupus anticoagulants and COVID-19 patients, have receded, too.

These issues seem less pressing for now in light of worries about VITT, Dr. Salazar says. He and his colleagues developed guidance for the lab, hematology, pharmacy, and infectious disease for patients suspected of having VITT. It was also a means of letting clinical colleagues know VITT could be a possibility. This preparation helped guide the diagnosis of and treatment for a patient with VITT who presented to Houston Methodist in early June, he says.

“We have to stay on top of the literature and this rapidly evolving field—and not just VITT but also COVID-19 in general.” Dr. Salazar calls this challenge exciting, interesting, and above all necessary for the best patient care.

It’s important to get that information out there, Dr. Smock agrees. “Even very reputable journals have prepublished [information] without extensive peer review, because [VITT] can be severe and associated with fatalities.

“There is,” she continues, “this kind of balance between public confidence in the vaccines, but also making people aware of these potential complications in a way that’s transparent. We need to figure out how to prevent it and treat it,” Dr. Smock says. “But the likelihood of having severe COVID-19 and having a coagulation complication related to COVID-19 itself is still far higher than any of these vaccine complications.”

Dr. Smock echoes Dr. Salazar in saying that this needs to be tracked and learned about. “You want to get a handle on exactly which patient groups are at highest risk because you might eventually be able to identify some groups that would not be candidates for certain vaccines. And of course you have to always be transparent with patients about the potential risks, even of something rare.”

Dr. Smock points to two useful documents on VITT from the ISTH: a two-page interim guidance (https://bit.ly/ISTH-VITT-guidance) and a flow chart (https://bit.ly/ISTH-VITT-flowchart).

In cases of suspected VITT, lab testing should start with a CBC. “You want to see what their platelet count is,” Dr. Smock explains. The immune disorder causes activation of platelets, which drives the downstream actions that culminate in thrombotic events. Thrombocytopenia is a signal, in this setting, of ongoing platelet activation.

Dr. Smock

“If it’s the right timeline postvaccination, if they have symptoms of a thrombotic event, or you’ve confirmed a thrombotic event, and they have thrombocytopenia, then you should be highly, highly suspicious that it’s this disorder. You should order other coagulation testing that can give you more information,” she continues, including D-dimer, PT and aPTT, and fibrinogen assays, to look for signals that there’s activation of the coagulation system.

“You should also draw pretreatment samples for this more specialized testing, like the ELISA assay that can pick up

the antibodies," she adds. Some treatments can interfere with testing, "so you'll need to get that sample to send to a lab like ours." The additional testing could help guide patient monitoring and will confirm the diagnosis.

Not only is more information helpful clinically, she says, "but when the CDC investigates potential cases, it helps them to have a complete profile."

As Dr. Smock indicates, evidence suggests certain lab tests might be superior to others in identifying VITT. Specifically, PF4 ELISAs that are complexed to polyvinyl sulfonate may be better at identifying PF4-specific IgG antibodies, Dr. Salazar says. He and colleagues have done their own evaluations and determined that such an assay was the best choice the lab could offer, though he says he's "not yet sure how much to make" of the PVS aspect.

ARUP Laboratories has also geared up for this testing, Dr. Smock reports. The complications are similar to HIT, specifically autoimmune heparin-induced thrombocytopenia. While not an exact match, she says, this is the pathophysiology it most closely resembles.

That's why the PF4 ELISAs are a good bet, she agrees—commercial ELISAs designed to pick up heparin PF4 antibodies in patients with HIT. "It looks like ELISAs from several different manufacturers also pick up the antibodies that are occurring in these vaccine-associated complications." The titers are usually quite high, she notes.

But the understanding is still incomplete. Interestingly, Dr. Smock says, some non-ELISA immunoassays (automated in-solution immunoassays on a coagulometer instrument) do not pick them up. "You might hear them called in the literature rapid assays, or automated HIT immunoassays."

Says Dr. Smock, "We're getting calls about hospitals that may have some of these versions of the test that are not adequate for this particular disorder, so they need to bypass their own testing and send ELISAs to another lab."

That comes with logistical difficulties. "You have to be able to identify when you're receiving testing from one of these patients versus a patient with HIT, because in one scenario your assay will work correctly and in one scenario it wouldn't."

Understanding the full picture entails one more layer of testing: platelet activation tests, such as the serotonin release assay. But these are much more specialized, with limited availability, and technically difficult. Nor are they necessarily sensitive.

During the surge in the Houston area last summer, the overwhelming majority of patients in the hospital and presenting to the ED, according to Dr. Salazar, were COVID-19 patients. "So the high likelihood was that the sample we received in the laboratory was from a COVID-19 patient."

That had ramifications for how the laboratory prepared. Dr. Salazar, like so many others, recalls the concerns he had about supply shortages and trying to predict volumes. "I can recall especially checking in with the lab to make sure we had enough reagents to run D-dimers, for fibrinogen levels, for anti-Xa assays, seeing that it was likely that many of these patients were going to be treated with some type of anticoagulation."

Thankfully, he says, supplies held out, but the laboratory continues to deal with the ramifications, including occasional shortages of blue-top tubes.

He's hardly alone. Dr. Wool says that on a recent conference call, a vendor reported significant rises in use of D-dimer reagents as demands for the test balloon. It's also been fueled by the high D-dimer rates seen in COVID-19-related coagulopathy—depending on how labs report values, this can require a second dilution. "Some places are even reporting above 20 microgram per mL FEU or the equivalent," which leads to those aforementioned offline dilutions and use of even more reagents.

Shortages have become a perpetual pandemic aftershock. As labs look beyond their primary vendors, that in turn

creates more shortages with new vendors. “It’s a major domino effect,” Dr. Smock says. UHealth is helped by its size, but smaller systems may be in more dire straits, she suspects.

And while the reference lab isn’t drawing patients, its clients are. “We don’t need to supply the blue-top tubes for all those patients, but our clients need to. So we’ve been having calls and conversations with them to discuss approaches and brainstorm ideas,” says Dr. Smock.

The recommendations of the CAP, including members of the Hemostasis and Thrombosis Committee, are at https://bit.ly/CAP_bluetop.

Marching alongside that search for supplies is the lab’s ageless quest for knowledge.

Dr. Wool’s group described its work recently in a paper on the impact of COVID-19 on platelets and coagulation (Wool GD, et al. *Pathobiology.* 2021;88[1]:15–27). “So we’ve gotten a bit published,” he says, “but some of my colleagues who’ve published these large, well-done cohort studies describing their work have been really valuable in the field. I take my hats off to them to be able to get these giant studies done during the pandemic.”

The pandemic has put a quantum mechanics gloss on research: *It’s only been a year!* and *We’ve had a whole year!* are both fair characterizations of the growth in knowledge.

“We want to practice based on peer-reviewed, properly designed studies,” says Dr. Goodwin. “And a year is often not enough to do it.” It’s even harder in the midst of a global pandemic.

Nevertheless, he applauds the solid science that has started to emerge from the roller derby that was 2020. “We’re learning a lot from it,” he says. “I remember reading articles that weren’t even peer reviewed, and you were making your best assessment of the data” to see if it could be applied clinically. The literature and data are being reviewed more rigorously these days, he says. “Finally.”

He, like the others, says he would love to understand more about the pathophysiology of COVID-19-related coagulopathy. “It has been proposed that this is some variant of a lupus anticoagulant or antiphospholipid antibody syndrome. Some have hypothesized that this is more like a heparin-induced thrombocytopenia,” a notion that has drawn revived interest because of VITT.

“If we can better understand the true pathophysiology, then the laboratory testing can mirror what needs to be done,” Dr. Goodwin says.

Dr. Goodwin

That knowledge would also help explain the testing mysteries he and others have encountered, including with viscoelastic assays, which did not detect expected fibrinolytic components. “That generated a lot of questions,” Dr. Goodwin recalls. “Is there something about the disease where they truly aren’t lysing—which was hard for us to understand, because the fibrin degradation products and the D-dimers were so high—or was the assay system itself not sensitive enough to detect that lytic component?” He echos Dr. Wool’s mention of D-dimer elevation in association with infections. “D-dimer, while sensitive to fibrinolysis, lacks specificity for fibrinolysis, meaning it is elevated in many other settings, including in association with infections,” Dr. Goodwin says.

At Houston Methodist, Dr. Salazar and colleagues continue to evaluate the potential role for viscoelastic testing. “More studies are needed,” Dr. Salazar says. “When we did viscoelastic tests in our COVID-19 patients, we saw that the maximum amplitude was usually quite high, consistent with the sort of hypercoagulable state that many

patients were in," as well as with their very high fibrinogen levels.

Houston Methodist also has a long-COVID clinic. "So far we haven't been getting a lot of coag-related questions, but it doesn't mean we're not going to get them in the future." The lab also offers an algorithm for ambulatory patients to determine whether they are at high risk for progression and to determine eligibility for monoclonal antibody therapy.

Knowledge, always the lab's strongest currency, offers the best chance for everyone to reattach to normalcy, even amid the always shifting demands of COVID-19.

Says Dr. Salazar, "The needs continue to evolve, even as we're hopefully progressing to a stage where we don't have an acute surge of patients." The vaccines have been immensely successful, he notes. "But we need a far greater percentage of the population to get fully immunized. Until that happens," he says, "COVID-19-related coagulation problems will continue to be problems."

Karen Titus is CAP TODAY contributing editor and co-managing editor.