SIBO SOS® Expert Update from Dr. Mark Pimentel

Shivan Sarna: I'm Shivan Sarna. We have Dr. Pimentel with us. For those of you who are familiar with him, I know you're going to recognize his incredible face, but here is part of his bio. He is the executive director of the MAST Program at Cedar Sinai.

Shivan Sarna: We're just happy that you're here.

This is an honor to have Dr. Pimentel here. Go ahead and share your screen again. You know what I always say about him… and that is that if anyone's going to find a cure for SIBO, he is the one leading the way. That is such an understatement of his originality of thoughts and expertise and caring and true devotion to this cause.

So, what I'm going to do right now is to let him take over so we can learn from him. And then, we have some very specific questions that have already been submitted, which we will be taking to him in the second part of this session.

We share the recording within seven days so log in to your SIBO SOS® library to find it in your Simplero portal if you have that access.

Okay, thank you. Dr. Pimentel, take it away.

Dr. Pimentel: Thanks, Shivan. It's always great working with you. And you're very professional. And I really enjoy doing these podcasts.

And also, the whole point of doing this type of podcast or broadcast about SIBO and its updates is to try and stay on top of the data because the data are now coming very quickly.

The second is to make sure that we're all on the same page, that we're not veering too much off course because a lot of times, when new things are emerging, you have got to put it into the context of what the clinicians who are studying SIBO are doing with the information. And I think that's why it's important to do this every now and then. And Shivan, thank you for allowing me to speak today.

So, this is a bit of an update. Some of you who've seen some of my previous slides will recognize many of the slides because, really, the introduction is the same. But there's some really new

things coming. And believe me, in a few more months, there's going to be an even greater number of updates because there is a SIBO guideline being published, which I'm happy to be a part of. And I can't talk too much about that until it's published obviously. But that is imminently published. So we're very excited about that as well.

And we can update you after that because there's going to be some changes in the nomenclature or naming of SIBO and it's and its aspects. So I'm excited about some of the things that we did to fix the problems in SIBO.

And again, sorry to be cryptic, but stay tuned!

For those of you who are novices to SIBO… what is SIBO? Well, *small intestine bacterial overgrowth* is a condition that's been coined for almost 50 years. Back in the 1960s, this was initially described. And what it was describing in the 1960s—and I'll spend a little time on this because it's confusing. In the 1960s, people were having surgery for ulcers. Nobody knew what caused ulcers. They didn't know *H. pylori* was the cause of ulcers. And they were doing reconstructions of the bowel to remove the parts of the stomach that have ulcers. And then, they created these blind loops of bowel. And without graphics, it's hard to explain the different challenges and the different changes they made to the bowel to help these ulcer patients at the time.

As a result of that, these patients were getting diarrhea, bloating, gas and distension. And they started to take juice from the small bowel. They found that the bacteria was extremely high in the small bowel. And that was the initial description.

But then, over the ensuing decades, nobody ever went through the effort of saying, "Okay, well what is the normal bowel bacterial content?" And so, SIBO became a little bit confusing because people were saying, "Well, it's got to be greater than 10^5 in the small bowel."

Well, 10^5 or greater than 100,000 bacteria per milliliter in the small bowel was what they saw in those very disturbed bowels with a lot of surgery. But normal people never have more than 10^3 in their small bowel or more than 1000 bacteria per ml.

[05:06]

Dr. Mark Pimentel: And so, these numbers have been redefined lately. And this will also be evident in some of the papers that will come up this year.

But normal small bowel generally has been believed to be less than 10^3 bacteria per milliliter.

Now, the other things that are coming down the pipeline—and again, I can't talk too much about it—is that's based on culture. Culture only cultures what we can culture because we don't know how to culture everything, all the bugs, in the small bowel. But there will be new definitions of SIBO based on sequencing probably by May. At DDW, we're going to present some of that work.

And so, that will be another interesting aspect of SIBO if you do sequencing instead of culture.

But there's always this entanglement—IBS, SIBO, IBS, SIBO. What is it? If it's SIBO, then is it really IBS or is it not IBS?

The way I like to frame this—and if you've heard this before, forgive me. But the way I like to frame this is like *H. pylori* and peptic ulcer disease. The term *peptic ulcer disease* was you had an ulcer in your stomach or in your duodenum. And that was peptic ulcer disease. Now, *H. pylori* was discovered. And *H. pylori* was discovered to cause peptic ulcer disease. Probably 60 or 70% of ulcers were eventually noted to be due to *H. pylori*. You get rid of *H. pylori*, the ulcers never come back.

And so, despite that, we didn't change the name to *H. pylori* disease. It's still remained peptic ulcer disease.

So, I think the same thing is seen here. So let me sort of say it in a different way. IBS is the overarching framework for people who have altered bowel function of unknown etiology. Of that group, we now think 60% to 70% of IBS with diarrhea, for example, are SIBO.

Now, you can have SIBO and not be IBS. For example, if you're on opiates, you're paralyzing the function or the motor function of the gut, or you have an adhesion, or you have other things that impair motility. It's of like *H. pylori*. You can have *H. pylori*. That could maybe cause your dyspepsia, but you don't have an ulcer (or cause other symptoms, but no ulcer).

So, it's the same story. It's just a modern version of the *H. pylori* story.

Here is sort of the breakdown of SIBO. And of course, there's many causes of SIBO. So, when you have a patient with SIBO, is it IBS or is it SIBO? It's important to know because if it's IBS, it's not cancer. I mean cancer can cause SIBO. For example, if you have cancer of the small bowel blocking the bowel, you're going to have SIBO. You treat the patient with antibiotics, they'll feel temporarily better. But boy, it's important to know that they have a cancer there.

And of course, that's a rare event. As you can see here, there isn't even a cancer pie, a piece of the pie. But the point is you sort of want to know why they have SIBO because it will aid you in how to treat the patient.

The classic example is the adhesions, the orange piece. If you remedy the adhesions, their SIBO is much easier to treat or their SIBO doesn't come back. And I have patients like that where successful treatment without any recurrence of adhesions… and the patients are cured.

Now in the framework of IBS, going back to that irritable bowel syndrome framework, there are three main types of IBS—IBS-C, IBS-mixed and IBS-D. But as we move forward, we actually think there are two types—that is IBS with constipation, and then the rest which have a diarrhea component.

And so, we know this because of what we're finding with some of the blood biomarkers because we don't find the blood biomarkers for autoimmunity in IBS-C. Therefore, the pathophysiology of IBS-C is different.

And we also know that IBS-C is associated with elevated methane and increased methanogens, which we don't see in the—

Again, separating these two conditions along these lines that you see on this slide.

We're working from this new map or framework that we've created, which is that we think that IBS starts from acute gastroenteritis. When I say *we think,* that was something we said about five years ago. I'm going to show you a data that says, "no, we now know that food poisoning causes IBS." So, we don't *think* anymore. We know that food poisoning causes IBS.

[10:01]

Dr. Mark Pimentel: We now know that the CdtB toxin is important to this process and that autoimmunity to a protein called vinculin (which I'll explain later) leads to gut nerve damage. It's gut nerve damage. It then leads to poor flow of the gut, and then the bacteria start to build up which is bacterial overgrowth. And of course, in this scenario, we call this irritable bowel syndrome because this is the path to the IBS piece of the puzzle that I showed you back on this slide. So, that middle part is, we think, formed by this sort of sequence of events.

So, let's focus on the bacterial overgrowth linked to IBS first. I could give a whole one-hour lecture on just that link, or even longer, because there's so much data now. There hasn't been a

meta-analysis of this, of breath testing in IBS, for quite some time because now there's probably 20 to 30 studies. So it probably warrants a second mega meta-analysis.

But nonetheless, this is a meta-analysis from 2010 showing that it's very clear that, if you have IBS, and you look at age and gender matched studies, that IBS, breath testing in IBS is much more abnormal than in healthy controls.

So, we knew in the early days of this—and of course, some of these studies were back from 1999. We knew in the early days of this that the breath test which was always used to diagnose bacterial overgrowth was abnormal in IBS.

So, what is the breath test? The breath test is you drink a sugar—and of course, some people use glucose, and some people will use lactulose (and we can discuss why one or the other are better. We prefer lactulose. And I can explain that in Q&A).

But anyways, the lactulose gets into the stomach, then into the small intestine. And if there are too many bacteria in the small intestine, within 90 minutes—that's the time the lactulose spends predominantly in the small intestine—you get fermentation, and hydrogen and methane are produced. That gets into your blood, goes to your lungs, and then we measure it in the breath.

Initially, the test, the breath test, was called the *hydrogen breath test*. But we have strongly guided the community of scientists to no longer call it the *hydrogen breath test* because of methane (and as you'll see soon, hydrogen sulfide). That's not the only gas produced. In fact, hydrogen, no matter how high the hydrogen was, in many studies that we've done, we couldn't predict symptoms.

So, if the hydrogen is elevated, you have overgrowth. That's clear. You had symptoms, yes. But if your hydrogen was 50, or your hydrogen was 100, your symptoms were no different. You were sick in both instances, but not *more* sick because the hydrogen was a hundred because hydrogen is a surrogate for bacterial overgrowth, but it is a fuel for other bacteria in the gut.

So here, hydrogen can be used by methane-producing organisms to produce methane. And the methane organisms use four hydrogens to produce one methane. So you don't need many methane producers to use up the hydrogen because they're converting at 4:1.

Sulfate-reducing organisms, or hydrogen sulfide producers, are 5:1. So you don't need as many of these to sop up a lot of the hydrogen in the environment.

And so, without knowing the three gases all at the same time in the human breath or in the human gut, you really don't understand what's going on with the gases in the breath test.

So, we now call it a *lactulose breath test* or a *glucose breath test*, not a *hydrogen breath test* because that's really too simplistic as to what's going on in this fermentation in the gut.

Okay, so getting back to IBS, this is a culture study by a Swedish group showing that, if you look at the number of patients who have greater than 10^4 coliforms—coliforms are colon bacteria like E. coli and Klebsiella—or greater than 5000 coliforms, a lot more IBS patients were positive using those cut-offs.

And so, the point was that it is true in this study from all the way back in 2007 that IBS patients have too many bacteria in their small bowel. We've gone on since then to actually define overgrowth as greater than 10^3. And using that definition, this is where we get the 60% to 70% likelihood of IBS-D being SIBO. So, about 60 to 70% of D-IBS is SIBO because 60% of patients who had their juice taken from the small bowel had SIBO using culture in this study in 2012.

[15:00]

Dr. Mark Pimentel: Now, it says 27% in the non-D IBS. But these were not healthy people. These were people going for scope who did not have IBS, but had other diseases. So, they could have had adhesions and other things. So that's why there is a little bit of an elevated rate. But it is statistically different in IBS, much higher and IBS.

A lot of work has been done. This is 2015. This was some of the earliest studies doing PCR—meaning actually trying to figure out what bacteria are in SIBO. And it's interesting because this was—I don't want to say *primitive* techniques, but *lower level* techniques because sequencing was really starting to take off during the time these studies were being conducted. And the techniques for sequencing were still in evolution.

But E. coli and Klebsiella were the culprits for those coliforms. So, those colon bacteria that were elevated in SIBO, it was the E. coli and Klebsiella in 2015. And I'll show you some more modern data that says exactly the same thing.

But here it shows that Klebsiella and E. coli are elevated.

Now, you look at the graphs, and you say, “Well, that doesn't look very different.” But on the y axis—this is log 10. So every number is 10 times higher number of bacteria. So it is different. And it's quite a substantially higher number.

A lot of our work—we have a number of areas of tremendous interest. But one of the mandates of the MAST program which I run, the Medically-Associated Science and Technology Program, is to try to get into the small bowel.

All of the 10 or 15 years of microbiome work (since the Human Microbiome Project was started and published in Nature) have focused on stool bacteria. And people have not focused or studied the small bowel bacteria. So we're one of the first initiatives worldwide to actually do this. This is called the Reimagined Study. And we're looking at it in various disorders. But we're taking consecutive patients who have endoscopy or are getting endoscopy in our GI lab, and we're getting samples from them.

We presented this at the DDW. It hasn't been published yet, but it's in the process of being published currently. But this was presented. So this is public information.

What I want to illustrate here is that the far right where you see this very large gray section and very large purple section a little bit higher, that stool, everything on the left is duodenum, jejunum. And FD means furthest distance of scope. So we took patients who had double balloon endoscopy which can almost get through the whole small bowel, and we got juice from the small bowel.

And what you can see here is that the small bowel based on just looking at the colors (each color is a different genus), you can see that the small bowel is completely different than the far left which is the large intestine or colon or fecal samples.

And so, studying stool and saying that represents the human microbiome is wrong.

The second thing that I now emphasize is that, if you think about it, people say, “Well, the stool is the largest collection of organisms.” I actually don't believe that's true anymore. I think if you look at the surface area, the colon is about three feet, the stool is a log. So the only thing you're exposed to is the surface of the log, not the contents of the log. So, the bugs in the middle of a stool aren't affecting you.

Now you, in perspective, take the three feet of colon, and then compare that to the 15 to 20 feet of small bowel with the villi and everything else, the small bowel, if you spread it out, is the size of a tennis court. So imagine spreading a thin layer of peanut butter on a tennis court and how many bacteria that represents—not to mention the small bowel is where food materials chemicals are more easily absorbed.

So, the influence of the bacteria in the small bowel are far greater than the colon in my view. And I think understanding the small bowel will be much more important to human disease than the colon.

So, let's move on.

Shivan Sarna: One question for you, Dr. Pimentel. You're just blowing a lot of people's minds who spent thousands of dollars on stool tests. I just want to go back and ask you about the little letters at the bottom. There was one that said FD.

Dr. Mark Pimentel: FD means *farthest distance*.

Shivan Sarna: Okay.

Dr. Mark Pimentel: So, we were able to get into the ileum from above with double balloon endoscopy in some patients.

But some patients, maybe we're not quite in the ileum. So that's why it's called *farthest distance*.

Shivan Sarna: Got it! Thank you.

Dr. Mark Pimentel: So, based on the most modern sequencing, this is what SIBO looks like on the right compared to non-SIBO on the left from the small bowel using the most sophisticated techniques. And already, you can see my cursor, enterobacteriaceae are coliforms. So, in essence, that's the E. coli and Klebsiella. And we now know Klebsiella is here. And this other light gray is E. coli. Again, we don't see them here. You can see, if you look over here, we don't see them here in non-SIBO.

[20:24]

Dr. Mark Pimentel: So, E. coli and Klebsiella are the villains of SIBO.

The other thing we were able to do for the first time—and this is probably the most important finding of 2019 for SIBO—is people have been questioning breath testing. This is lactulose breath testing. So, for once and for all, we have validated that the lactulose breath test absolutely means SIBO in multi-dimensions

So, we were able to show that, absolutely, the 90-minute time point is the best time point for lactulose for a rise of 20. That correlated with elevated gammaproteobacteria (which are the E. coli and Klebsiella classes).

It also correlated with a reduction in firmicutes (which is something we see in SIBO because, when these guys go up, these guys go down). It also correlated with excessive gas. And it also correlated with finding that the bacteria metabolome in the small bowel was favoring hydrogen production.

So, the small bowel bacteria had changed to a composition of bacteria that was promoting hydrogen production pathways by those bacteria.

So, all of it now fits together. And we can now say definitively that the cut-off of 20 at 90 matches the symptoms, matches the microbiome, and is the most accurate end point for using the breath test.

Okay. So, I want to talk a little bit about methane, and then get to food poisoning before we wrap up and go to Q&A. But methane is important for constipation IBS.

This is a meta-analysis of methane and constipation. Again, 2011, there's probably 20 or 30 studies here as well, all showing the same or even more clear picture that, if you're methane positive, you're constipated.

We knew from a study we did in animals that, if you put methane into the small intestine, you slow transit by almost 70%. So, methane was the active ingredient that was causing constipation.

So, we thought if you can get rid of methane, you can improve constipation.

And we did that with a study with neomycin and rifaximin. And we're working on something new now, which I hopefully will show you here in a couple of slides.

But again, reminding you of how this works, hydrogen fuels methane production, but it also fuels hydrogen sulfide on the bottom.

So, we now have a new device that's in play. This isn't the device. This is the initial prototype. The new device is much more pretty. But basically, this was the study we presented in 2018 showing that if you have hydrogen sulfide in your breath test of greater than 1.2 ppm, you had more severe diarrhea among the patients who had symptoms.

So, we now know methane is associated with constipation. Hydrogen sulfide promotes diarrhea. Hydrogen sulfide is pretty toxic to the epithelium. And we think it's actually a direct toxic effect. But it's not a hundred percent clear.

Treating SIBO though, it goes back to this New England Journal of Medicine paper where we showed Rifaximin for the IBS and we believe for the hydrogen SIBO (maybe even the hydrogen sulfide. I can't know for sure yet because we haven't done enough hydrogen sulfide tests in clinical practice). But this clearly shows that rifaximin improves diarrhea, improves bloating, improves stool consistency and the new FDA endpoints, and formed the basis for the approval of rifaximin for irritable bowel syndrome.

We did something. We studied the progress of IBS with diarrhea over a decade. In other words, how many patients were coming to a tertiary care hospital for the condition IBS with diarrhea? And since the use of rifaximin, we've seen a 30% reduction in referrals to clinical medicine at tertiary care hospitals for IBS diarrhea and mixed IBS.

So, that's a good sign. That means that sort of like *H. pylori*, maybe we're reducing the burden of IBS-D in the community, or maybe doctors are getting wise to rifaximin and are starting to treat it at their level, at the primary care level, and seeing the response. And therefore, we're seeing less patients.

[25:18]

Dr. Mark Pimentel: This slide needs to change slightly because the paper was just published last month. And this is probably the most important connection of SIBO to IBS because in the target three trial of rifaximin, breath tests were done in a subset of patients. So follow me as I explain each column.

The overall response to rifaximin in target three was 44% of patients got better. But if their breath test was negative, only 25% of patients got better. If the breath test at the beginning of the study was positive for hydrogen, 56% got better. That's a lot more patients better. But if the rifaximin made the breath test go away, now the breath test is negative, 76% of those patients met the FDA endpoint for improvement.

This is the first study to prove in a very large scale FDA-approved trial that if you did a breath test and it was positive, you're more likely to respond to rifaximin and have a clinical benefit. In other words, if your microbiome is messed up based on the breath test, that's the patient who best responds to rifaximin. So first study ever to show this.

For the methane side, we published the small double blind study which is comparing neomycin and placebo to neomycin and rifaximin. And if you give neomycin and rifaximin together, you had less constipation after the trial. You had less bloating after the trial.

But this was the most important slide or finding in that paper. And that is that if you had methane, and your methane dropped to less than 3 ppm because of treatment, that's the person who was best improved as a result of the treatment.

So, in other words, it's not about the neomycin plus rifaximin. It's about can you get the methane less than 3. And if you do, that patient will feel the best.

So, as many of you know, we've been working on using lovastatin. So, this is based on a number of years of work that we've been doing to test lovastatin because lovastatin lactone is produced by Aspergillus which is a fungus in swamps and wet areas that secretes these granules that contain the lovastatin.

Now, the lovastatin in these granules were released in the environment. And lovastatin actually isn't for cholesterol. It's to reduce methane in the environment of the Aspergillus, so that these methane doesn't accumulate because it makes the Aspergillus sick—or at least that's the theory.

And so, we said, "Well, maybe lovastatin can reduce it." And we tested this in the lab. And lovastatin was the only statin that could do it because anytime humans changed statins to one of the more modern forms—so you took the molecule, you broke it apart to make it better for cholesterol, you ruined the effect on inhibiting methane production.

So, we got to go to the old school lovastatin to make this work.

And so, SYN10 is lovastatin but delivered in a specific way in multiple sites in the gut where we think the methane is most prominent to be produced. And it's non-absorbed. It's created to be non-absorbed so it doesn't get into your bloodstream. So, it's not going to help your cholesterol, but it will block methane in the gut.

This is the first study. And if you focus on the red line, the red is the number of laxatives taken. The least number of laxatives was taken in the subjects who were taking the high dose SYN10. And then, in between, for the blue line; and then the patients who took placebo were taking a lot of laxatives because their constipation was quite bad.

We're right now in the middle of a double-blind randomized phase II trial. We're still looking for patients. We're pretty close to more—we're more than halfway enrolled. We just have to complete the trial. We're hoping to complete the trial in the next six months. So, if you have patients, we're also paying for some of their travel. We have patients coming in from all over the country. But we're hopeful that we can get this trial done and get this thing close to market in the near future.

So, this is the ongoing phase IIB trial. Methane has to be there. They have to be less than 65 years of age. They have to have constipation. It's a three-month trial. And the primary outcome is clinical improvement of constipation.

So the final part of this is, let's go back to the beginning which is, "Well, how did this all start? And can we fix the root so that we don't have to deal with treating SIBO, but preventing it or curing it?" And that's really a lot of our focus in the lab right now, focusing on this first part.

[30:13]

Dr. Mark Pimentel: Remember when I said *we think* IBS is caused by food poisoning? This study says, "no, we know that IBS is caused by food poisoning," this meta-analysis by the Mayo Clinic with 45 trials, 45 outbreaks of gastroenteritis around the world.

2019… food poisoning causes IBS full stop. It's not all of IBS is caused by food poisoning. Of course, that's not true. But again, 60% to 70% of IBS we think is caused by food poisoning. So you were fine *until* you had that food poisoning episode.

The risk factors are the severity of the food poisoning. So, if you've had one week of bloody diarrhea, you're more likely to develop IBS. And if you've had one day, interestingly, if you're a woman, you're more likely to develop IBS. We can touch on that because we think IBS is an autoimmune disease because of food poisoning. And women tend to get more autoimmune diseases.

So, it's not because you're a woman. It's because there's something physiologic about women getting autoimmune diseases more likely. That could be the important factor here.

So, if you've seen this, sorry to show you this again, but this is the basis for our new work back from 2008. But basically, we infected rats with Campylobacter on the right, placebo on the left. And these rats who got Campylobacter developed bacterial overgrowth. Twenty-seven percent of them develop bacterial overgrowth from the Campylobacter.

And so, we knew in 2008 that if you get food poisoning, you will get bacterial overgrowth as a result in animal studies.

Not only that, these animals on the right, the pink bar, if they got Campylobacter and they got bacterial overgrowth. 80% plus of those rats had weird stool. So basically, they developed IBS and increased rectal lymphocytes which has been found in humans with post-infectious IBS.

So again, this is the first animal model using a bug that causes IBS in humans, Campylobacter, showing in animal model that replicates what's going on in humans.

Now, using this animal model, we started to look at, well, what could be the cause because toxins are very important to bacteria in terms of how they hurt humans. Now, Shigella, Salmonella, Campylobacter, E. coli, you go back to this list very quickly, there were Campylobacter outbreaks, there were Salmonella outbreaks, there were E. coli outbreaks, Shigella outbreaks. And they all lead to IBS. So we knew all four of these organisms lead to IBS. But only one toxin was common to all four of these organisms. And that was *cytolethal distending toxin* with B being the active toxin.

So, we then did a study where we repeated this experiment, except we gave a third arm where we gave a Campylobacter where we diluted the toxin. And the rats didn't get IBS.

So, we were believing that CdtB played an important role in the development of IBS.

I don't have time to go through all the steps. But fast forward to this study, we took recombinant—meaning we purified CdtB. So we injected it into rats, just CdtB. They didn't get food poisoning. They just got the toxin. And then, a month later, we gave them a booster. And then we saw what happened. And lo, and behold, the rats developed IBS. But they developed antibodies to CdtB because if you don't see it, you don't have antibodies. You can see on the left, no antibodies. And then after CdtB, more antibodies to CdtB. But look what happened to vinculin. They developed autoimmunity to vinculin as well.

So, exposure to CdtB led to elevations and anti-vinculin and autoimmunity in these rats.

It's hard to understand this. So I'll just explain it. The higher the anti-CdtB, the more bacteria was found in the duodenum and the ileum. The last vinculin was found in the gut—because vinculin was being mucked up—the weirder the stool was because the stool wet weights were altered.

And then, there was also very unusual changes in cytokine expression, some of them going down (for example, anti-TNF which is an anti-inflammatory marker. It was actually less in IBS. 1beta, an inflammatory marker, was less in these IBS rats.

So, anti-CdtB does something very particular and very interesting to the host. And while I can't tell you, we have expounded on this in a very elaborate study in humans that you'll see in DDW in May that actually explains a lot of what you're seeing and why rats and humans are identical in their phenotype of IBS. And that will be very interesting.

[35:14]

Dr. Mark Pimentel: But it allowed us to generate a blood test for IBS using anti-vinculin and anti-CdtB. And we're now at generation two of the blood test. So, I'm not even showing generation one because it's obsolete. Generation two improved these proteins. Vinculin and CdtB are very finicky. And so, we found a way called epitope optimization (which I can't just discuss) to allow the epitope to be better exposed so that the antibodies combine. And as a result, we get even better separation between IBS and IBD.

If you want a blood test, you want to be able to separate IBS from another disease that causes diarrhea. I don't care about healthy people because they're not coming to my clinic. I have two people with diarrhea in my clinic. Is there a test that says you have IBS?

And you can see the summary of this new data from this past year, that if both markers are positive, there's a 98% chance you have IBS. And that it's because of food poisoning.

It's important to look at this [likelihood ratio], not to focus on specificity and sensitivity. Specificity of this test is set very high. The sensitivity is sacrificed because, remember, if you took a hundred patients with IBS, only 60 might have been from food poisoning. So, the best sensitivity you could ever get is 60. So, it's not important for sensitivity. Specificity is more important because that will save money and get patients to an answer and likelihood ratio because this gives you your post-test probability.

So, this is how you calculate post-test probability. So let's say you think they have IBS at a 50% rate. And the likelihood ratio is 5.3. So you draw a line between here, 5.3, and you get to 85% or so; and at 6.0, it goes up to over 90%.

So, the bottom line is we're getting a very good post-test probability.

This is medical certainty at the top. Eighty percent or higher is a medical certainty.

So, what we did recently is we looked at every test that's ever been developed for IBS-D. I know we're talking about SIBO, but let's just try and focus on IBS-D for a moment because not only are these tests re for SIBO. There's a number of tests that have been described, but only anti-vinculin and anti-CdtB, and if they're both at the highest specificity or post-test probability for IBS-D and pinning it down that this is due to food poisoning.

So, the conclusions of my talk are IBS is related to SIBO. I think the new breath test study just published last month confirms that breath testing is not only important, it suggests that rifaximin—it implies who's going to get better on rifaximin.

Secondly, we now have proven through culture, deep sequencing, and relating that to symptoms and hydrogen production, and within the bacteria themselves in the gut (in that one presentation from DDW) that we're now able to define that lactulose breath test with a 90-minute cut-off of 20 ppm is the correct cut-off. And that's what we go with now.

That vinculin is a really important part of this process. So if your vinculin is positive, you're more damaged, per se, maybe less ICC cells, maybe less motility, and therefore the SIBO may be harder to treat. That's what we see in the clinic.

The patients I really liked are the ones with anti-CdtB only. Vinculin is tougher.

There are many causes of SIBO. We went through some of them, but of course not exhaustively. Hydrogen sulfide and methane are linked to specific symptoms in IBS and SIBO. And hydrogen sulfide is linked to diarrhea; methane to constipation.

New therapies are emerging to treat SIBO, and in particular, SYN10 for constipation and methane. And so we're very excited about that as well.

Shivan Sarna: Thank you.

Dr. Mark Pimentel: So that's the MAST team there. And thank you very much for your time and attention. Hopefully, we've got plenty of time for questions.

Shivan Sarna: Oh, thank you so much. You know, while we have the MAST slide actually up in this transitional moment, can you tell us the best way to donate to MAST?

Dr. Mark Pimentel: The best way to donate is to go to—if you go on Twitter (and my Twitter is @MarkPimentelMD), there is a link there that goes straight to the donation page for MAST. All the money goes straight to the research. Nothing is skimmed off.

[40:15]

Dr. Mark Pimentel: We recently were amazingly blessed by a generous donor who was able to get us a new piece of equipment that allows us to identify down to the species and strain level what bacteria are involved. So we're super excited about putting that into play.

So, all of this helps. It helps a tremendous amount. And we just put it straight to patients and straight to helping them find the cures.

Shivan Sarna: Great! Thank you so much. Okay, so go find him on Twitter and find that link, everyone.

Thank you! That was fantastic.

In that one graph that you had that showed the farthest reached in the large intestine and all that, it just showed the *E. coli*. It didn't show Campylobacter. Is that what it's called? It just showed the *Klebsiella* and the *E. coli.* Is that just because that one person didn't happen to have the *Campylobacter*?

Dr. Mark Pimentel: Yeah, this is a very important thing to make clear. If you get *Campylobacter*, it's a food poisoning. So you get it—in the rats or in the humans, you'll get it—and then, you'll get rid of it. It's gone after about a month. So it's just for that episode.

But the damage it caused since then leads to the stasis of the bowel. And then, your natural *E. coli* and *Klebsiella* are like, "Hey, they're weeds."

Think of it like weeds in a garden or weeds on the grass. If you don't mow the grass, the weeds will win. Mowing the grass keeps the weeds in check. I think that's the analogy that I like to us.

So, what it shows is that *E. coli* and *Klebsiella* are elevated in the small bowel in contrast to the colon.

Shivan Sarna: Okay, thank you. In the beginning, you said that the nomenclature for some of the traditional terms that we use associated with SIBO are going to be changing. Will that also coincide with a treatment change?

Dr. Mark Pimentel: So, treatment changes depend on double-blind randomized control trials. Once we finish the SYN10 trial, and knock on wood, it works, then obviously we'll have a paradigm change in treatment for constipation.

But until then, rifaximin is what we've been using. And of course, there are other treatments for hydrogen and other antibiotics as well.

Shivan Sarna: Is SYN10 used for other ailments?

Dr. Mark Pimentel: SYN10 is a brand new formulation, never been on the market. It's not available. You can't find it in another country or anything like that. It's just in trials right now.

Shivan Sarna: How much of the IBS population *isn't* SIBO?

Dr. Mark Pimentel: …isn't? So, if you were to look, for example, at IBS-D, the way I showed the pie, about 30% of people with IBS-D, it's not SIBO (at least depending on which study you look at. One of our studies was up to 80%, so maybe 20% are not SIBO).

But yeah, I would think 20 to 30% are not SIBO. And then, you have to look for other things like gluten sensitivity, bile acid diarrhea… and there's many other things that are on that list.

Shivan Sarna: Okay. Okay! The other thing I saw on one of your slides about what MAST also does, and it had to do with hormone homeostasis…?

Dr. Mark Pimentel: Yes.

Shivan Sarna: Well, how does hormones relate to these digestive disorders?

Dr. Mark Pimentel: So, Dr. Mathur is part of the MAST Program. So, we have multiple clinician scientists by PhDs. And they all have their areas of interest and focus. But Dr. Mathur is an endocrinologist. And she noted that polycystic ovary syndrome, for example, is more common in IBS. In fact, there was a recent paper just last month confirming her work.

But we do know that some bacteria in the gut produce testosterone. And we do find that in polycystic ovary patients, that these production of testosterone can be elevated. That's already been published by our group and by her.

So, we're trying to seek answers as to whether the bacteria of the gut can contribute to hormonal disease since they can produce some of these hormones.

And *hormonal disease* is a really bad term. But the point is that hormones can affect human physiology very dramatically. And if the bacteria can produce a particular hormone that can have a significant effect on the host.

[45:01]

Shivan Sarna: And Dr. Mathur also I know has been studying about insulin resistance and the gut as well.

Dr. Mark Pimentel: Yes.

Shivan Sarna: Is she finding a correlation between the two of those?

Dr. Mark Pimentel: So, there has been a lot of work we've done that she's headed in terms of relating methane to obesity, methane to insulin resistance. And that work continues to show the same effect. Higher methane, in the presence of high hydrogen—so you have to have not just the coal factory, but the coal… that analogy and these environmental issues currently. But you have to have the coal and the coal factory. You can't just have the coal factory. The same thing here. You have to have the hydrogen fuel and the methane. And that scenario is linked to obesity in three studies we've done.

And the second thing is getting rid of methane resulted in an improvement in glycemia. So we think it's important too, glucose metabolism, in some way (that we're still working on).

Shivan Sarna: So, there's a lot of people in the Facebook group — which is now over 13,000 people, very exciting—that have been talking about how they take a test, a SIBO breath test, and they have maybe hydrogen; then they do it again and the numbers go to methane; and then they do it again, sometimes, it's a flat line. They're just all over the place!

Dr. Mark Pimentel: I understand the question so, let me say that in 23 years of doing this work, I've seen one patient convert from hydrogen to methane. So that conversion almost never happens. You're a methane colonizer, or you're not. And if you're not, you're not going to be unless, somehow, you get colonized over years.

But the true statement you said initially… we do see patients, for example, they're 40 ppm hydrogen, you give an antibiotic, and all of a sudden, they're 80. So we think, based on what we know already, that those are hydrogen sulfide producers, meaning they have hydrogen sulfide, the antibiotics kill the sulfate-reducing bacteria, all of a sudden—

Remember, five hydrogens to make one hydrogen sulfide. You get rid of the hydrogen sulfide producers, hydrogen shoots up. So, if you don't kill them both, you will get a release of hydrogen that's higher.

But what's interesting, which is why hydrogen is so confusing, is that using that by itself is, a lot of those patients say, "I feel better, but my hydrogen went up." It's because the hydrogen sulfide is causing the diarrhea, not the hydrogen. So, the diarrhea got better, even though the hydrogen went up, and then that's confusing the doctor, the patient. The patient says, "Well, maybe it's

not," but it's more because of a lack of understanding because we don't have all three guesses on breath testing.

Shivan Sarna: And when do you think that the hydrogen sulfide test will be available for the masses?

Dr. Mark Pimentel: I'm saying 100% this year.

Shivan Sarna: Oh, good for you! In MAST or just if we come to Cedar-Sinai?

Dr. Mark Pimentel: No, it should be on MAST. But stay tuned! I keep saying stay tuned.

Shivan Sarna: It's okay, it's okay. We like that song. We like it when we say tomorrow, but we'll live with it because we know it's coming. I mean, it's so close.

Dr. Mark Pimentel: It's very close.

Shivan Sarna: I wanted to mention to the audience who are quite impatient—and I understand—or we hear that you've proven and shown that you now know exactly what the bacteria are or that you've confirmed the breath test, as a patient, sometimes, I'm with other people, and we're going, "Well, that's great. How does it impact my treatment? Good for you!"

But I wanted to just express to the community that it's so important. It's an important step as a scientist as Dr. Pimentel and Dr. Rezaie, Dr. Mathur because it gives them more research money to advance all of our treatment. If I read another article with just the most insidious headline questioning the validity of all this, and in actuality, all they're doing is writing a great headline, and then just going, "You know, you should really find the underlying cause," I feel like just going like, "Everyone agrees with you."

Sorry! Anyway, I just wanted to express that to the community because, a lot of times, I hear like, "Well, that's great. What does it mean for us?" It means we're getting closer, but also, those steps of validity and credibility are incredibly important in order to make this progress.

Dr. Mark Pimentel: What you said is so true. In science, it's kind of frustrating because when you know the answer, it doesn't mean everybody else knows the answer.

For example, we have answers (which I can't talk about here) that will take us out three to five years. But we can't just… it's not that simple. It has to be scientifically proven, scientifically and

rigorously tested. It has to be done credibly because, otherwise, doctors will blow it off and patients will be frustrated.

[50:22]

Dr. Mark Pimentel: So, if it's done responsibly, slowly, meticulously and repeatedly—it's not just one experiment, it has to be shown in this way, and then that way, and then…

Believe me… doing the work is a lot more frustrating perhaps than hearing the work because it requires a lot of resources, as you said, but also a lot of time and energy to get these papers out.

Each paper is a month's worth of work in writing and editing. And so, it's tedious, but it's necessary. Otherwise, the work is marginalized.

Shivan Sarna: That's the word, it gets marginalized. And when it gets marginalized, that opens up so much vulnerability for lack of progress and for setting the science back. So… alright!

"Okay, what do you think is the cause of bloating immediately after or very soon after eating or drinking when it's clearly too soon to have the item already begin to ferment the gas?"

Dr. Mark Pimentel: I love that question because we see this quite often—not all the time, but quite often. Somebody says, "Well, I wake up in the morning, I drink a glass of water, and all the sudden, I'm already starting to feel bloated."

Because you have SIBO, you generally—whether it's first thing in the morning, of course, it's much worse after eating later in the day. So what I would do in clinical practice, I would say, "Okay, let's get back to that in a second. If you eat, does the bloating get a lot worse?" The answer is always yes. "If you eat through the day, is your bloating getting worse as the day progresses?" The answer is always yes.

I say, "Okay. Now, let's get back to that water you drank in the morning. You have more gas in your small intestine because you have SIBO. And that gas is sort of distributed everywhere. But as soon as you drink water, you're turning on the motor of the gut. And then, all of a sudden, that gas starts to conglomerated in one area, and you feel the pressure of it because the gut is now moving and moving things in weird directions. And then you get a collection of gas all of a sudden just from that water."

So, it's not that you're fermenting faster, or you've suddenly fermented a whole bunch of gas. It's that it's coming into one loop of bowel, or it's moving to a section that causes more distension for you. And that's what I think is happening.

Shivan Sarna: Like cranking the motor basically.

Dr. Mark Pimentel: Yeah, it's like turning on the hose, but you haven't opened the spout yet. The hose starts to stretch and…

Shivan Sarna: Okay, that's awesome. That's great.

"What is the difference between food poisoning and stomach flu? And are the impacts of the chances of getting IBS the same?"

Dr. Mark Pimentel: So, there are viruses that cause "stomach flu." I think that's what people refer to viruses as. But the problem is you can't tell. So you can call it a stomach flu, but it could actually be Campylobacter or salmonella because salmonella can cause vomiting. So you can't tell the difference.

There are viruses out there. Let's say you meet somebody, a good friend of yours, and they've been saying, "Oh, I've had the stomach flu," and then, you hug them, and you don't eat the same food, and then you get the flu… that's generally a virus. But if you're talking about food poisoning, that's usually a bacteria.

Now, viruses potentially can cause IBS. We don't understand why. But Giardia can cause IBS. And Giardia has a ton of vinculin. So I think that might be an interesting connection.

Parasites have vinculin as well. As you're killing them, you get exposed to it. So it's possible that there's a relationship there as well.

Shivan Sarna: Can you define vinculin for me again please?

Dr. Mark Pimentel: Oh, vinculin is the protein in cells that help the cells move. Humans have it in our cells, especially in the nerve cells of the gut, the particular form we're talking about. And so, antivinculin antibodies we think are causing IBS and SIBO. So, if you're exposed to parasites, it's possible that's how you get your antibodies.

Shivan Sarna: Ooh, that's good. That's really interesting because I haven't ever heard that. I've heard that parasites can be associated with SIBO. And a lot of people who have SIBO have

parasites. A lot of people have parasites that don't realize they have parasites. But I didn't realize that about the vinculin.

Okay, so this is a really big question that so many people have. And it has to do with this label of having an autoimmune disease.

There's some people, when they hear autoimmune disease, they have a very severe gut reaction to it and are feeling like they have just been given, in some way, to be dramatic, a death sentence. Do you feel that way about calling it an autoimmune disease and having it be as severe as maybe lupus or something like that?

[55:12]

Dr. Mark Pimentel: So, of course, lupus is a much more severe autoimmune disease. And autoimmune diseases are, in general, varied in their intensity.

What I experienced in my clinic is that I do the test, the test comes back positive, the patients feel liberated because they say, "Finally, I have an answer. I know what caused my IBS. I may not have remembered the food poisoning. But I now know I had it and that this is what's going on." And now, there's some optimism for future treatment because we now know the target to treat. We just have to figure out the nuts and bolts of how to treat it.

I get more frustration with patients who have a negative test because they feel like, "Damn! I wish I was positive because then I knew the answer." And they still don't know the answer. So, I get more of the negative on the other side.

But I can see where people who, for example, have autoimmune disease in the family, and they see the ravages of autoimmune disease, that this might feel very devastating. And it is a little frustrating with vinculin because vinculin, at least in our initial use in clinic, seems to portend a more severe illness.

It's important to know though. I mean, knowing is better than not knowing in my view as a doctor; but also, for patients I think.

Shivan Sarna: So, autoimmune diseases are on a spectrum, right? Some are more severe than others.

"Okay, so let's say you have taken the test, positive for antibodies—I don't know which one—what is the proper treatment? Prokinetics only? Have you seen people get better and how?

Do the antibodies impact IBS-C, IBS-D and IBS mixed?" I think you've already addressed that today—"in your observation beyond what is proven by the studies?" Are you with me?

Dr. Mark Pimentel: Yeah, I'm with you.

Shivan Sarna: Okay. Basically, what are you seeing in your clinical observations? "For example, I have positive antibodies, and IBS-C and SIBO."

Dr. Mark Pimentel: Yeah. So, the antibodies are less likely to be positive in C. But about 25% of C patients are positive. So, in that case, it's likely that the antibodies are contributing .

The antivinculin as I've mentioned is the more significant antibody in the IBS smart test and second generation test.

But I have a patient—let me give you an example. I have a patient. He came in. He got food poisoning in a very unusual way which I won't describe. He got food poisoning. And then, ever since then, he developed IBS.

We measured. He was anti-CdtB positive—not antivinculin, not the autoimmunity. And then, we treated him. He was getting treatments. They were working. It was a struggle, but they were working.

Six months ago, he stopped all therapy. He felt great. I measured his anti-CdtB again, and it's normal.

So, CdtB antibodies will go away. The reason this test is important in my practice—number one, first to identify it's food poisoning. Okay, great. Secondly, vinculin means more likely prokinetic (to answer your person's question). But you still need to take antibiotics first.

Third is, please, if you have these antibodies, avoid places where you think the likelihood of food poisoning is higher. I'm not saying don't travel. I am saying be much more cautious.

This man was instructed to be ultra-cautious. And as a result, his IBS is gone. He's taking nothing now.

And so, if you're CdtB positive only, I love that because I think, in two years, it will all be gone if you just avoid food poisoning. But if he gets food poisoning again, he forms these antibodies. So it's not over for him as long as he's ultra-cautious, which is what our instructions were.

So, it gives us better instructions for patients I think is how I see it.

Shivan Sarna: If you are showing that you have the issue with your vinculin, then [00:59:40]?

Dr. Mark Pimentel: Yes. I mean, we still try to wean people from prokinetics. Our goal is not to keep you on drugs forever. But our goal is also not to use antibiotics over and over and over again. So, if we can use a prokinetic even at a tiny dose to prevent the need for repeated antibiotics, I'll do it because we're trying to prevent bacterial resistance and so forth.

[01:00:03]

Shivan Sarna: I know we're wrapping up. But for methane, when you are given rifaximin and neomycin, how do you feel about the neomycin "wiping out" your microbiome? No rifaximin stays in the small intestine. It has a totally different experience there. But with neomyosin, for that vision of like a nuclear bomb in your gut, which is what a lot of people really strongly feel about that…?

Dr. Mark Pimentel: Neomycin is not a nuclear bomb like Cipro or Augmentin or some of the more systemic antibiotics. And neomycin also is not absorbed. It's 95% non-absorbed; 5% only gets into your blood.

I mean, it's not the same as rifaximin. You do get resistance to neomycin. There are many resistant genes to neomycin in bacteria. So, you don't want to use that one over and over by itself (that's what we used to do back in the early days). But it's non-absorbed. So, it's almost similar to rifaximin.

Shivan Sarna: You just said that, in all your years, you haven't seen one particular set of circumstances where—can you repeat that for me one more time?

Dr. Mark Pimentel: So, we went back and looked at all the breath tests before and after treatment and so forth. And we were only able to identify one patient that I saw, that I personally saw—there are others, but it's very rare. What I'm saying is that your hydrogen, your first breath is hydrogen, you take an antibiotic, and you're now methane… that almost never happens. That's very, very rare.

Shivan Sarna: So, we do have someone who's saying that that happened to her.

Dr. Mark Pimentel: It happens, but it's very, very rare.

Shivan Sarna: It's very rare… and maybe a suggested re-test just because you have seen it so infrequently?

Dr. Mark Pimentel: I think so. I would recommend that.

Shivan Sarna: Okay. I have one more question for you that came in from Dr. Allison Siebecker here.

Dr. Mark Pimentel: Always the toughest question.

Shivan Sarna: Always the toughest. The very first question that you liked was also hers. Let's see. Let's see. It came in this weekend? I hope I can find it very quickly. If not, we will just go.

What else do you think I should be asking you, sir? Is there like some other thing you're super excited about? You have a lot to be excited about. But is there anything else that you're like, "Everyone needs to know about this."

Dr. Mark Pimentel: You know, honestly, I think 2020 is going to be a very big year for patients with IBS-C dif, I want to share optimism about a trial. You know, obviously, we don't know who's getting what. And we don't know what the trial results will be. But I'm very hopeful for SYN010. I want to give some hope this year because I think we're in the year where we know.

We're also in the year where we know exactly how the vinculin is working. You'll see that at DDW. We're in the year where we're finally starting to see hydrogen sulfide which will give more clarity to patients who are frustrated, saying, "My breath test is negative, but it's really not."

And so, we're going to have a lot of things clear up this year, a lot more things than any other year I think in the past.

So, I'll leave it on that note. I think that's really positive.

Shivan Sarna: I think so too. Thank you so much, Dr. Pimentel. As always, we really appreciate you. And of course, you know I'm going to be knocking on your door to hear about what's going on either at DDW or right after, so you can share it directly with the community.

Dr. Mark Pimentel: That sounds exciting. I look forward to that.

Shivan Sarna: Thank you so much. And I will also post the link so everyone can check out the IBS smart test because, of course, now that we have an even deeper understanding of it, I know that a lot of you are going to want to take that test. And they've made it easier than ever by

having it be available in Canada and having the ability to have—not even necessarily you'd have to go to a doctor's visit to get the test. It's all on their website.

Dr. Mark Pimentel: It's also available in Switzerland, Spain, the Middle East, Mexico, Canada… soon Australia. So it's starting to grow. So that's a good thing.

Shivan Sarna: That's fantastic! And also, please everyone, consider donating to the MAST program… as well as tell all your friends about food poisoning and the awareness that they need to have. So many people I know with gut issues, I asked them if they've had food poisoning, and they just look at me like, "Yeah." We just need to get the word out.

So, thank you, Dr. Pimentel. Thank you all very much for being here. And we will keep you posted and see you next time.

Dr. Mark Pimentel: Thanks Shivan.

Shivan Sarna: Thank you.

[01:04:52]