SIBO Research Masterclass with Dr. Mark Pimentel
Part 1

Shivan Sarna: Dr. Mark Pimentel has such an incredible bio. We'll give you his abbreviated version. It's an honor to have him as I've said. The executive director of MAST, a research program created exclusively to study the causes and treatments for SIBO, IBS and the microbiome-linked diseases at Cedar Sinai.

Is it pronounced "mast" or M-A-S-T, Dr. Pimentel?

Dr. Mark Pimentel: It's "mast."

Shivan Sarna: It's "mast." It's very exciting. This is a big deal, you guys. We need to get funding for this program. I believe that if anyone can find a cure for IBS, it is Dr. Mark Pimentel. Welcome sir.

Dr. Pimentel: Thank you very much. It's a pleasure to be here. We're going to do an amazing job today. We're going to try to take you through all the new data from DDW. So you're going to get literally up until one hour ago almost data. So it's very exciting.

Shivan Sarna: I know we've been working on it for a really long time. And it's finally happening. So the microbiome and IBS, take it away.

Dr. Pimentel: Thank you so much, Shivan Sarna.

Look, the microbiome and IBS and SIBO and all these connections are really coming together. And this particular DDW—DDW stands for *Digestive Disease Week*—it's a week where all the scientists get together to discuss a variety of topics. But one of the parts of this meeting is new data on the microbiome, new data related to IBS, new data related to small intestinal bacteria overgrowth. And a lot of really exciting things came out of the meeting.

But more importantly, the acceptance of all these different ideas has just grown immensely in the last year. And I'm very pleased with that.

The MAST Program, the Medically Associated Science and Technology Program is really a gift because it gives me the opportunity now as the executive director to really guide a group of individuals—which I'll show you at the end in a very nice picture—to get to the bottom, figure out the causes, figure out some diagnostic tests, things that will help patients directly.

And the whole point of the MAST program in a sense is to say, "Look, we see patients. We know what the patients want. We're here in the trenches doing it. We're not a pharmaceutical company. We're not trying to pick things that are going to generate revenue. We want to pick things that are going to help the patients."

And that's really what the goal is, is to find the cure.

And I've dedicated 22 years doing that. I'll do so for as long as my family allows me. And I'm very pleased to continue that.

How do I get to the next slide?

Dr. Pimentel: I think some of you may have seen this slide before. But it's really sort of a spoiler in a sense that it tells you what I'm going to tell you. We now, in 2018, believe that food poisoning triggers this change in the microbiome we call SIBO which is, in essence, IBS to some extent, that the toxin *cytolethal distending toxin*, creates this autoimmunity, the vinculin. This changes the nerves of the gut. Breath testing and culture are proving that this is in the sequence of what this event, this first food poisoning event triggers.

So, again, we're going to focus first on the breath testing because that's where it started, the breath testing culture, what substantiates this connection between the gut bacteria or SIBO and IBS. So that's where we're going to start. [04:12]

I tried to create this year some fancier graphs. You'll see some of those as we go through. They're a little more clearer to see.

But the small bowel is 15 ft. When it gets distended, believe me, you'll know you're bloated. When the colon gets distended, almost half the

colon is behind the cavity. It's called *retroperitoneal*, if it dilates, you don't get protrusion of the abdomen.

So this notion of bloating being caused by colonic gas never quite added up because this thick membrane overlies most of the colon. So the colon can't really cause protrusion of the abdomen like the small bowel can.

But the small bowel is 15 ft. long. And it generally has no bacteria in it. We've been kind of discussing this over the years, that the number of bacteria in the small bowel should really be about a thousand per milliliter. That's okay. Anything less than that is okay. [05:15]

Dr. Pimentel: But the colon, half the weight of stool is bacteria or 10^{th} to the 12^{th}. We can do the math, but it's a lot of bacteria there. And people have focused on looking at the stool (and I'll get into why that's a problem later).

And patients get this confused. Doctors don't know how to settle this in their mind. So is it really IBS or is it really SIBO?

And the way I'm handling this now to make it very clear for people is SIBO what's happening, IBS is the disease. So it's like saying you have ulcers, but the ulcer is caused by H. pylori. It's not H. pylori disease. It's ulcer disease.

The reason I say that is because SIBO can be caused by many things (as you'll see in the next slide). And so we don't want to say SIBO = IBS because SIBO could be adhesions or it could be something else.

SIBO is the downstream event that causes the symptoms.

But what caused the SIBO? That's what we're trying to get a handle on. Why does this SIBO occur? And if we know that, we can cure it. But there are different causes of SIBO.

That's why, on this slide, I say, "Not all of SIBO is IBS. And not all of IBS is SIBO."

But we're seeing that 60% plus of all irritable bowel syndrome patients, particularly diarrhea type, are SIBO. And that's really important because we're finding the

major cause of irritable bowel syndrome. And it is SIBO. But why?

I already mentioned that IBS is the bulk of SIBO. But there's a lot of other things that can cause SIBO. Somebody on morphine for two weeks, a normal person—this was done in the 1990s—will get SIBO. So the opioid epidemic that we're having in the United States, all of those people who are opiates, develop SIBO. They get bloating, distension and of course constipation as a side effect. Adhesions, pseudo-obstruction, diabetes are very common causes of SIBO.

In fact, we just did an analysis of all the breath test we've ever done. And diabetes is a clear risk factor for SIBO. And we know that for two reasons.

First, diabetes impacts the nerves of the gut over time (just like it impacts the nerves of your feet, and you get that neuropathy when you get really long-term poorly controlled diabetes). So the gut is affected.

But also, the gut loves sugar. High blood sugar means high sugar everywhere. And high sugar everywhere means bacteria that are very happy.

All of that probably contributes to the development of SIBO and diabetes. And then, there are other minor or less common conditions. I know there's a lot of questions these days about Ehlers-Danlos syndrome because it's increasing milder forms of EDS, and not the old textbook, really hardcore forms. These milder forms may be part of the problem as well or part of immune-recognizing of SIBO. [07:52]

But when you go back to IBS again, IBS breaks down into, really IBS-C, IBS-D and IBS-mixed. But I'm going to try and argue that there really is only two categories. There's the mixed and D.

And this is based on that IBS is an autoimmune disease. IBS-C doesn't appear to be autoimmune. It appears to be a bloom or an increase of methane organisms or methanogens (and we'll touch on that later). But the IBS-mixed and –D, they have these antibodies. And we think they stem from food poisoning.

So really, we're capturing M and D based on the origin of the illness which is food poisoning. So M and D, food poisoning; C, maybe it's just the methanogens.

So, basically, what we do is a breath test. So here's a sugar. And we use, in our center, lactulose. But others use glucose. I personally prefer lactulose. And I will argue that lactulose provides a greater understanding of who has overgrowth, number one.

The second part of it is we know from new rifaximin data—which I'll show you later—that if you did glucose, 25% of people would miss the opportunity to know that a drug would work for them if they did glucose. And if we want to, we can address that in question. But I would strongly now, based on new data, recommend lactulose over glucose because of the types of treatment that could benefit the patient.

The sugar then goes into the small intestine. And then, the gases permeate through the blood to the lungs. And then you see the breath test.

So this is an example of a breath test. This is extracted from North American Consensus. And this is a positive hydrogen breath test because, by 90 minutes, the gases rise to greater than 20 ppm. And that is the new gold standard description of what bacterial overgrowth means on a breath test. [10:12]

Dr. Pimentel: Now, the North American Consensus is an extremely important paper published for a lot of reasons. The first reason is nobody had established what is the real definition of SIBO based on breath testing. Nobody had gone through all the literature to talk about what the best way to do to culture the bowel. So we wanted to say, "Look, this is what the data in the published world says. This is the summation. This is what, after all that, the experts feel should be the general guideline of how to interpret these tests."

"If you want to prove that your new way of interpreting the test is better, do a head-to-head comparison. Take this criteria from the North American Consensus, do your breath test, use your criteria and say they're better. And then, another consensus will come in a couple of years, and we'll

change it"

But everybody has to be doing the same thing. You have one study saying 12 ppm, another study saying study, another using glucose, another using lactulose. You can't compare trials. The numbers are all over the map because of these poorly correlating or discrepant methodologies.

We said, "Everybody use the same methods. If you want to use a different one, then you have to show this method and then your new method."

I hope that's clear.

Anyways, breath testing though—this is 2010. This is a very old study now in my view. We were a part of this—it showed that, look, IBS patients had more positive breath tests by a factor of 10. So it's really significant that IBS patients have this abnormality.

And why? Why are they having this?

So, people argued and said, "Well, the breath test isn't accurate. It's not this. It's not that. Culture is the best."

The problem with culture is how do you get the organisms? And I'm going to just speak to that a little bit when we get to the deep sequencing because we're doing a trial right now. That's been just amazing as we unfold the results.

But here's a culture study. This is from 10 years ago. And they showed absolutely that IBS patients in yellow had more coliforms.

Coliforms, for those of you who don't know, are the bacteria that belong in the colon. They don't belong in the small bowel— Klebsiella, E. coli, those types of characters. They're only in the small bowel. They're too high too often in IBS based on this very large and very challenging study because they had to do 165 aspirates (which is very expensive) from the small bowel.

We then did a study in collaboration with some of our very good

colleagues in Greece. And they provided us samples. We did the sequencing of these. These are duodenal aspirates from Greece. If you have IBS, you have more E. coli, you have more Klebsiella. Again, here's that E. coli, Klebsiella or coliforms.

Now, you look at this bar, and you say, "Well, not a big difference here." You have to look at the Y axis. Every point there is 10 times higher bacteria. So we're talking 10 to 20 times more E. coli, Klebsiella. It's a factor of 10 for each number. It's not a small difference.

The point is that these are the characters that are overgrowing in the overgrowth of IBS.

But the problem with overgrowth is it's really complicated. Everybody says, "Well, order a breath test. You plug it in. And you just get these amazing results. There are mailed kits. There are all sorts of ways to do breath testing." Some people have a machine that only measures hydrogen. Some people have hydrogen and methane. Some people don't even have carbon dioxide. In 2018, that's not as common. Now, most people have a machine that measures all three. But I'm going to argue that a machine that measures four is even better. And that's coming in a few more months. Here's some data on them.

But the predominant gas produced by the gut that isn't carbon dioxide by bacteria—humans produce more carbon dioxide than any bacteria in the gut because that's our fermentation byproducts. We can't use carbon dioxide to measure bacteria. Carbon dioxide, we produce a ton of them. So you have to use methane because humans don't produce that. Hydrogen sulfide—which we'll talk about in a minute—humans don't produce hardly any of that. There is a tiny bit that's produced by cells, but hardly any.

We can distinguish bacteria from human by using hydrogen, methane and now the new hydrogen sulfide. And it works like this.

Hydrogen is produced. The problem, every time—we've ran studies for 20 years. And it always frustrated me because I would see a hydrogen breath test of 200 ppm, I'd see a hydrogen breath test of 40. Both people are sick,

both people have bloating. Both have some form of diarrhea. But it didn't matter whether you're 200 or 40, they both had diarrhea. And the diarrhea was similar. So we couldn't use the hydrogen as a thermometer for the intensity of symptoms. [15:14]

Dr. Pimentel: And so, we always got stuck with this. We couldn't understand why hydrogen was never proportional. So even if you produce 200 ppm of hydrogen, you didn't have more bloating than the person with 40.

Now, if you started with 200, and then we gave you an antibiotic, and you went down to 80, you were feeling better. The breath test would be showing reductions. But it was never quantitative at the beginning. And this was sort of somewhat frustrating.

But also, because of that point that I just made, that the amount of hydrogen didn't correlate with symptoms at baseline, people were doubting that the breath test made sense because, well, if somebody is producing 200, they must be sicker. And we could never prove that. But I'll show you why. So, hydrogen-producing bacteria feed hydrogen to the methane producers if you happen to have these characters in your gut. And as a result, you produce methane.But look at the numbers on the bottom right—four H^2's to make one CH^4. So it's sort of like saying you're going to eat four In-and-Out burgers to make one something. And you're eating more than you're making.

And so, when methane is there and the methanogens are there, they're eating a lot of hydrogen to make methane. And sometimes, you get a flat line breath test because they're eating it all up. They're just consuming it more than they're making the methane. But they get energy from this. And this is how the methanogens sustains themselves. They get energy from that.

Now, the sulfate-producing bacteria, the new characters that we're looking at, 5 hydrogens on the right to make one hydrogen sulfide, using up all the hydrogen. And this, we were always speculating, could be the flat line breath test because the flat line had no methane, no hydrogen at all, patients were having diarrhea and they were sick.

And so we now realize that there's another gas we haven't done.

This is basically the way it works. But remember, these two organisms, the methanogens and the sulfate-producing, they're fighting. You've got two mouths trying to feed on hydrogen. And one is going to win the battle. And the one who's winning, that's the symptom that you get.

And I'll show you later that the sulfate-producing bacteria for hydrogen sulfide is the cause of diarrhea and linked to diarrhea. That's why we could never figure out the hydrogen level. The hydrogen level was never proportional to diarrhea or bloating or any of that. Methane causes constipation. The sulfate-producers cause diarrhea.

This was presented at the DDW a few weeks ago. We validated completely a new instrument that measures all four gases. They're showing three gases here, three lines here. But let me explain.

This is to show you how the hydrogen is being eaten. The blue line is hydrogen. This is patient or this is the group of patients who had no methane, no hydrogen sulfide. And of course, the hydrogen is high.

As soon as one gas other than hydrogen is present—either methane or hydrogen sulfide—hydrogen is lower. When there are two consuming gasesa—meaning this patient had all three, hydrogen, methane and sulfide—the hydrogen is the lowest. So they're eating hydrogen. It's sort of proof that they're eating hydrogen. And this is what it looks like. This is how much lower. You're 56 ppm lower hydrogen when you have one consuming gas and 106 ppm lower if you had two consuming gases because the hydrogen are being eaten up.

This is probably the most important slide because it shows that if your hydrogen sulfide is present on this machine in the number that we determined that this machine detects, basically, you had more diarrhea and more urgency with your bowel movements. And you also have some more abdominal pains, but the numbers didn't reach statistical significance—again, for the first time, showing that those are significant.

Another way of looking at this is the fight, the fight between methane and

hydrogen sulfide. So let's start with the hydrogen sulfide side because I've already mentioned that and that's simple. If you have hydrogen sulfide and nothing else, your diarrhea is higher.

Let's go to the left side, the red's. You have methane. You tend to have a lot less diarrhea, meaning you're constipated.

But look who wins the battle when they're both there. Methane always wins. So if you have methane, eve if you have hydrogen sulfide, methane trumps the situation, and you're constipated or you have less diarrhea. [20:08]

Dr. Pimentel: And that's what we've been seeing in the breath test and part of the data we presented at the meeting.

We'll get back to that in a little bit because the breath test becomes important again later in the presentation. But I want to talk to you about rifaximin. Of course, rifaximin is really the only microbiome drug that's approved by the FDA because it doesn't really work like an antibiotic typically because it doesn't damage the colon bacteria. It doesn't reduce bacteria in the colon. We've studied this in animals. We've studied this in humans. The big TARGET 3 study looked at this. No change to stool bacterial composition.

However, the small bowel is reduced because it's not absorbed because it's not water soluble. So all the bile that's in the small bowel gives it some solubility. It works in the small bowel, and then precipitates in the colon, giving you this benefit.

This is the New England Journal of Medicine paper that we published that shows that rifaximin works. And rifaximin work for pain. It worked for global symptoms. It worked for bloating. It worked for the new FDA end point. It worked for stool consistency. It worked for everything no matter it was the first trial (called TARGET 1), the second trial (TARGET 2). We're combining the data. No matter how you looked at it, it was statistically significant.

But for the first time in this massive trial, these two trials, we showed that

you gave something and people stayed better for three months. Again, everything was—all but a few time points, it was statistically significant, meaning we effected a change through therapy that was sustained.
This is what I was getting to earlier about the breath test. This is new. This is from ACG 2017. It's just about to be published. But because it is public, we can describe it here.

Everybody was questioning the breath test and whether it had a relevance to treatment. And the answer is… *absolutely.*

So, look at this. In the overall TARGET 3 trial, 44% of people met the FDA end point. Now, the FDA end point is really hard. You have to have proven pain, you have to have proven stool consistency and they have to happen exactly the same week or forget about it, that week didn't work. So that's only why 44% responded because it's such a tough end point.

But if your breath test was negative in that trial day one, only 25% responded. Look at the final column, if your breath test was positive in this trial before you started rifaximin, 76% of those people responded to rifaximin using this very challenging FDA end point.

The bottomline is a positive breath test predicts response. Number two, get the breath test normal. That's your best response.

So the breath test is a thermometer. And we have to be using it better to understand the response of patients with this condition.

I love this slide. Out of all the deck, this is my favorite slide. And the reason it's my favorite slide is I've been working with rifaximin for 15 or 20 years and what I really want to see is "What am I doing? What's happening in the world because of rifaximin?" And I was hearing this from all my colleagues across the country. "Oh, we don't see much diarrhea IBS as we used to. We see more constipation." And I said, "Well, let's look at that. Let's study that."

It turns out that over the 10 years that rifaximin has been available, there has been a 30% reduction in people being sent to programs like mine because of diarrhea IBS because rifaximin is making it go away at lower

levels of healthcare.

Lower levels of healthcare also mean cheaper levels of healthcare because as soon as you're coming to me, you've already had a CT scan, two colonoscopies and all the other stuff that was all unnecessary because it was all negative.

And we're making an impact, and you could say, in a sense, curing 30% of people from having to come all the way to Cedar Sinai or to these big centers because the doctors in their community are getting it. And as a result, patient are getting better before they need to get all these advanced care. I'm very thrilled with this slide because this is really such a meaningful and important finding.

Now, let's go to the C-IBS. Forgive me for the title. Look at the great title, *Tertiary Care C-IBS*, constipation IBS. Do you know how many drugs are on the market FDA-approved for constipation? There's a whole bunch! However, despite all those drugs, there's a 35% increase in patients coming to my office who are C. And a lot of them are methane. And because people don't know how to do the methane thing yet, we're seeing more of these patients now than we ever did. And that's across the board too. So, bear this in mind as we go through the methane data. [25:06]

Dr. Pimentel: What I've tried to do in this presentation is try to give you some really cool, new stuff. I know place talk about diet and all of these. And this was at the DDW Utah. It's 15 volunteers. They did small bowel microbiome analysis before and after a high fiber diet.

SIBO increased to 80% on a high fiber diet with consequent digestive symptoms and resolved with resumption of a normal diet. Why is this important? Because I tell everybody fiber means more SIBO… fiber means more SIBO. And that's what this says right here. They put people on fiber, and they got worse. The microbiome got worse. And when they resumed normal diet, things went back to normal.

So I've been saying this for years, and I'm going to say it again. And this data substantiates that.

Now, let's shoot over to methane. Methane is really important and I want to take you through this. This is a slice of the literature. At the time I could do this step, it's going to be more impressive because it's probably three times more papers. But the data is essentially saying the same thing. If you have methane, you're constipated.

This was a study at the DDW. And we've talked about methane probably on Shivan Sarna's webcast before. I don't want to belabor the methane point. Methane causes constipation. But this is a study, again, hot off the press from Mexico saying if you're C-IBS, your methane is way higher than if you're mixed or D. if you're methane-positive, your BMI Is higher.

I've been telling people this. We've been having data showing this. More methane, more BMI. And I can address this in the Q&A. We don't have all the time in the world to go through that. But we have a tremendous number of studies, the largest studies on the planet, done here at the center that show that if you're methane, your BMI is higher. We know that you can harvest more energy because of that. And that's a problem that's maybe part of the obesity epidemic. And we can discuss that later.

So, let's talk about methane and C-IBS, treating it. And so we had already put some stakes in the ground in 2014 with this. This was a double-blind, randomized control trial. It's a small study. But let's give an antibiotic with the SIBO, and let's give neomycin, the antibiotic, with rifaximin.

Now, we chose this because we already knew neomycin had some effect. But the second reason we chose this is because we tested this bug in test tubes at a special lab in UCLA that can grow these methanogen.

And we showed that when you give neomycin or rifaximin, we got a really good effect on these organisms. And so we said, "Let's try this in humans." And sure enough, the best effect was combining neomycin and rifaximin.

The problem with neomycin and rifaximin is this issue that four weeks after this trial, everybody seemed to be back to the way they were. The methane came back. And antibiotics don't seem to have a long-lasting effect on methane as it does for rifaximin treating the hydrogen. If we're

treating the hydrogen, it stays away for a long time. But the methane is a tougher beast.

Remember, antibiotics were designed for bacteria. Antibiotics were not designed for archaea. Methanogens are archaea. They are a different cluster of organisms. And so we didn't develop antibiotics for that.
So, one of the things we've been working on is this Lovastatin notion because it's been known that this enzyme in the middle, F420, which is produced by bugs like *Methanobrevi smithii* take hydrogen, but Lovastatin locks that by getting in that molecule and just stops the methane production.

As a result, hydrogen build up. And the bugs that produce hydrogen don't like all that hydrogen. It may settle down too because it inhibits them. So you kind of kill two birds with one stone if you can block this F420.

We've been working with a company. And they built Lovastatin designed to stay in the gut in the original form that it came from the fungus, Aspergillus (because that's the only form that seems to work. Aspergillus produces this in its environment to block methane).

And if you look at the red line here, this is the laxative use. If you're on a high dose of this, you're hardly using any laxatives at all. And you can see the rescue medications are way down if you're on the drug, meaning your constipation is relieved and you don't get diarrhea.

This is not a diarrhea drug. It is not a laxative. This is treating the cause of constipation.

In the last part of this talk, I want to touch on this food poisoning issue because this is the most important and exciting area where we could look at perhaps causes, cures and things that can settle this rather than just this antibiotic approach that we've been doing. [30:00]

Dr. Pimentel: This is a study from last year. This is basically willing to do any more meta-analysis. A meta-analysis is you take all the trials that are out there, and you say, "Okay, does food poisoning cause IBS?" And you can see, I put this in all caps, "FOOD POISONING CAUSES IBS." End of story.

This is four or five studies by the Mayo Clinic. And they basically say, "Look, out of 100 people, 11 are going to get IBS if they get food poisoning." And we have determined that this is a major cause of IBS and could account for all two-thirds of IBS in the country based on CDC data.

This is *a* cause of IBS and *the* most important cause of IBS. And how that fits with SIBO is what I'll describe now.

These are the risk factors. If you get food poisoning for three hours, no big deal. If you get food poisoning for a week, you're more likely to get IBS. If you're a woman, you're almost twice as likely to get IBS. And that may be why more women have IBS. We don't understand why women are at risk.

Antibiotics because you're in the hospital with your food poisoning, more sick.

So, almost all of the risk factors are related to the severity and the intensity of the food poisoning.

So these bugs, Shigella, salmonella, E. coli all have one thing in common, and they all cause IBS. And the only one toxin in common is CDT, *cytolethal distending toxin*. And we've spent the last 10 years trying to figure out CDT. [31:33]

It culminates in this study. And I have probably a whole 1-hour presentation just on CdtB. But it culminates in this trial. We basically have proven that CdtB from campylobacter is the trigger.

But what we did is we said, "Oh, let's just get the toxin—toxin only, no campylobacter, no E. coli, just the toxin—and inject it, like a vaccine, in the arm of a rat. And we did this. and what happened was the rats developed antibodies to CdtB. They also developed the antibodies to vinculin which we already had developed and understood was a test for IBS.

So, exposure to CdtB equals development of anti-vinculin antibodies. And

as a result of just putting the toxin in these animals, they get higher duodenal bacterial counts, higher ileal counts, reduced vinculin production. Their stool weight changes because they're getting IBS. And there were all sorts of cytokines and inflammation in the gut. It changed because of just getting CdtB in the arm.

So this toxin is, we now believe, the cause of IBS from food poisoning.

It seems that the toxin creates an antibody called CdtB. It cross-reacts with a protein called vinculin. And I'm trying to point out vinculin here. Green arrows are actin (which are the lines in the cells. They're like the skeleton of the cell or the scaffolding like you might see in a building that kind of holds it in position.

The red tufts at the end (which the red arrow points to) is vinculin. It's right at the end of actin. And it acts like a mortar to stretch the little fingers of the cell out to reach to the next cell to connect it.

And this is really important for nerves because nerves need to reach out to each other, grab on to create the wire network that connects your brain, your spinal cord, the nerves to each other to keep the system integrated. And we know from animals that don't have any vinculin, they don't develop a nervous system in the gut at all.

We know that this protein is critical for nerves.

And after a series of experiments—and I'm sort of skipping steps, but this is what we know happen—cytolethal distending toxin exposure from food poisoning. This toxin is in E. coli and campylobacter and so forth. Getting that exposure, it binds to parts of this toxin. And because of binding to parts of this toxin, one of these parts of this toxin is very similar in appearance to vinculin.

So now, the antibodies that were reacting to CdtB see you as foreign and react to you. Hence, the change in the nerves of the gut and the stasis and the resulting bacterial overgrowth.

We developed a blood test. This was a study that were published in PLoS

ONE in 2015. Almost 3000 patients. And we looked at IBS, we looked at IBD. We looked at Crohn's and ulcerative colitis. We looked at Celiac. We looked at healthy people. And the bottomline is this graphic.

If you have a positive test for both anti-CdtB and anti-vinculin, there's a 95% chance you have IBS—95%.

Now, why is it important for a couple of reasons. First, I'll show you a study—I think I have it in here—again from Mexico showing that the blood test, if it's positive, means you're more likely to respond to treatment. That's fine. But more importantly, it tells the patient, "Okay, food poisoning caused it." [35:16]

Dr. Pimentel: I can tell you in my clinic, when I use this technique, I'm keeping people away from food poisoning. I'm doing everything I can when they travel to give them good advice and prophylaxis. If we can keep food poisoning away for a long time, IBS will disappear as these antibodies continue to decline.

It's kind of like getting a tetanus shot. You get a tetanus shot, you need one in 10 years because, over 10 years, you don't get the tetanus. And so if you don't get food poisoning, over the time, antibodies will diminish, diminish, diminish. And your IBS will get better and better and better.

Now, we want to jumpstart this obviously. We'd like to get these antibodies out of the bloodstream. And we've done that. We actually did plasmapheresis in three patients. We filtered the blood of all the antibodies including this one. And the IBS disappeared for a month.

Please don't go and do that. I mean it's like dialysis. You can't have dialysis every week for IBS. It's just not the right thing.

But it proves the point that I'm trying to make. These antibodies, if you clear them out, IBS is gone. And we need to figure out how to get rid of the antibodies to make people better today and not wait 10 years for this to decline.

Blood tests are both negative, you drop down at 24%.

I think about my kids when I look at this graph because if I had a 20-year old daughter—and I have lots of patients in my clinic where the parents come in, their 20-year old daughter or son has IBS. They come in and I say, "Oh, yeah, a colonoscopy and a second colonoscopy and a CT scan and an ultrasound. All of it is negative." If I had a test from day one that told me that they had IBS, why would I let my 20-year old son/daughter to have had two colonoscopies.

I'll tell you from personal experience, I just had a colonoscopy. My first, I was 50 years old. So now you know my age. And it's not fun. The prep is not fun. To thank of my 20-year old going through that *twice*, and it's negative at 20 years old, why not have something that tells me what I have now, tells me why I have what I have, and also tells me how to protect myself from the future by preventing food poisoning and being more diligent with my food choices and where I eat. If I knew that, maybe I could self-help.

This is the Mexican study that basically show that if you're anti-vinculin, anti-CdtB positive, you're more likely to respond to antibiotics. And again, the formatting is a little messed up. But that's the bottom line here.

Here's the study that we presented at the DDW, this animal study. We basically put CdtB antibodies into the vein of the rats. And we wanted to see where these antibodies go. At the bottom is IgG. So it's just immunoglobulin. It goes everywhere. You can see it's lighting up.

These animals are alive. They're not harmed by this. They're just going through our camera that measures fluorescence. And as soon as you put it in the vein, they see where the fluorescence is everywhere.

If you give vinculin, it's going right to the gut; anti-CdtB, right to the gut. And then, when the bowel is removed, it's in the gut.

These antibodies are going to your gut and causing damage. We know that from experiments like this. So we're getting to the bottom of where it's going and how it's working every day as we're doing these experiments.

This is the sequence—E. coli, campylobacter, shigella, salmonella, C. diff. They have this toxin. Then you get this anti-vinculin. Then you get this damage to the nerves. We know it goes straight to the nerves of the gut. And then you get this breath test positive because you have SIBO. And antibiotics are beneficial today because that's what we have in 2018.

I'll tease you with more information here because this is something that's relatively new. Let's look at IBS. Now let's say you have IBS, why did campylobacter do this to you? Why are you damaged? And the answer to that is in a study. I asked my good friend at the US Military Research Institute in Bethesda. I said, "Can you look at the people deployed to Iraq or Afghanistan because we've been working with them on these big studies," and I said, "If you already have IBS, and you get deployed, does it increase your chance of food poisoning, meaning does the nerve damage and the gut moving slower mean that campylobacter likes that? It can grow faster? It can get into you and cause an infection?" [40:11]

Dr. Pimentel: And that's what I think was happening. So I wanted to know could that be true.

And campylobacter is really a nasty organism. We deal with this in our lab. That's what we use for experiments. We have very strong precautions on how we use it. But the point is it can kill children under two. It's a very harsh infection.

And so, what we did in that trial is we said, "If you already have IBS, and you're traveling to a high risk area such as combat deployment, is it higher to get a real food poisoning?" And the answer is "It's three times more likely you're going to get food poisoned."

It comes back to my point about having a blood test. If you have a blood test that says you have this, you're three times more likely to get food poisoning when you go to somewhere.

I'm not telling you not to go anywhere. I'm not telling patients to stay home. I'm telling patients, "When you go places, and you're going to places with a high risk, you better take additional precautions"—and we can talk about that in the Q&A—"because you are the ones at risk. You

have a higher chance of getting more food poisoning, more antibodies, more food poisoning, more antibodies. And this just keeps the situation going."

We're working on this aspect as well which is that we know food poisoning like C. jejuni has this toxin. And it creates this autoimmune enteric neuropathy—in kids! You see these kids from developing countries. They're bloated, they're distended. They're unwell. They don't grow well. And basically, that's bacterial overgrowth. That's called *tropical sprue bacterial overgrowth* in developed countries. They get distension and vitamin deficiencies because, at that time, they get so many of these infections that the bacteria area eating all their nutrients and they're quite ill. But they're also the spreader because they keep getting the food poisoning because of this damage these antibodies do.

We have a very nice study we're doing with a community of African children. And we have their blood in our lab. We just got to run that next week because we think that these antibodies is predicting this malnutrition.

But you can't fix this. This is a political problem. And so it's hard to do that. So what we want to do is could we immunize. Remember what I told you. If you immunize with CdtB, it's a bad thing. But if you immunize with a part of CdtB that's responsible for the vinculin, we might be able to fix this.

And that's what we're doing now. I'm not showing the actual data, what we found. But we now know the parts that are good and the parts that are bad. And we are going to figure out a way to prevent IBS from ever happening and also maybe even have some benefits in under-developed countries.

I'm going to finish with—and I know I'm supposed to stop 1:45. Three fecal transplant studies. This was at DDW.

Three double-blind, randomized control trials, does fecal transplant work for IBS, otherwise known as SIBO (because 60% to 70% of IBS and SIBO)? And this was a study that I was long-awaiting because I know

Caterina Oneto very well, and she was part of this trial. She's was part of this trial. They're using capsules to deliver fecal transplant material to IBS patients.

The bottomline is not statistically, but the placebo did better than FMT. So what does that mean? Well, if placebo does better than FMT, maybe the FMT is making you worse because why would placebo do better. It wasn't statistically better, it's almost 20%, 15% to 20% better, the placebo.

Again, sorry for the format. Next trial is fecal microbial transplant in patients with predominant abdominal bloating. This is a double-blind study from Belgium. This trial suggested there might be some benefit. But it was a very small trial of 22 patients and placebo 42 in treatment.

And the third trial, fecal microbial transplantation alters gut microbiota in patients. It's a double-blind trial. And the results are: patients receiving fecal transplant had an increase in biodiversity, but not statistically different from placebo. No improvement in the IBS or even after three months. And there was a little bit of improvement to their quality of life in placebo, again, compared to FMT. [44:49]

So here again placebo worked better than fecal transplant suggesting fecal transplant may be making people worse rather than making them better. [45:07]

Dr. Pimentel: In conclusion, I think randomized control trials are now showing that fecal transplant may not be helpful for IBS, for bacterial overgrowth for that matter. I've been saying that for a long time because, rationally speaking, if you have too much bacteria, please don't add more. It doesn't make sense. It's not logical at this point.

So, my new worksheet, we call it *5.0*—I can't wait until I get to 10.0 because I'm going to have the cure hopefully. Let's hope for that. But diarrhea mixed IBS, you have acute gastroenteritis, you get this immune response to this toxin, anti-CdtB which, through molecular mimicry, gets you these autoantibodies to your own vinculin, changing the gut motor function.

Then you get this poor motility. Then you get SIBO at the bottom. And if you get hydrogen—and I'm going to add hydrogen sulfide to that—you get the diarrhea and mixed.

On the flipside, it's not autoimmune. You get a bloom of methane or methanogens, M. smithii. You get excessive methane, you get slow transit, and you get the constipation side. And that's how we see the story, as it stands, in the light 2018.

This is my team. We have 16 people. We have one, little baby who comes to our lab every now and then though they're not allowed. But they are in this picture off to the right. That's Christine's baby. The baby wouldn't stay in the carriage, was crying for mom. So he ends up part of the MAST team.

But we have 16 people all dedicated to trying to discover the microbiome links to human disease, obesity, IBS, the SIBO connection to all these things.

I wish I could tell you some of the stuff we learned this week in our lab. Because we have a big team now, we almost get new data and exciting data every week. And so I'm excited to keep sharing with you a lot of the developments as they occur. Thank you. [46:53]

Shivan Sarna: Oh, my gosh! You were amazing. Amazing! That was awesome! Awesome, awesome, awesome. Thank you so much. You totally blew my mind as usual. Congratulations!

Dr. Pimentel: The journey continues, let's just say that. There's a lot of new things. And I'm very thrilled to soon have a breath test that actually measures everything. That's simple to patients because patients are being told, "Oh, you have nothing. It's the flat line breath test." No, it's not something—hydrogen sulfide, we've now shown that.

We got to keep advocating for the facts. And that's really what we're all about here.

[CONTINUES WITH Q&A PART 2]