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CXCL9 Then and Now

Voiceover:

A rare, deadly disease that leaves patients in critical condition and doctors searching for answers. Knowing the signs can save a life. This is Investigating HLH, a podcast about hemophagocytic lymphohistiocytosis, brought to you by Sobi Inc.

Kari Magee:

Hi, and welcome to another episode of Investigating HLH. I'm Kari McGee. Today, we're here with a group of physicians whose work has shaped HLH. So today, we're actually going to talk about the story of CXCL9 and really where it started, how we understand how it's evolved, and what it might look like in the future and how it can help with diagnosis and treatment. We're here with Dr. Michael Jordan from Cincinnati Children's. Welcome.

Dr. Michael Jordan:

Thank you.

Kari Magee:

Sarah Nikiforou from Dana-Farber Cancer Institute, and Joseph Rocco, or Joe, from the National Institute of Allergy and Infectious Diseases. If you guys would just take a minute and introduce yourself. Dr. Jordan, go ahead.

Dr. Michael Jordan:

Yeah, I'm an investigator at Cincinnati Children's Hospital. I've, um, uh, I'm a physician scientist and I've been studying, uh, HLH and how it develops for, uh, a number of years, uh, and have been very interested in interferon gamma biology.

Sarah Nikiforou:

So I'm Sarah Nikiforou. I'm a hematologist oncologist in Boston at Dana-Farber Cancer Center, and I treat not just HLH, but other inflammatory syndromes in adults. Sometimes taking them through hematopoietic stem cell transplantation. And in adults, we, for the past decade, struggled to get people to recognize that HLH does occur in adults as opposed to the primary genetically driven HLH syndrome we see in pediatrics. And that has come a long way. But our current challenge is now connecting some of the diagnostic and treatment algorithms developed in the pediatric setting and translating them with new information into how do we treat adults.

Joe Rocco:

And I'm Joe Rocco. I'm a clinical investigator at the National Institutes of Allergy and Infectious Disease, where I run an HLH program focused on clinical translational science and trying to learn about the biology at the bench and then apply that to taking care of patients at the bedside.

Kari Magee:

Thank you all. So really, when we think about HLH, it kind of— it started with the mouse model study that you did in 2004, and it really defined the role of interferon gamma in this disease and how it works. So would you mind, Dr.

Dr. Michael Jordan:

Dorden, just kind of giving us that information. You know, before the genetic sort of roots of primary HLH were identified, right, there was work in humans with HLH indicating that there were a whole variety of inflammatory mediators that are elevated. And, you know, not surprisingly, when people would look at almost any inflammatory mediator, it would be elevated in patients with HLH. But there was no understanding of what was actually causing the disorder, what was causing the illness. And so when perforin was discovered as the first genetic etiology of HLH, we began to kind of think about how could we recreate this disease experimentally to understand what were

actually the causal factors. And so based on some, also some preexisting understanding that the perforin-deficient animals had very unusual immune responses, we were able to sort of really sort of elucidate what was going on and were able to identify it as HLH. And, you know, to our surprise, we actually thought TNF would be really important, but it really had no role that we could identify. But so, to our surprise, we found that almost all of the illness was driven by interferon gamma in the animal model.

Kari Magee:

And Dr. Nekiforou, how do you think that this work really impacted the field of understanding of HLH?

Sarah Nikiforow:

Sure. So HLH is difficult to diagnose in general. But I think in adults where it can be multifactorial, as we will discuss, the diagnosis can be incredibly challenging. And some of the diagnostic criteria we've been working with over the past decades have either been primarily in children, like the HLH 2004 score, even an H score derived for adults didn't really cement a diagnosis. It just said these are people you should investigate the diagnosis of HLH in further. And when there were specific concrete markers So things like ferritin and soluble IL-2 receptor, soluble CD25, those are up in any type of inflammation. So historically, these diagnostic criteria and even some specific testing was hard to cement a diagnosis with. So understanding that we might have reliable biomarkers that really help us diagnose, not just is this a syndrome that we'd like to put a moniker on, but what's driving and thus what treatment modalities might we reach for next that are going to be most successful and targeted. I think that was a huge shift in how we think about diagnosis and treatment of the disease.

Kari Magee:

And I'm glad that you brought that up because really the way that we diagnose HLH is so difficult. And before we get into biomarkers, we can take a step back. And there is one piece of, um, area of study that has gone on with the threshold model. And I would get— I'd love to know what your thoughts are on the threshold model.

Dr. Michael Jordan:

Yeah. So I think different people mean different things by the threshold model. So probably 15 years ago, colleagues and I put out a sort of a review article, you know, just trying to understand the variation we see in patients with HLH. So we know that some of the most severe mutations will sort of inevitably present early in childhood, even at birth or prenatally. And yet, groups like the Italian registry reported a series of families where the patients were young adults practically, or teenagers, young adults with genetic HLH, with mutations in UNC13D, for instance. And so we're trying to understand why is there such variation? What's the relationship between primary and secondary? And it really, as we look at the observations, we say, well, the most severe mutations are probably the ones that present early in life, and it's really all genetic determinism, and maybe milder mutations, you may be fine until you catch some infection that you're unfortunate. And then even at the opposite extreme, if you go back to the—I was thinking about this recently, the avian flu from the 1990s, right? Not any of the recent ones. It has a super high case fatality rate.

And investigators in Hong Kong showed that a lot of these patients that were dying really had a picture that looked very HLH-esque. And yet these are people that don't have any apparent genetic etiology. So that would be sort of an extreme example of an all-environment, nothing genetic, right? And we were speculating that the patients with malignancy were probably also on that far end, at least not sort of germline genetic, but they're maybe okay except for their malignancy is driving this abnormal process. And so this is an interpretation, and I think it makes sense still, but it needs some data, I think, to back it up.

Kari Magee:

Dr. Rocco, how do you think it affects you and your adult population?

Joe Rocco:

Yeah, I think this is one of the biggest questions in the adult HLH field because these, you know, adults are complicated. Not that children aren't complicated, but, you know, these patients are coming in and the sort of classic teaching of this, there's primary HLH driven by genetic causes and secondary HLH, which has to be from a malignancy, autoimmune disease, Or infection, where in reality we get patients coming in with maybe an underlying malignancy, B-cell lymphoma, and then maybe they get acute influenza and that's what tips them over. And if you just find the acute influenza, you don't do them any justice. So really thinking through all of the secondary triggers, which could all be present in one patient, because if you don't address those, you know, you can keep them alive, you can dampen their hyper-inflammatory response, You have to treat every single underlying potential trigger in order to prevent this from coming back and improve survival. And we had no good data on that until now. I think all of us at this table are working hard to establish these cohorts of children and adults to really try and classify the drivers and how much each thing can contribute in every situation. And of course, I think we're finding that it's way more complicated than, you know, we may have thought in the beginning.

Sarah Nikiforow:

Right.

Kari Magee:

And that's a great, that's a great way to go to talk about CXCL9 because we've had all of these diagnostic tools along the way. And one of the things that's emerging is the biomarker CXCL9. And so Dr. Jordan, Cincinnati Children's was one of the first to start using CXCL9 in the diagnosis of HLH. So would you guys, I mean, all of you guys can just give me an explanation of what you think about CXCL9 and the diagnosis of HLH. HLH and how that's kind of affected your practice.

Joe Rocco:

Yeah.

Dr. Michael Jordan:

So the CXCL9 was a valuable marker. It was a pharmacodynamic marker that was used in a clinical trial, NIO-501-04. And, you know, based on what we saw in that trial, really the clinical lab and I really— I was really pushing the clinical lab at Cincinnati Children's to adopt this is a useful measure. And the clinical lab was responsive. And so, like I said, we've been measuring it for some years. And the observation is that essentially everybody who has HLH, either clear familial HLH or probably almost everybody, I think, and actually Joe can speak to this, I think, with much more authority, that has— it looks like they have any kind of HLH spectrum disorder, will have elevated CXCL9. So it's a very useful marker for, you could say, confirming a diagnosis, also ruling out a diagnosis. If you think somebody has HLH and they don't have elevated CXCL9, you're probably wrong.

And we found that it's super useful for monitoring response to therapy regardless of the modality that you're using.

Kari Magee:

You know, one of the things that I was thinking about was back to your mouse model with interferon gamma is the lead for HLH. So CXCL9, explain how that fits into why CXCL9 is important?

Dr. Michael Jordan:

As a biomarker? Yes.

Joe Rocco:

Yeah.

Dr. Michael Jordan:

So, well, we might get deep in the weeds here a little bit, right?

Kari Magee:

It's all right, get in the weeds.

Dr. Michael Jordan:

So quite some years ago, probably now getting close to 20-some, you know, over 20 years ago for sure, we started looking at serum samples from patients with HLH. Based on our mouse studies, we thought in the mice, we can actually see super dynamic, like, hundredfold shifts in interferon gamma in the blood when they're infected with a common mouse virus called LCMV. And so we thought, oh, we'll see something similar in samples from patients. We were actually very disappointed when we did this because we could see that the majority of patients had elevated interferon gamma, but it was just this, what I would call a super wimpy elevation. So the normal depending on how sensitive or how really precise your assay is, is maybe something like 10 picograms, which is a really small amount, right? A lower limit of detection. The patients might have 30 or 50 or 120, right? And so we looked at a whole series of them and we were kind of like, well, this is— maybe the mouse model isn't that good because we were super disappointed. But then we saw some actual data originally from, to give credit where credit's due, from from Novimmune, a European-based company, where they were looking at CXCL9 and CXCL10 as well in these patients, and they were seeing 100,000 picograms, whereas normal may be several hundred picograms depending on your assay. They were seeing these huge dynamic ranges and very big shifts, very obvious, easy to appreciate.

So we recognize that this is obviously a much better biomarker in this context.

Kari Magee:

Dr. Rocco, you recently published a paper titled CXCL9 as a Novel Prognostic Marker to Identify High-Risk Adults in HLH. Would you mind sharing about that, kind of what your goal was and what your findings were?

Joe Rocco:

I want to start launching it off of exactly what Dr. Jordan was saying is, you know, residents always come to me and say, why CXCL9?

You tell us this is an interferon gamma-driven disease. Why can't we just measure interferon gamma? So I've been down the rabbit hole as well. And I think a very important point about interferon gamma is evolutionarily we've evolved for, you don't want interferon gamma circulating in your bloodstream. It's a very inflammatory cytokine. So it's broken down very quickly. And if you look at the most conserved portion of the interferon gamma protein, it's actually the portion that binds it into the tissue. So it's released in the tissue and it wants to stay in the tissue.

And CXCL9 has the benefit of being one of the few chemokines, if not the chemokine, where its entire production is almost driven by interferon gamma, whereas others have lots of different inputs. So it provides this really reliable circulating biomarker based on the biology of what I would call HLH, which I say is pathologic T-cell activation making too much interferon gamma. And what we found, you know, we built this very heterogeneous broad adult HLH cohort. And, you know, we did loosen the criteria a little bit. I think, you know, I love a diagnostic criteria as much as the next person, but the criteria that exist, they're not super specific. And indeed, they're actually quite strict. So in this cohort that we established, it was very systematically collected. We had all of the clinical labs.

It was across multiple centers, including CXCL9 testing, which didn't always return quickly, but we had it checked at the time of HLH suspicion. And in the patients that met full criteria or a little loosened, 4 out of 8 HLH 2004 criteria, we put all of their clinical labs into a machine learning model and we said, tell us what combination, any combination we can use to figure out who is at the highest risk of dying of HLH in this hospital admission. And remarkably, the only lab that was selected from that model was a CXCL9 level. of, you know, about 16,000. And when we dug a little bit deeper into the weeds, what we found was, you know, ferritins are actually very skewed by whether the patient has an underlying autoimmune disease or is post-transplant. Soluble IL-2 receptor, soluble CD25, a T-cell activation marker, was actually very skewed in patients that had underlying malignancy. Kind of makes sense. They're probably chronically immune stimulated, hopefully driving some T-cell response against the malignancy.

So they couldn't give you a good sort of read on how sick is this patient right at this time. Remarkably, CXCL9 showed no bias based on the underlying driver of the disease. And I think that is what gave us the CXCL9 as sort of the prognostic model in these patients. And of course, everyone loves a good threshold cutoff. 69, 16,000 is so easy. But of course, everything is more complicated than that. But maybe the most important point of CXCL9 in our cohort was not 16,000 bad versus good, but the higher it is, the actually the worse it is. And for patients, every 5,000 increase in CXCL9, their 90-day mortality or 90-day odds of mortality actually increased by about 4%.

So coming in at 80,000 or 90,000, big problem versus coming in at, you know, 16,000 or 20,000. And I think that's where we can actually start to translate some of these findings to the bedside to take care of patients.

Kari Magee:

And so, Dr. Nikiforou, one of the things that's interesting about this data and is great for you is it's in adults. And you just discussed how the pediatric interpretation is a little bit difficult. So how does this affect your practice?

Sarah Nikiforow:

So that's a— there's going to be a long answer forthcoming, right? Because as we've been alluding to, there is diagnosis. There is, does it help you identify the underlying trigger or what treatment decisions do you make? And then the fact that you can actually use this to track inflammation, right? So there's so many different ways to use this, but I want to preface it by saying where you practice is going to depend, is going to impact when you get these results back, right? So I work at a—

Dr. Michael Jordan:

Unfortunately.

Kari Magee:

Yes.

Sarah Nikiforow:

I work at a major world-class center, but as of today, we do not have an in-house platform for CXCL9 and some of the other indicators we mentioned. Ferritin comes back quickly, soluble IL-2 receptor does not. We get IL-6 back quickly, but things like IL-18 we don't, right? So, I think some of this has to be tempered by the reality of not every center is going to have this to make decisions right off the bat, right? So, that's just step one. And 2, I do think that we are going to continue, even if something is primarily interferon gamma fueled, I don't think we can ever get tunnel vision and look in a complex inflammatory syndrome at just one marker, right? I think this is a more predictive, prognostic, non-biased marker than we've had before, but I would caution people, especially medical interns, who are wanting to focus on one thing, right? And say, okay, A equals B, so I'm gonna treat with C. I think we need to continue to gather the data because this really is a complex network of inflammatory stimuli and processes that go on. But in terms of diagnosis, in my hands, I'm gonna have to have a high suspicion for diagnosis and probably start treating with anti-inflammatory agents before I have all this back, right? So, it will help with diagnosis, but I can't say, oh, I'm not going to treat for sepsis, right? Or, I'm not going to keep looking for the

underlying malignancy. And Joe, as you mentioned, CXCL9 reflects the inflammation, not the underlying trigger, right?

Joe Rocco:

Yep.

Sarah Nikiforow:

So, it can help with diagnosis when it comes back, diagnostic certainty, but in my mind, as you look at things evolve over time in a critically ill patient, You figure out in these therapies which have anti-inflammatory properties, which are good to protect organ function, keep people stable, lower liver and kidney numbers, you can give curative chemotherapy. They also potentially increase your risk of infection, right? This is just a given if you smack down the immune system. So it allows you with these biomarkers, including CXCL9, just to be more targeted in terms of where do I think I'm going to get the most bang for my treatment intervention.

Joe Rocco:

And can I just—

Kari Magee:

Please.

Joe Rocco:

—tail on top of that? Because I think that's such an important point. And this is where I think actually the analogy to something like sepsis, not talking currently about how they overlap, which is another fascinating discussion, maybe we'll get to it. But when you think of sepsis, you know, there's 2 treatment components. There's keeping the dying septic patient alive, which is usually just fluids and vasopressors and supportive care. And then, you know, finding out why they have sepsis, the underlying trigger, usually a bacterial infection. And you have to treat both or else you're not going to take care of it. HLH, I look at this, or interferon gamma-driven hyperinflammation, the CXCL9 sheds a little bit of light of how sick they are, how, you know, in a sepsis model, how hypotensive they are, maybe, even though HLH patients are often not hypotensive for very interesting reasons related to interferon gamma biology. It gives you a little insight into how sick they are, but doesn't tell you anything about what the underlying drivers are.

So dampening down that hyperinflammation is often required as well as—

Sarah Nikiforow:

As a prelude to, yes.

Joe Rocco:

Exactly. And some of those things may overlap. Steroids may help an underlying autoimmune disease, and it might help dampen down the hyperinflammation. You might need a little etoposide eventually to dampen down a very severe hyperinflammatory patient, which may also knock off some, some malignant cells. But you got to figure it out or else you're going to be missing things and the patient outcomes are still going to be bad.

Sarah Nikiforow:

And I would say that one of the ways I use CXCL9, and this pulls in the threshold model as well, is especially in a 60-year-old patient who turns to me and I'm thinking the same thing, why me and why now? Right? The idea of having additive factors, some that are static, Potentially the genetics, others that are going to change, and helping address what kicked them over this threshold and where are they going to be once I dampen the inflammation, right? How close to that line that I may need to keep them on anti-inflammatory modalities. Joe and I have calls every week to discuss patients in terms of, right, do we stay? Do we go down? But I think understanding what portion of their presentation is inflammatory. And what portion are some of these other factors helps you really modify your therapy, both in the moment, the first few weeks, but also long-term, right? And that's something that the other inflammatory ferritin-soluble IL-2 has given us some hints at, but is not as helpful at distinguishing. Did I phrase that correctly?

Dr. Michael Jordan:

No, totally. And I would just add that, you know, I like to tell, you know, trainees this, that, you know, inflammation's like ice cream. There's a, gazillion different flavors, right? And CXCL9 really does help you to understand, oh, this is what immunologists would call a type 1 immune response. And it's not necessarily specific, right? In a complex sort of inflammatory response, a lot of mediators may be turned on. If we could measure them all, we could profile them and say, oh, this is skewed more this way or that way. We oftentimes can't measure all the relevant things. So, but it is useful. To give you an idea of, of what kind of inflammation is this.

And that obviously has therapeutic implications as well.

Sarah Nikiforow:

But so, Joe, I think you were the one who said, or no, maybe it was you, Michael, an HLH-like syndrome, right? And so, especially in the

adults, I see things that I would say, oh, this is sort of classic HLH or malignancy-associated HLH. But then we also have new acronyms like IECHS, right? Which is immune effector cell HLH Like syndrome. So even the acronyms we're coming up with emphasize that, one, there's a spectrum of hyperinflammation. Two, there's an incredible overlap in the diagnostic possibilities when you're looking at these and even treatment modalities potentially. So in the commentary to Joe's CXCL9 article, I posited that, along with Nancy Berliner, Putting an acronym in a clinical syndrome is probably in the future going to be less important than understanding the biology, which we're going to do by having readily available biomarkers. And that's going to be way more important than what title we put on it, because we're even seeing HLH-like syndrome. I mean, I see 40-year-olds with primary HLH after EBV infection. Right? They get treated totally differently than someone who's had chimeric antigen receptor cell therapy.

And you even get, after checkpoint inhibition, which we use now all the time in solid tumors in oncology, some of those patients present, even post-bispecific antibodies. So really understanding inflammation rather than all sitting around and bickering about whether HLH applies to this patient or not.

Kari Magee:
Right.

Sarah Nikiforow:
That's becoming very, very different.

Joe Rocco:
And I think that's actually a great comparison because let's say someone comes in, gets checkpoint inhibitor therapy, they go into this HLH-like syndrome. You see their CXCL9, very similar to cases we've had recently, it's 400,000. Maybe you can treat them with very targeted therapies attacking cytokines or intracellular signaling pathways, and that may be all they need as they get further away from the checkpoint inhibitor therapy.

Sarah Nikiforow:
Right. Because in that threshold model, huge jump was the medication. their underlying inflammatory milieu is pretty low.

Joe Rocco:
Just bring them back down. Whereas we have other patients recently come in looking very similar, very critically ill. We treat them with very targeted therapies and they get better, but not completely better. And then you see other markers. CRPs are still very elevated. CXCL9 is virtually normalized. And this is where, again, the residents and medical students trying to learn this lose their minds. It's like, Dr.

Rock, you told me the CXR9 was high, get it down, and that'll be fine. It's like, well, it will be fine, but that's where the trigger comes back. And it turns out this patient actually had underlying Still's disease. We got rid of this hypergamma-driven activation, but the Still's disease was still simmering along and you needed to knock that out. Once we knocked that out, they completely got better. CRP normalized.

Sarah Nikiforow:
And that's from IL-18 and macrophages, right? So this is where the multifactorial component to an end stage that we call inflammation is so important to back out. Where were you going to pivot? Oh, I was just going to, uh, a different way or same way.

Dr. Michael Jordan:
Uh, just, yeah, I realized we need to back up a little bit where, when you were asking us, Carrie, about what is CXCL9, we, there's some basic biology we kind of just skipped over. We went straight to a lot of clinical kind of utility, which is important, but I was just pointing out that, you know, so CXCL9, there's a, it's a, it's basically a member of a, of a class of chemokines. And I think it was Joe, you mentioned this, that typically myeloid cells, when they are exposed to interferon gamma, will make CXCL9. And interferon gamma is also something that we see good evidence that it's produced and consumed locally in tissues. But when macrophages or other myeloid cell types are exposed to it, they make just buckets and buckets of CXCL9. And that actually diffuses very broadly. And it's actually because it's a chemokine that tells inflammatory cells, come here, there is something going on here. Right.

And so that's why I think there's really, it's easy to measure in the blood because that's not where the action is. The action is probably in lymphoid tissues or other tissues depending on the context, but you can measure it in the blood unlike interferon gamma with good sensitivity. But it's a, Like I said, it's part of a family of chemokines. When a T cell makes, or an NK cell makes interferon gamma, and many cell types will make it, but myeloid cells are like the champions. They'll make huge amounts of it. When they sense interferon, they'll make large amounts of this, and that'll draw in other cells. And that sort of perpetuates a local immune response. But of course, we're looking at a systemic problem.

It's just part of why it's a problem is that you get this sort of systemic immune activation. And so we see You know, super high levels. But, you know, somebody with a child in the ER with a cold, right, will have elevated CXCL9, but it's going to be just a little bit, you know, the normal level might be 650, maybe it's 1,000, right? But somebody walks in with 10,000 or 100,000, there's probably something very unusual going on in that patient. And it's very likely, especially when you get further and further up, it's probably in the spectrum of HLH physiology. And so, you know, I'd say it's remarkable how variable even in the classic primary HLH patients it can be. It's always elevated, but it's usually like 30,000. But our lab will dilute up to 600,000, and we've seen patients with like greater than 600,000 picograms of CXCL9, which is amazing considering that's, you know, that's 3 orders of magnitude higher than like the normal upper limit, right? So, But I just wanted to point out that this is very much, it really does, I would say, implies that there's a lot of myeloid cells sensing a lot of interferon gamma when you see these tens of thousands of picograms of CXCL9 in the blood. So that bit of biology I think was worth pointing out.

And as Joe, I just back up what Joe said, that CXCL9 is very, not perfectly specific, but very, very specific to interferon gamma. CXCL10 is pretty good too. Um, but you had to pick one. We're not going to measure both. So we went with 9. I think 10 is probably a little less specific. 11 is definitely skewed towards different interferons, like interferon alpha and beta. But, um, so yeah, just a little bit of biology I thought we, we missed over.

Oh, and the other aspect of all this is that the cells that make interferon gamma, um, actually express the receptor for CXCL9. So you can see these are reciprocal loops, right?

Sarah Nikiforow:
Right.

Dr. Michael Jordan:
So in other words, the T cell makes interferon gamma, the macrophage senses it, right? Produces CXCL9. CXCL9 binds a receptor called CXCR3, which is on the T cell that makes interferon gamma. In fact, you can identify T cells that are prone to make interferon gamma by their expression of CXCR3. So again, you can see this sort of reciprocal handshakes here saying, you know, come here. And especially all you guys that make interferon or gals that make interferon gamma, right? So yeah.

Sarah Nikiforow:
And I was going to tangent off that for a second, getting back to Testing for this. So you had mentioned that you do look out up to, and one of the things that I have found in working with our laboratory send-out group is for any of these markers, even soluble IL-2 receptor, you may get companies that you send this to and they say, oh, it's over 4,000.

Dr. Michael Jordan:
Hmm.

Sarah Nikiforow:
And it's incredibly important as a clinician to know, is it 400,000 or is it 4,002? Right? Because as Joe has said, that has prognostic implications, right? You may think, and I would, because every medication we prescribe has some downsides, I may say if it's 4,002, I will start with a therapy at a lower dose than if it's 400,000, right? So I would be very careful when you are contracting with who is going to give you this incredibly vital information. fast enough, it also has to be very quantitative because it really does drive care. It's not a binary yes/no for any of these markers.

Kari Magee:
And one of the things that often comes up too is, and you've brought it up as well, you don't have that lab and those capabilities to test CXR yet.

Dr. Michael Jordan:
Today.

Kari Magee:
Today. And there are a lot of people that are trying to get it. It's coming, but you don't have it at this point. So how do— oh, that's funny. So how do you manage that? Because there's some people that will say it takes a week to get back information. And so if you're waiting a week on this extremely important biomarker, how, how do you handle that?

Sarah Nikiforow:
So one, I know the bat phones to call to get the preliminary info, which is not what everyone would have. But I do think that the reality is many of us treating HLH, and we were talking about this, um, where we are talking at extra-specialized tertiary centers, that's not where every patient is going to present de novo. Right? So that most of these are going to be diagnosed at centers that will have to wait. So

unfortunately, you do need to do the kitchen sink, right? So that's where some of the older diagnostic criteria are still the best we have to go with. You have to keep your feelers up and suspect. But I think that training the staff to say, okay, well, there are certain targeted therapies, we need to wait and show that that target applies. I don't know that that's the way we can practice, right? I think we have to actually act earlier and then refine if you're in the situation that I am where that data comes back a little later.

Joe Rocco:

And I think that's so important because it's a multifactorial problem as well. You know, knowing the bat phones as a clinician actually is very important no matter where you practice because there is ways to get this done and understanding its importance. However, The other thing on us specifically is to get the data out there that actually shows how useful this is.

Sarah Nikiforow:

And then you convince the laboratory leadership to say, yes, this is worth investing in having an in-house platform, right? So it builds upon itself.

Joe Rocco:

And when you look at the labs that we use, there are very few of them that are, if this is high, you can be reliably certain. that these targeted therapies, again, they won't fix it, but they will help. And I think CXL9 provides that rare utility, even if it's not a perfect gauge of how sick someone is. It almost certainly tells you— this is preliminary data, I'm yelling at myself and not getting this out there yet— targeted therapies hitting associated cytokines and pathways can stabilize these patients and give you time to sort of figure out what's going on.

Sarah Nikiforow:

Unlike a ferritin where it doesn't help you at all, right? In terms of how you need to treat in isolation.

Dr. Michael Jordan:

Agreed. It's probably the best example of this, honestly. I mean, people will use things like CRP to say, should we inhibit IL-6, for instance? And that certainly has utility, but I think this is even more impressive that it really points to, you need to be thinking about this pathway as part of why the patient is so sick.

Kari Magee:

And so, I love that we've had these discussions and it seems like this is important and exciting so that we can find these. Exactly. So, moving forward and looking, if you had a crystal ball and you could say, this is what I would love for HLH and biomarker testing, what would that look like for you?

Dr. Michael Jordan:

Perhaps point-of-care testing would be really amazing, I think, in some contexts, not in every context, but even working at Cincinnati, sometimes they get so many samples, I can't get the same-day result. And that can be really a problem for some patients. So we oftentimes will call ahead and say, make sure it makes it onto today's run. I have to know. And it still takes Till the afternoon if we draw it at, you know, 5 in the morning, right? So, I think that point-of-care testing, I think, would be, for a subset of patients, would be extremely helpful.

Sarah Nikiforow:

I have at least 2 thoughts. So, one is, as I had said several times, throw the acronyms out the window, right?

Kari Magee:

Yep.

Sarah Nikiforow:

I think that what I would love to see is this type of point-of-care testing. I would love to see multiplex ELISA assays that get you several cytokines back at once. So, for instance, IL-18, soluble CD25 receptor, those 3 clustered together seem to be crucial, but even IL-1, IL-6, if you've got a chimeric antigen receptor patient. But I would love to see this applied to all types of inflammation, right? In a way that we are doing cohort studies like Joe has done, and really track what are the profiles. And I'd love you to comment on flow cytometry as well, but What is really the core panel we need to distinguish these types of inflammatory syndromes? And thus, what you can do is say, okay, we're going to identify high-risk patients for eligibility on studies for particular interventions, right? You'd also have a panel that you can check quantitatively their responses, right? HLH has been incredibly difficult to study. Because how do you define a PR? How do you define a CR, right? Meaning partial response and complete response. So I think that having a panel, which obviously has to include CXCL9 in multiple different inflammatory syndromes, and then track that into our clinical trials around treatment, that's where I would like to have it seen with point-of-care testing going to make that all possible.

Joe Rocco:

Oh, and I think that this has sort of a bright side and a sort of side to be more aware of. The bright side is this is not hard sort of studies and data to acquire. These are complex patients, multifactorial problems, and starting with everyone's help at the table and beyond, putting together these complex cohorts and finding this CXCL9 looks to be an interferon gamma syndrome and dampening that clearly seems to be useful clinically. But what are the downsides? I think there's, there's 2 big ones in that setting. Um, and you tied it to the biology of the flow cytometry. You know, it's remarkable how well specific populations, uh, you know, first, uh, published by the Cincinnati group with, uh, Dr. Jordan involved, these activated T cells that are making the gamma that are driving the syndrome and dampening the hyperinflammation clearly helps, but it doesn't necessarily get rid of those T cells. It shrinks them.

But doesn't get rid of them. That goes back to the, that's not fixing the problem. You got to find the underlying problem and treat it. It's not completely fixed. The second side is trying to expand this on multiplex assays and looking at other cytokines. And that is, you know, that's the golden goose. If we can do that, figure out this equals this, this equals that, I think that's on us to prove, but it's not necessarily going to be as easy. And I think The IL-6 cytokine maybe brings that to light.

You know, people would think, oh, IL-6 high, let's target IL-6. Well, it turns out in some situations, CAR T cells being one of them, that actually works very well. In other situations, I think SARS-CoV-2 is maybe one. There's probably others where blocking IL-6 actually doesn't necessarily seem to help. In some cases, it may even make things worse. So IL-6 biology is actually much more complex than interferon gamma biology. There's pluses and minuses of IL-6. So if we're going to start doing things like this, keeping the focus also on the biology and understanding really how things work before we start translating.

Exactly. Exactly. So, um, there's a lot of work to do. Uh, and, um, but I think it's a promising start. Um, but we don't want to put the cart before the horse in some of the situations. We got to get the data out there as well.

Sarah Nikiforow:

And do you mind commenting, Mike, this is to cement it in my head as well, on some of the flow cytometry? I know it hasn't been as easily quantifiable, right? And as portable as some of these ELISA-based assays, but how do you use flow cytometry to generate some biomarkers, quote unquote, that might help guide diagnosis and treatment?

Dr. Michael Jordan:

Yeah. Yeah. So, so there's lots of different ways. So, let's see, when people say, for instance, people say inflammation, they mean a lot of different things. Like, right, we talked about it's like saying ice cream, right? Lots of flavors, right? So when people say activation of a T cell or any kind of cell, right, people often mean a lot of different things. And so Rafi Ahmed's group back in the sort of early mid-aughts, I think, did some really remarkable studies where they gave volunteers live viral vaccines and they tracked the T cells responding to the live virus. And they did a whole bunch of stuff. Probably they didn't show us all their data, but they said, oh, look, these, the cells that are actually responding to the virus right now, right, while they're viremic, while there's antigen we know being presented, they have really high levels of CD38 and HLA-DR.

And that in fact was just a beautiful sort of, you know, 2 markers that created a nice separate population. And it went up with sort of antigenic activation and it came down, right? And it can show the kinetics. So we said, well, we know in the mouse model that this is, kind of the distinguisher, right? In addition to tons of cytokine, that tons of cytokine is coming, is being driven by tons of antigen presentation from many, many more activated T cells. So, so, uh, and so these markers don't work that way in mice. So it just doesn't work. Uh, but, uh, but we started looking in humans and sure enough, we can see the same population. And essentially in an untreated child with primary HLH, they're like super high, you know, like a, uh, a very high fraction of circulating CD8 T cells will be 38 bright. So T cells mostly express 38, it's kind of dim.

So it goes up as a higher level and DR positive. And as they get treated, it comes down nicely. So, um, uh, and that's, uh, in other groups like, um, um, Shan Chandra Kausan's group has shown nicely that you see this, um, you know, in EBV infection, not just, you know, HLH, but even regular. So we think that probably any significant viral infection will give you some of that. We see this in patients with cancer and an inflamed phenotype, for instance. But I think that this is always— and we actually, what we published, we said, well, there's no magic threshold, right?

Sarah Nikiforow:

Mm-hmm.

Dr. Michael Jordan:

We see it, it's quite high. It does vary, it's variable enough. So we said, well, let's compare it to another sort of severe inflammatory syndrome. And so our ICU group had a really nice study looking at patients with sepsis, almost all of which were gram-negative bacteremias. And so we just compared to those patients, and they had a notable complete absence of these T cells because T cells

were not involved in the acute sort of gram-negative bacteria sepsis. And in that comparison, having even a small number of activated T cells was enough to distinguish HLH from sepsis. I think the number was like 7%.

But honestly, most patients, if they come in pretreatment, it's going to be You know, half of their CD8, at least primary HLH, you know, half or more of their CD8 T cells will be, will express this kind of recently, I like to refer to it as antigenically activated phenotype, right? You know, activation, you can, you could dump, um, you know, a cytokine on a T cell that, that may have the receptor for that. And you could say I activated it, but, but really it's referring to they're seeing antigen, right? With their T cell receptor, uh, and they're getting activated by the antigen. And they develop this phenotype. It doesn't happen immediately. It's not hours or minutes, but it's within, you know, as they begin to expand and develop within days, you might say, of at least in the experimental human context of vaccination.

Sarah Nikiforow:

I can see our ICU colleagues wanting all of this information right up front because it's really critical.

Dr. Michael Jordan:

And if I can do a little advertising, our clinical lab has had an activation, T-cell activation panel for years. And it has markers like FAS and, um, oh, I'm forgetting all the different markers on there. Um, a variety of different markers. CD25 is on there, which we can talk about later, but strangely is not useful in flow and HLH in the blood. And I've been hounding on, hounding them for years to, you know, to change the panel. And it actually is now does include 38 and DR.

Kari Magee:

Mm-hmm.

Dr. Michael Jordan:

So we can actually, um, identify those cells on a clinical basis. And I think it literally just went live like That's great.

Joe Rocco:

And I think to dovetail off of that, you know, exactly what you're saying, it, the quantitation of activation is probably important to some extent, but again, in an acute EBV, you get a huge quantitation. What we have found tracking these longitudinally in patients is the most important thing is actually the second thing you said, which is it comes down. And actually the thing that defines HLH more than anything using this panel is it doesn't come down. It goes up, whether it's by EBV or malignancy or something. And actually it either keeps going up or it stays at this really high quantity. And the best model we had for this was actually the CAR T-cell model, because in the CRS setting, the T-cells become activated, they kill tumor, and then they come down. The thing that separates the quote unquote IECHS patients, the immune effector cell HLH-like patients, is It doesn't come down. And in fact, it stays high for weeks, even months.

And these patients develop this severe HLH-like syndrome. So, um, again, everything's more complicated than we wish it was, but I think those 2 factors, the quantitation of how activated is it and the does it persist. So, um, my description, the antigenically, the persistent, instead of saying antigenically, I say pathologically persistent, pathologically activated T cells. is one of the major factors that sort of defines the HLH syndrome, I think.

Sarah Nikiforow:

And as a clinician, again, using all of this information and having it readily available, this helps me say, great, you're done. I'll see you in 2 weeks, but it'll be pro forma, and I don't expect to need to follow you long-term, versus patients who, if we say sayonara, end up in the hospital 2 months from now, right? So again, these are very different than diagnostic criteria, but using for response? And in that threshold, are we totally damped down? The analogy that I use for explaining it to patients is you've got a pot of water on the stove. It may not be boiling, but is the burner off or is the burner on medium? And as soon as you crank it up one more level, you're going to burst into a boil, right? And this, these biomarkers help us more than fever resolving or other things like that.

Kari Magee:

Well, I, I really appreciate you guys really discussing this. It just really shows the fact that this is not an easy disease state. This is not an easy syndrome. And I appreciate that you took the time to realize too that CXCL9 is a very important biomarker and that it really has helped and it just continues to evolve the HLH, um, diagnosis and treatment. So because we've covered so much, I, one thing you want someone to walk away from this podcast today.

Joe Rocco:

I think the, the big take-home point is, is going to be a long one. No, I'm just kidding. Um, the positives and the negatives of CXCL9. We have a specific marker that seems to identify this interferon gamma-driven hyperinflammatory patient. Um, using it in that fashion can be very clinically meaningful. But also appreciating what it doesn't tell you and the fact that it's still not giving you what the underlying cause is. Searching for that underlying driver is still so important, but potentially you have some clues now to dampen down the inflammation,

keep the patient alive while you search for that underlying driver. Those are the take-home points, I would say.

Sarah Nikiforow:

And as a clinician, we, as I mentioned at the start, we've struggled for decades with recognize HLH. Right? Now we're saying you got to be more nuanced.

Kari Magee:

Yes.

Sarah Nikiforow:

It's not a checkbox. We have so many more tools to be able to not just diagnose, but to target our therapy and see who needs the longer-term follow-up. And especially with Joe's paper, and something we didn't mention that I should, um, tout, uh, Michael and Adi Lorenz for, is the optimized HLH inflammatory index, which combines, um, ferritin and soluble IL-2 receptor, right? But recognizing that some of these biomarkers actually have prognostic implications, and that inflammation itself, whether you meet the criteria for HLH, whether you actually have lymphoma, inflammation is deadly.

Dr. Michael Jordan:

Yes.

Sarah Nikiforow:

Right? And so I think we go way beyond recognizing, do you meet 5 out of 8 criteria, right? To, you have inflammation, I'd like to understand it better. And we need to treat it fast.

Dr. Michael Jordan:

So the points I would add to what the great points you guys said is that just really practically is that CXCL9 is, maybe this is more than one point, but is really our best way to measure interferon gamma activity in a human being. And because interferon gamma gets turned on in a lot of different contexts. I think that be careful that an elevated CXCN line is not necessarily specific for HLH-like physiology.

Sarah Nikiforow:

Mm-hmm.

Dr. Michael Jordan:

Like I, as I mentioned, you know, anybody with a coming into the ER with a cold, what, you know, might have a modest elevation. So it's not necessarily all that specific. It might be specific. And I think this is something that people might need to sort out. It's complicated. Once you get high enough, like tens of thousands or certainly hundreds of thousands, that you have HLH-like physiology, but that it's a very sensitive, extremely sensitive measure, I think, of somebody with HLH-like pathology. And so of course that means if it's normal, then you really are barking up the wrong tree if you're thinking about HLH. So I think that's probably the practical thing I would add is that Yes, it's, it's a great measure of interferon gamma.

Interferon gamma is not necessarily restricted to HLH physiology except in extreme levels. Right. And so it's super useful in that regards in terms of is this, you know, looking like HLH or not.

Kari Magee:

Thank you guys so much. I truly— this has been such an honor for me to sit and listen to you and learn from you and just the dedication that you have in treating HLH patients. And just trying to learn more is just very admirable. So, I appreciate you guys. Thank you.

Sarah Nikiforow:

I'd like to tout the HLH field because frankly, the learning that we all share and the amount that we all communicate on clinical cases is really heartening. And I think that one of the things I've learned is that you can't practice this alone. You need to have a multidisciplinary team established immediately. But also everyone in this field is open for phone calls and very, very willing, as you can tell from the past discussions, to disseminate their information both about biology and patients. So please access those of us here in the room and not to be able to gain that experience because none of us can see every patient with HLH and know all the presentations.

Joe Rocco:

And to add on to that, open—

Sarah Nikiforow:

clearly we're excited—

Joe Rocco:

open to trainees that are interested in this because I think we do all realize that this is an evolving field that is sort of rapidly expanding in some ways that, you know, anyone that's interested in it, we're very reachable, as you said.

Sarah Nikiforow:

And touches multiple different subspecialties. So it really is an opportunity for learning.

Kari Magee:

Thank you, guys. Thank you, listeners. And don't forget to follow us so that you can be updated on all the new investigative Investigating HLH podcast.