TLC Sessions

Episode 58: Breakthrough Biomarker?

Interview with Bhupesh Prusty

Transcript - 1:13:50

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Long Covid, IgM, ME CFS, ME CFS patients, patients, proteins, cells, reactivation, viral reactivation, infection, auto antibodies, SARS-CoV-2, fibronectin, B-cells, plasma, herpes virus, develop, produced, virus, mitochondria

TLC hosts
Emily Kate Stephens, Noreen Jameel

Noreen Jameel 00:00:06
Hi my name is Noreen Jameel and this is….

Emily Kate Stephens 00:00:08
Emily Kate Stephens.

Noreen Jameel 00:00:10
Both of us have been diagnosed with Long Covid

Emily Kate Stephens 00:00:13
And we've created this podcast dedicated to the condition. Welcome to the Long Covid Sessions.

Noreen Jameel 00:00:25
This is a special episode, we've given our platform over to Bhupesh Prusty to present his paper, that's been eagerly awaited by both the ME CFS community and the Long Covid community.

Emily Kate Stephens 00:00:36
This episode is an opportunity for Bhupesh to present his findings.

Noreen Jameel 00:00:41
He was keen to share it with us because we can offer him time and the platform when giving a speech often doesn't convey all the nuances.

Emily Kate Stephens 00:00:50
He's actually presented it at two ME CFS conferences in the past month, and he was given 15 minutes and 30 minutes respectively. So we wanted to provide him with a platform that he was able to talk through his full paper, which has now been submitted for publication. Here we're presenting someone's science that has not yet been peer reviewed, but it has been validated by numerous other scientists and the cohorts of people with whom he has been working. And as he says at the end of the interview, this is for you to take and consider. Do feedback to us. Please

use our comment section, use our contact section from our website and feedback to us on social media. Tell us your thoughts.

Noreen Jameel 00:01:33
The big thing is the news of a potential biomarker, and that's something I think really needs some vigorous investigation and some peer reviewing.

Emily Kate Stephens 00:01:42
In presenting his findings here today. What we are trying to do is something of a call to arms to disseminate this research and get other people on board to look at it and take all of the various elements into further studies so that if this is a biomarker, we might then be able to start moving towards a potential treatment.

Noreen Jameel 00:02:07
This is a very exciting day for us because we've been looking forward to this for a few weeks now since we last spoke.

Bhupesh Prusty 00:02:13
I'm happy that we're doing this. I felt a little bit awkward after our first conversation. I tried to hide everything and it was a little bit uncomfortable to me also to talk about something without telling what I'm talking about exactly. So today we are here so that we can talk about everything freely.

Emily Kate Stephens 00:02:31
Yeah. And just to clarify that there was a bit of a backlash after your presentation at the ME CFS conference and you weren't trying to tease people or hold back but actually what you've done is you've spent the past three weeks going back over, re-evaluating your results. You've added new results into your paper so it was more of a sort of due diligence process that you were trying to go through rather than trying to tease people or withhold information.

Bhupesh Prusty 00:03:03
Yeah, I mean the other issues also, for example, here we have been working with multiple different cohorts of patients, which were managed and organised by different clinicians and we have to go through individual cohorts of samples, get the approval before we go out and talk about it. And there are a lot of bureaucratic protocols behind this, which took time because there are a lot of quarters in the paper and everyone has to approve the publication. I took my time to go through the data and add more questions. In between we also spoke to some of the leaders in the field of immunology, Professor Akiko Iwasaki and others and we got some nice advice from them to test some controls and things like that, and we have to do that and we are ready. This week we are going to submit the paper. We are going to talk about it at the invest in ME conference in London.

Emily Kate Stephens 00:04:02
You are due to submit your paper which means that it's not yet been peer reviewed or published. But just to clarify, you mentioned that you have worked with a huge number of co-authors on this paper. Can you just give us an indication of the sort of breadth of people that you've been working with because it's not just within your university and within your usual research team? Is that correct? You've been working further afield as well.

Bhupesh Prusty 00:04:29
Yes. So, part of our paper is based on detecting herpes virus antibodies in ME CFS patients, Long Covid patients and this work is done in collaboration with Professor Marshall Williams and Maria E Arizal from Ohio State University. So, we have a long-term collaboration and we are

working together on these Herpes virus dUTPase proteins. Then we have collaborations with a Carmen Scheibenbogen from within Germany. We are in collaboration with a BMBF project which is sponsored by the German government to work on ME CFS and Long Covid and we have collaborations with two different separate cohorts of patients. One is called Covidom, which is a central German government consortium where our university is also involved as a separate group or cohort of samples. And then we have a healthy control cohort called snap cohort, which belongs to the city of Wuerzburg.

Noreen Jameel 00:05:24
This is what's really interesting about your work is that you're using not only these different cohorts, healthy, sick ME CFS but you're also distinguishing between ME CFS and Long Covid and then mild, moderate and severe and then men and women. It's so detailed that people can't really at this point so that you haven't covered every base.

Bhupesh Prusty 00:05:48
Yes, when we spoke last time, we had a large cohort of SARS-CoV-2 positive samples, but we were not in the stage to differentiate which is basically recovered or completely healthy again after the first SARS-CoV-2 infection and who are still suffering from mild and severe symptoms. Now, within the last couple of weeks, we have managed to differentiate them using a scoring system which was published last year in a peer reviewed journal. And now we have three subgroups within the Long Covid patients. Large number of patients who were positive for SARS-CoV-2 infection and after six to 12 months they are more or less having normal health. So we call them the 'No Long Covid' group. We have a 'Mild Long Covid' group where the symptoms are not very severe for this patient and we have a 'Severe Long Covid' group who more or less overlap with ME CFS patients with the majority of their symptoms. Good thing is that we are able to biologically provide evidence of the disease differing from No Long Covid group to Severe Long Covid Group, which in my opinion is one of the best things to happen at this stage.

Noreen Jameel 00:06:53
I just make the very obvious point, to date we don't have a biomarker for Long Covid and your paper may show us that we do.

Emily Kate Stephens 00:07:04
So, can you explain to us what that is?

Bhupesh Prusty 00:07:09
Yeah, to clarify here that the cohort of samples that we are going to talk about now called the Long Covid group are within the first six to 12 months of Covid infection. Therefore there is not really a huge difference between the No Long Covid Group and the Mild and Severe Long Covid Group. We are talking about a potential biomarker here which is more than 85% accuracy rate. And we believe that when we go to the cohort of patients who are into their second or third year after the first Covid infection and still suffering with Long Covid We might see the real difference between the No Long Covid group and the Severe Long Covid group. This work is in progress and we hope that we will be able to add this data during the review process or the revision stage. I would like to start systematically with the many different stages of the paper instead of directly going to the Long Covid biomarker part which probably will be helpful for the readers to understand. The first question that we asked in the beginning of our studies is; if there is any way that we can validate or we can prove that herpes viruses are potentially involved in the process of disease development for ME CFS as well as the Long Covid. We were looking way to search for signatures of these herpes viruses not only for HHV6, which we frequently study, but also EBV and other herpes viruses like HSV1 HSV2 and Varicella Zoster virus, and during this time we came across the previous work being done by Marshall Williams

and Maria E Arizal from Ohio State University. They have been working with these dUTPase proteins from the viruses and these proteins are very early proteins and we develop strong antibody responses against these proteins. It seems that these antibodies can be a very strong marker for herpes virus reactivation. So, we started working on this and we looked into the randomly collected healthy population samples we looked into ME CFS patients No Long Covid Group, the Mild Long Covid group and the Severe Long Covid group to find if there is any signature of these antibodies against these herpes viruses. Now let me make it clear that the presence of antibody against particularly the IgG against herpes virus doesn't mean that the infection is still going on but we argued that if we find any signature of these antibodies in the Long Covid patients in comparison to the No Long Covid patients, which is a gap of six months to 12 months. That tells us that during this time, the virus might be reactivated because the antibody response will only develop when the virus it's active. That would suggest that at some point of time there is viral reactivation that happened and that can contribute to the disease.

Emily Kate Stephens 00:09:59
Is there a timeline on how long after an infection or an activation that that antibody signature will be present?

Bhupesh Prusty 00:10:07
So these are basically immediate early early proteins in the virus life cycle. So as soon as the virus reactivates or infects the proteins will be produced to my knowledge, there is no such studies being done to say how much time it takes to develop these antibodies.

Emily Kate Stephens 00:10:21
But they remain present.

Bhupesh Prusty 00:10:23
Yes, they remain present till the viral proteins are there somewhere. As these are IgG immunoglobulins they will be there for a little bit longer time beyond the infection period. But their presence suggests that reactivation has happened a couple of weeks or months before. It was very interesting to see that the antibody against EBV, the Epstein Barr virus dUTPase herpes virus 1 dUTPase proteins and HHV6 dUTPase is frequently high in ME CFS bases. They are significantly higher in ME CFS patients than the Long Covid patients, particularly the EBV dUTPase is extremely high in ME CFS patients. If we look into the three groups of Long Covid Patients, the No Long Covid group, Mild Long Covid group and Severe Long Covid group, we see that particularly the IgG response against HSV1 which is type one herpes viruses significantly higher. EBV is also reactivated in Long Covid patients BUT the antibody response is not statistically that significant against the healthy population. Of course, the amount of antibody response is still higher, unfortunately, or let's say the most peculiar result what we found is that the HHV6 antibody response against HHV6 dUTPase proteins, goes down Long Covid patients.

Emily Kate Stephens 00:11:48
Which is a differentiation with ME CFS isn't it?

Bhupesh Prusty 00:11:51
Exactly. This is a key point even though this is not a positive signature. I think it is interesting and also tells about potential differences. In T cell and B cell response during these conditions. Now we have to understand that the ME CFS patients that we are studying here are chronically ill for a very long time. They are not within the first one year of their disease. So there is a difference between the disease state here between the ME CFS and the Long Covid patients. So which is not very peculiar to see such a huge difference but um this tells us that during the initial stages of infection or reactivation, a lot of changes probably going on in the T cell and B cell response and HHV6 particularly is a study for T cell specific virus so it is very interesting to

see that IgG responses these viruses are actually suppressed are going down and there is a inverse correlation we see here also that severe Long Covid patients have more or less no antibody response or very mild antibody response left. This was the first part of the project which told us that, yes, there is a herpes virus reactivation going on in ME CFS patients, as well as Long Covid patients. People have tried before to find that the signature of herpes virus reactivation in the form of DNA RNA in the blood, viral DNA which they could not find. We believe that type of reactivation is probably going on in specialised tissues and localised tissues as there is a humoral response developing against the vital proteins there has to be viral proteins present and the viral protein is there in some part in an amount which is enough to create this humoral response. So, we try to understand what exactly the viral protein can do because we know from previous studies from our collaborators that these viral proteins, the dUTPase proteins these viral proteins are capable of doing multiple different immunological changes in the body. We are more focused on understanding what can be their effect on mitochondria. So we created vectors, a construct which we can put into cells, different cells, human cells, where they can express his viral dUTPase proteins inside the cell and then we try to understand the mitochondria. So, what we found is that once these viral proteins are expressed inside the cell, the cell starts to use a completely different morphology of mitochondria which is called hyperpolarized and hyper fused mitochondria. This type of mitochondria is being observed in many different types of neurological diseases or under different type of chemical stimuli also, the mitochondria becomes fused and clumped together in one part of the cell. What we also showed that there is an inhibition in apoptotic for the mitophagy pathway, so the mitochondria are not really healthy, they are not recycled properly. The mitochondrial energetics is also compromised particularly in the presence of EBV dUTPase. And moreover, what we found very interesting is that whether this is HSV1 dUTPase or it is HHV6 dUTPase, or it is EBV dUTPase, it is all capable of interacting with the cellular cytoskeleton means the entire backbone of the cell, the network that keeps the cell intact and by doing that they are capable of changing actually the mitochondrial morphology plus the mitochondrial distribution and mitochondrial morphology is heavily dependent on the cell cytoskeleton. During these experimental approaches, we were able to show that the presence of these proteins have a big consequence on the health of the cells in terms of the mitochondria mitochondrial energetics. And we hypothesised or we predicted that during these early times of the viral reactivation in Long Covid patients as well as in ME CFS patients, proteins are contributing in localised tissues by changing the health of the cell or the morphology of cells as well as the physiology of the cell, where they are reactivating.

Emily Kate Stephens 00:15:37
This is in both Long Covid and in ME CFS.

Bhupesh Prusty 00:15:40
Yeah, so we cannot look into the tissues of the Long Covid patients but as we see this effect happening in human tissues and we have a Long Covid recent slowing the significant division we can only predict that wherever the virus the reactivating whether this is epithelial cells of endothelial cells or any other cell type for the virus is reactivating there has to be strong physiological effect of the virus or the reactivation on the cells. What is the context of discussing all these over here that we know that herpes virus reactivation, particularly prolonged and leaky herpes virus inactivation of EBV as well as herpes virus type one known to cause autoimmunity and autoimmunity is a very widely discussed topic in ME CFS as well as in COVID-19 positive patients. So here we are bringing the potential link of autoimmunity to the disease context in terms of the virus reactivation. So, what the virus reactivation is doing besides this is something which we will study later, but here what we are trying to focus on is that can these herpes virus reactivation be linked to the high level of autoimmunity that we see in what group of patients. Now, we will come back again to the topic of autoimmunity here because it is already known in the field of acute SARS-CoV-2 infection that the patients with

acute infection have a high level of auto antibodies. In the recent past we have also seen several papers showing that autoimmunity is also signature in Long Covid patients. We know from the literature that in ME CFS patients we also have auto antibodies. So, we try to understand if there is a very specific auto antibody which is contributing to the disease or there are more than one auto antibodies which in combination have the capacity to alter the physiology of the cell or they can contribute to the disease.

Noreen Jameel 00:17:28
You have identified the specific dUTPase that come with reactivation of the herpes viruses or EBV that are causing the damage to the cells and hence the mitochondria. It's not the same as SARS-CoV the actual virus itself doing the damage because you've identified a certain signature dUTPase, is that correct?

Bhupesh Prusty 00:17:52
Yes. So what we're talking about here is the herpes virus dUTPase we are not talking about any viral protein from the SARS-CoV-2 what is the hypothesis from the very beginning that after the SARS-CoV-2 infection there is a strong reactivation of herpes viruses which we also see here. So what we are trying to see is what is the contribution of the herpes virus, the disease progression process rather than what is the direct contribution of the SARS-CoV-2 proteins, for example, the spike protein which is widely a topic of discussion, we're not looking into that we are looking into the contribution of the herpes viruses, but the later half of the paper we are going to talk about a bigger contribution from SARS-CoV-2rather than herpes virus but that will come later. So we did a very small study with the autoantibodies in ME CFS patients particularly we do not do anything with the Long Covid patients because as you might realise funding is very limited. So we had the opportunity to do a proof of concept study with a very small number of patients. What we did here is that we tried to look for IgG and IgM responses against 120 well studied auto antigens, they are known to be the antibodies produced against these auto antigens in many different autoimmune diseases. So we took those panels as well as we looked into 120 Different pathogen associated auto antigens. Some or other diseases have been associated with the antibody response against these antigens. So a very simple question based on the expression pattern of these autoantibodies. Can we differentiate healthy controls from mild and moderate ME CFS or severe ME CFS patients? And the answer is that it is not that easy. The IgG response was not clearly sufficient to differentiate healthy controls from severe ME CFS. It was very interesting that the IgM response in auto antigen has the capacity actually to differentiate healthy controls from mild and severe ME CFS. We looked into out of the 120 different auto antigens, which are the best 10 auto antibodies or auto antigens, which are actually contributing to this differentiation between the healthy control and severe ME CFS called auto antibodies against CRP which is the C reactive protein, collagen five, six single stranded DNA, double stranded DNA which are classical signatures of autoantibodies in Lupus, or other multiple sclerosis, theses type of autoimmune disease. One thing which was very interesting: all these autoantibodies are actually high against these antigens, part one, which was actually decreased in ME CFS patients, particularly the severe ME CFS patient was against fibronectin. So, in our last conversation, I told you that we knew some data from one year before so this is the data that we knew, long time before actually coming to the stage, the fibronectin, IgM response to fibronectin goes down in severe ME CFS patient but there was no way we could understand why this is happening. So we wanted to know if the virus is reactivating in localised tissues deep inside some of the tissues how come that affect the entire body of a patient, the ME CFS patients and the Long Covid Patients are actually suffering from multiple different clinical symptoms. How can a localised tissue basically affect the outcome of a disease? and the only way we can think about it is it leads to localised virus reactivation or any metabolic change that is going on there can produce certain changes into the extracellular fluid like blood or serum that can actually pass on the information or the clinical feature to the other part of the body. As we know from the previous work of Ron Davis and Øystein Fluge from Norway that there is something in

the serum and we have also seen in the past if you take serum from ME CFS patients and put it into healthy cells, you can pass on the mitochondrial morphology or mitochondrial metabolism differences to the healthy cells. We try to understand if the immunoglobulin which is produced after the autoimmunity or auto antibody response has the effect on the health of the cell as well as mitochondria. So we basically purified the immunoglobulins from a large group of healthy individuals. As well as ME CFS patients. So we're talking about 30 ME CFS patients and 30 healthy controls. We established a protocol to purify very enriched immunoglobulins. Then we put these immunoglobulins into different types of healthy cells, primary cells as well as continuous tumour cells to see if there are changes in the mitochondrial morphology. We found a very strong effect of immunoglobulin particularly the IgG which has been isolated from severe ME CFS patients on the mitochondrial fragmentation in primary human endothelial cells. So we do not see much effect in other types of cells that we studied, but what we saw is very interesting that IgG isolated from severe ME CFS patients have the potential to cause mitochondrial fragmentation and change the health of cell endothelial cells, primary endothelial cells. We could show in the form of quantification of the mitochondria as well as we could show that this mitochondrial fragmentation is happening because of a decrease in the expression of mitofusin 1 which is a protein that keeps the mitochondria intact, stick together. These proteins are going down, as well as there is another protein which is called PLD 6 or mito PLD these proteins are also going down both mitofusin 1 and PLD 6 are actually responsible for causing mitochondrial fusion, they bring the mitochondria together to keep the healthy mitochondria intact. They are going down as a result of which the mitochondria is fragmenting. So this is a very interesting aspect, I'm not telling that immunoglobulin is the only factor which is causing the mitochondrial fragmentation because we have also have a hint that mitochondrial DNA probably is also responsible, not very significantly, but probably is a contributing factor. There might be other contributing factors like some of the metabolites that people are studying. At this moment, we are only focusing on the immunoglobulin aspect and what we could show is that the immunoglobulin in the severe ME CFS patient has the capability to target the mitochondria and cause mitochondrial fragmentation. Now the question comes, a very basic question you can ask okay, you are taking the immunoglobulins purified from the patients and putting on the healthy cells. So how do you know that it is the immunoglobulin which is causing the mitochondrial fragmentation, there are some other proteins which are binding to the immunoglobulins and going inside and doing the job? We took those immunoglobulin fractions that we have been purifying, and we did a mass spec analysis. Mass spectrometry is a technique in which you can identify the proteins, any protein which is there in your preparation, we found all the immunoglobulins, the different types of IgG that we purified we also found some monomeric forms of IgM, which we purified also. Together with that, we also found the immune complex proteins like the complement proteins, C1, C1Q C1R, C3 C5, C7, all the other complement proteins in our preparation, but we did not see any big difference between all these proteins, the IgG, the IgM, or the complement protein between the healthy controls and the ME CFS patients, there are differences. For example, the C3 protein there is a trend to be more in these immune complexes, but this is something which cannot really differentiate the healthy controls from the ME CFS. What we found is very clearly three proteins, which were decreased in these immune complexes in ME CFS patients, than healthy controls. It was not more decreased in severe patients or mild moderate, but they were constantly decreased in ME CFS patients than the healthy controls. These proteins were the serotransferrin, short form is TF and then A2N the alpha two microglobulin and fibronectin one. Fibronectin 1 is one of the most significantly decreased within these immune complexes. So we focus more on fibronectin. Why it is very important because we know from the past literature that fibronectin is a protein which is incorporated into the complement complex, it binds to several antigens and also it binds to C1 and C3 complement proteins and participate in the complement activation process, which basically is required to fight against several infections, particularly bacterial infection. As well as viral infections like HIV and things like that. That's why we focused on fibronectin one, and it was very interesting because the fibronectin was actually decreased within the immune

complex, suggesting that probably ME CFS patients have a compromised complement activation process, which can allow opportunistic infections as well as viral infections.

Emily Kate Stephens 00:26:48
The FN1 is reduced in the ME CFS patients?

Bhupesh Prusty 00:26:52
Reduced within the immune complex, that means the immune complex which is created whenever there is a requirement for immune activation, these proteins are not there in that complex, but this doesn't mean that these they are not there anywhere else. So this was the question came to our mind that why these proteins are not there in the immune complex, does it mean that these proteins are not produced at all or they are decreased in the patients or are they are there in the patients where they're not able to incorporate into the immune complex? There are two different aspects. So to answer this question, we tried to measure the fibronectin protein in the circulating fibronectin protein in the serum of healthy controls and ME CFS patients the obvious doubt in our mind was that the proteins were not produced in enough amounts, we could see the difference. It was very interesting to see that the protein is actually produced or is present in a significantly statistically significantly higher amount in ME CFS patients' serum them than in the healthy controls. That means that the protein is produced, it is there in the serum, but it is not incorporated into the immune complex, because of some reason that we still don't know. When we looked into, if this protein, this amount of the circulating fibronectin in the serum can actually associate itself with the severity of the disease. That means if we take healthy control patients, severe ME CFS patients with the Bell score of roughly between zero to 20 or higher Bell score of 30 or higher can we see a difference in the presence of this fibronectin amount in the serum and our data shows a very clear difference in the association of these circulating fibronectin proteins with a disease severity in ME CFS. That means that severe ME CFS patients having a Bells score of zero to 20 have a significantly high fibronectin amount in their serum in comparison to the healthy control or the patients with the Bell score of 40 to 50. This difference in the fibronectin amount itself is not a biomarker because it can differentiate ME CFS patients particularly severe ME CFS patients from the healthy controls with 80% accuracy which it is acceptable, but it is not really very good. We looked into the different cohorts of the Long Covid patients and what we found is statistically no significant difference between the healthy control and the no Long Covid group that means that SARS-CoV-2 positive patients who do not have any major clinical symptoms anymore. They do not so any big difference in the circulating fibronectin amount but when we move to the mild Long Covid or severe Long Covid patients see a strong difference. A higher presence of circulating Fibronectin in the serum of these patients in comparison to the healthy controls. What's very interesting here is that two differences between male and female individuals what we see in in general in the healthy population, the males have significantly lower amount of circulating fibronectin than females. If we look into both ME CFS patients as well as different groups of the Long Covid patients we see that the increase in the fibronectin amount in males is significant statistically very high because they are coming from the very low amount to a high amount we can call it as a pathological amount, the amount that can probably be associated with the disease, this difference is very high. So there is a very high statistically significant difference between the amount of fibronectin if we define a certain amount of fibronectin circulating in the serum as a amount which should be pathological because this is the amount beyond which is always detected in these patients and then this amount of the circulating fibronectin is extremely high in the male in comparison to the base level. This is very statistically significant. in female individuals the difference is not very high in the pathological amount and the amount which is normally present. So this gives an impression that reaching this pathological concentration probably is easier in females than males. So probably male individuals need much more stimulus much more disease influence to change the fibronectin amount to reach to that stage, but female individuals or female patients can reach this stage a little bit easier, which

probably is again it is just a case of probably is one potential difference by the ME CFS as well as Long Covid is more frequently seen in females than males.

Emily Kate Stephens 00:31:13
And just to clarify it in the control groups, there was a clear differentiation between males and females.

Bhupesh Prusty 00:31:22
It was it was a statistically significant difference in the healthy male and female, yes. There was a clear difference in fibronectin amounts Yeah, was actually more than 99% accuracy.

Noreen Jameel 00:31:35
So women have more. In general men have less Yeah, and then to cause a detrimental effect on the body. Men have to be producing a lot more fibronectin women the threshold is much lower. So in order to cause us to get sick, we need a small jump, men need a big jump.

Bhupesh Prusty 00:31:51
Exactly right then we try to understand why this fibronectin is going up. So if you look into the fibronectin it is a very interesting molecule. This is a marker of inflammation. So when whenever there is chronic inflammation going on the fibronectin is normally increased there are two different types of fibronectin. One is plasma fibronectin and one is cellular fibronectin. Plasma fibronectin is basically produced by only hepatocytes, these are not produced by any other cell types. But the cellular fibronectin is produced by many different cell types, endothelial cells, monocytes, macrophages and all these different types of cells. Both are present in the blood serum. Their job is a little bit different cellular fibronectin has most important job of the wound healing after the tissue injury, they participant, they're the major component of the extracellular matrix, and they are essentially very much required to do the tissue regeneration of tissue building after the injury. In comparison to that the plasma fibronectin is actually quite interesting from the point of view of immune modulation, the immune activation, participating in complement proteins and playing a role in platelet activation, Mast Cell activation and also amplifying the inflammation signals so that anti-inflammatory cytokines can be produced during the tissue injury or infection. They are actually there to do a positive work. They are not bad

Emily Kate Stephens 00:33:11
When you talk about tissue rebuilding but they also play a role in clotting?

Bhupesh Prusty 00:33:16
Exactly. But they themselves fibronectin by itself has a very minor role in clotting process. But in combination of other proteins for example, fibrin is another protein from the complex clotting pathway. When fibrin comes in contact with fibronectin then random clotting or other the uncontrolled clotting process starts. We try to understand why this fibronectin is changing. Is there a change in the composition of the fibronectin, how much of plasma fibronectin is changed or how much of cellular fibronectin is changed? What we found is that there is basically no difference in the composition of plasma fibronectin and the cellular fibronectin. Both plasma as well as cellular fibronectin are increased whenever there is increasing the total circulating fibronectin amounts are increased in these patients. We started to dig into the literature and we found very interesting observations. For example, let's take a paper where Trypanosoma infection. It has been shown that when mice are infected with Trypanosoma they see that during the early few days to week of the Trypanosoma infection the circulating fibronectin in the blood goes up but at the same time there is a decrease in the IgM and IgG response against this fibronectin. It is an inverse correlation: the IgM against fibronectin goes down in these mice infected with Trypanosoma whereas the circulating fibronectin goes up in the blood. We try to

understand the logic behind it. And there was another very interesting observation here was that some very old papers they tried to look into IgM against fibronectin in healthy populations. And they found that healthy individuals normally without having any pathogenic infection or anything have already very high level of IgM against fibronectin normally you find such type of high IgM response against certain cellular proteins, our own proteins only when these proteins or these IgMs belong to the natural IgM category. There are two different types of IgM. One is natural IgM. Natural IgMs are produced directly in the foetus. They don't require any antigen signal for the antibody to be produced so they are positively selected during the development process. Their main job is scavenging and helping so if the cell dies inside the body, their job is to remove the dead debris from our own cells. So that autoimmunity doesn't develop against these dead parts

Emily Kate Stephens 00:35:51
They're kind of the road sweepers, they come through and clear out the debris.

Bhupesh Prusty 00:35:55
Exactly. It was very interesting for us that autoimmunity is very clearly there in SARS-CoV-2 infected patients as well as Long Covid patients and in ME CFS patients. Then we asked the questions, does it also mean that when we see the high amount of plasma fibronectin or circulating fibronectin in these patients the IgM response against fibronectin goes down? So we tried to develop an assay here where we can look into IgM response against fibronectin and IgG response against fibronectin. Coming back to the old result of microarray the IgM result against fibronectin... I told you that in severe ME CFS patients we saw that IgM response against fibronectin was going down in our microarrays, it was a small study. It was a proof of concept study, but it was a clear difference, the top 10 candidates showed fibronectin as one of the candidates, which was negatively regulated. That was pretty surprising for us. That gave us first hint that something is going on with the IgM response to fibronectin. So, we developed Elissa assays in our lab and try to look into the IgM response and the IgG responsiveness to fibronectin. And what we found was very very interesting. If you combine the entire ME CFS group that means the mild, moderate and severe ME CFS group, and you really don't see much of a difference between IgM and IgG response against fibronectin between healthy controls and ME CFS. The moment you stratify these groups into severe ME CFS or mild, moderate ME CFS groups you will see that the severe ME CFS patients with Bell's score 0- 20 have significantly lower amount of IgM against fibronectin which is again, not the case with the mild and moderate patients. We are talking about patients who are sick for a very long time, so their body has adapted to these changes. We were very keen on looking into these Covid positive patients. These patients are within the first year of infection. So we looked into these and No Long Covid Mild Covid and Severe Long Covid groups. Here we found in general the first thing is that all the three groups had significantly lower IgM and IgG response against Long Covid (fibronectin?)which was itself enough to differentiate from the healthy consultant. Let me make it very clear here. If you take a person who was infected with SARS-CoV-2 six to 12 months before but has no major clinical symptoms at this moment they also have a significantly high amount of decrease in their IgM and IgG responses against fibronectin. The same is also true for mild and severe Long Covid patients but if you compare how much difference is there between this No Long Covid, Mild Long Covid and Severe Long Covid basically see that a gradual pattern the severe Long Covid patients have even lower amount of these IgM response against the fibronectin. So when we were discussing these results with professor Akiko Iwasaki I argued that probably this IgM against fibronectin belongs to the natural IgM category it is not yet known. or not yet being discussed that this IgM is a natural IgM category. I argued that as this is very highly present in most all the healthy individuals and their amounts are decreased after the infection I argued that this might belong to the natural IgM category, so she asked me the question: what happens to the other known natural IgM's? For example, IgM against phosphoryl choline IgM against a malonic aldehyde or MDA. These are the known natural IgM's. So during the last three to four

weeks, when I was not present in public, we were trying to test these IgM molecules in these different groups of Long Covid patients so that we can clarify whether it is only the IgM against fibronectin is going down or is it almost all the natural IgM against fibronectin going down. What we found is very interesting that it seems the entire natural IgM population is going down after the SARS-CoV-2 infection and there is not a single correlation with herpes viruses here. So we argue here that after the first SARS-CoV-2 infection somehow there are certain immune modulations happening or the SARS-CoV-2infection is reaching very easily to the primary hematopoietic cells, like spleen, the lymph nodes, the bone marrow and there the plasma B1 B cells, which basically are the major cells who produce these natural IgM's an and there they are affected or they're modulated in some way that they are not able to produce this natural IgMs. We see a clear difference or clear patterns, statistically significant difference between No Long Covid groups Mild Long Covid groups and Severe Long Covid groups. Which suggests that this difference is actually recovering in the No longer during these 6 to 12 months of the first Covid infection probably they are coming up that's why I told in the beginning it will be very interesting to look into two to three years post Covid infection patients if they have recovered back into the normal level. But the Mild and the Severe Long Covid Patients are still having this extremely low. You can call it almost depleted amount of these natural items.

Emily Kate Stephens 00:41:06
And just to clarify, that's all natural IgM rather than just your original hypothesis, which was initially you will just looking at the IgM against fibronectin. In your findings you have now found that it is actually wider than just the IgM against by fibronectin.

Bhupesh Prusty 00:41:23
Exactly, so we found at least not all again, we tested three at this moment and two of them are the most widely discussed natural IgMs. Their presence is extremely high in healthy individuals. So that means that we are safe to predict that most of the natural IgMs are depleted after the SARS-CoV-2 infections.

Emily Kate Stephens 00:41:41
Is that what you would then be able to assess, have a test and use as a potential marker?

Bhupesh Prusty 00:41:49
Yeah, so there is a little bit of a bottleneck here, as I mentioned that we are working with a group of patients at this moment who are into the six to 12 months after the first Covid infection this time period is clearly not enough to completely differentiate the No Long Covid patients from the Mild and Severe Long Covid patients. We have to look into the longer time period. But if you compare between the healthy controls and the severe Long Covid patients, so if we combine the total amount of circulating fibronectin together with IgM against fibronectin or IgM against any of these natural IgMs then we have an accuracy of more than 85% differentiating between healthy controls and severe Long Covid patients by compare the accuracy rate of a healthy control versus severe Long Covid In terms of only the natural IgM the I get 99% accuracy of difference, but this accuracy of differences also 95% against No Long Covid groups that is the bottleneck at this point. So we have to look into a little bit longer time post SARS-CoV-2 infection to see the clear difference between the individuals who have recovered after SARS-CoV-2 infection, the individuals who are still going on with persisting symptoms and things like that.

Noreen Jameel 00:43:13
Basically, something is happening to the B1 cells and they're not able to produce the natural IgM?

Bhupesh Prusty 00:43:20

That is what our hypothesis is at this moment whether it is happening because of the reactivation of the herpes viruses, which is also possible because we look into our previous work from our co-workers from Ohio State University, we see that they have done some mice experiments where they show that expression of the EBV dUTPase in the mice affects the marginal zone cells where the plasma B1 cells are actually located in mice and that affects the health and the functioning of the plasma B1 cells and other cells which produces autoreactive antibodies, so it is quite possible that the herpes virus reactivation can have a direct influence also. But I'm not able to come to any conclusion at this moment regarding the link to herpes viruses here because clearly the herpes virus, at least the three herpes viruses what we studied is not reactivated in all severe Long Covid patients. Combinedly it goes more than 60% it might be reactivated. For example, we did not check the Varicella Zoster virus HSV2 or CMV like that in our study, so if we probably combine them, we'd reach to the 100% and then we can say that maybe the herpes virus reactivation is playing the role, but at this moment, it seems that it is the effect of the SARS-CoV-2 because all the patients have constantly similar downregulation SARS-CoV-2 probably is playing a major role at this moment here.

Emily Kate Stephens 00:44:35
Going back to our previous conversation to do with the bone marrow are we still talking about whether it be the reactivation of herpes virus or the active SARS-CoV-2 virus, it's an assault. It is happening in the bone marrow to cause that depletion in the natural IgM?

Bhupesh Prusty 00:44:56
I still believe so. As a scientist I always argue with myself and I still believe that the depletion of natural IgM which is very specifically produced by plasma B1 cells, there are very few other cell types which produce a minor amount of these natural IgM's, majority is produced by these plasmaB1 cells and the bone marrow is the primary source. Later on, you can see them, plasma B1 cells in other parts like the secondary hematopoietic tissues like spleen, lymph nodes, the marginal zone cells below the gut mucosa and the peritoneal cavity in mice and things like that, but there has to be something going on in these tissues or in these specific types. And this brings us to the initial observation that we have a decrease in antibody response against Herpes virus dUTPases, and also the EBV dUTPase antibody response in severe Long Covid patients. There is probably something happening with the T cells and B cells because these viruses are very specific to T cells, EBV in particularly acquires long term latency in plasma B1 cells. It doesn't reactivate very frequently in plasma B1 cells but it reactivates in the circulating B cells in the body it might be possible that the virus the reactivation is happening in plasma B1 cells somehow which requires further work. It is very difficult to work with the plasma B1 cells particularly in human being because you don't find so many cells in circulating blood and I am not a immunologist per se so, I cannot really look for such plasma B1 cells in patients after the SARS-CoV-2 infection so this is an opportunity to the co-workers in the field of immunology they can look into this and take this further to understand if there is something going on with the T cell B cell repertoire in this primary homeopathic organs whether it's bone marrow, lymph node or spleen and things like that. I still believe that the clue lies why the virus infection is influencing these natural IgM so dramatically, so drastically after the Covid infection, as we know that with ME CFS patients, at least the majority of the ME CFS patients that we have here are before the pandemic so they did not have some SARS-CoV-2 infection. They still have a very low amount of IgM against fibronectin. We did not check the other IgM so far but it also says that probably the herpes virus reactivation is doing something or there are other viruses, which is capable of doing these changes in the plasma B1 cells and their ability to produce these antibodies.

Noreen Jameel 00:47:24
So could we hypothesize if the reactivation is happening in the bone marrow where the majority of the plasma and B1 cells are being produced that your disease severity is going to be worse

than the ones that are sitting in your spleen or your lymph nodes. So perhaps people with milder versions of Long Covid had infection in the bone marrow reactivated there but in the kind of in the other areas.

Bhupesh Prusty 00:48:05
This is a hypothesis we can definitely think about. We discussed before normally the virus reactivation is not frequent in the bone marrow. THE Bone marrow carries very specific type of latent viruses.
If we look into the previous studies the presence of herpes viruses in the bone marrow is up to 10 to 30% of the individuals healthy individuals, Parvo Virus B 19 is present in 60% of healthy individual bone marrow as latent virus or DNA is detected. So the reactivation is not very often the bone marrow particularly. So it is quite possible that some patients they reactivate the virus probably throughout the body, but the reactivation reaches to the deep tissues of the primary hematopoietic tissues like bone marrow very rarely and these patients who reactivate in the bone marrow or in similar type of tissues, it's a complicated and controversial topic because most of the natural IgM studies have been done in mice and very few studies have been done in human being and there is not clear overlap between the human and the mice studies. The reservoirs are different that is why I'm telling you to be cautious when talking about bone marrow or other primary hematopoietic tissues. Clearly there is a difference coming from these primary hematopoietic tissues.

Emily Kate Stephens 00:49:11
Essentially, whether it's in the bone marrow, there is something that happens to the B1 cells, which reduces the amount of natural IgM being produced and then causes an overgrowth in the fibronectin?

Bhupesh Prusty 00:49:31
Yeah, the fibronectin is again, an immune inflammatory response. So the hypothesis here is that you start losing the natural IgM, immediately there is no bad effect because it's like it's a humoral response. The body is not in one day going down with everything. The autoimmunity develops over a period of time several weeks to several months remaining natural IgM they try to do the scavenging, sweeping out the business. But at some point of time, when there is a large amount of inflammation going on or other type of viral reactivation is going on and there is cell damage, cells are dying or apoptosis is happening. Now cell debris and the cell debris has to be removed from the body and that is not enough amount of natural IgM to do this job. Then, we started developing IgG responses against these cellular debris that develops over a period of time which leads to the development of autoimmunity. And as I said it is a chain reaction. It's a vicious cycle. You develop autoimmunity, these auto antibodies not one or two, they have a different job to do in different places. And this then starts affecting the endothelial cell function, and other types of issues that we see in ME CFS as well as Long Covid patients. The most interesting thing here is the last part of our paper where we try to understand, so far we always talk about overlapping between ME CFS and Long Covid In terms of clinical symptoms, okay. Clearly various different types of clinical parameters or clinical features which brings the ME CFS patients close to the Long Covid patients so can we really differentiate or can we really find overlapping or similarities to how much extent between Long Covid and ME CFS patient so we did some multivariate logistic regression analysis in our paper and found that you can basically say that severe ME CFS patients and mild and severe Long Covid patients look similar in terms of the circulating fibronectin amount together with in terms of how much of IgM against fibronectin they have if these two parameters alone combined. Then the ME CFS patients, particularly severe ME CFS patients, look more or less similar to the Long Covid patients not the control but the mild and severe Long Covid patients and the approximate parallel is around 84/85% of accuracy. If we look into the total ME CFS patients and the total Long Covid patients the Mild and severe combined, is roughly around 81% similar depending on the expression

patterns of these two proteins and if you look into the severe ME CFS and severe Long Covid patients, there is basically no difference. They are more or less similar.

Emily Kate Stephens 00:52:09
And what will be really interesting is once you compare because of the longer term, exam situation of the ME CFS. Once you compare the two or three year Long Covid people, that is going to be a very interesting thing to study. What happens, do they converge because obviously the people with ME CFS have been suffering for much longer.

Bhupesh Prusty 00:52:32
I would like to point out a very interesting feature over here we'd spoke about women in the main and how these diseases are very frequent in women. We looked into how much of natural IgM is there actually in women, male and female - what is the difference between them. And if we look into the natural IgM against fibronectin, the IgM, against phosphoryl choline and IgM against the MDA, we see that female, women have more natural IgM than male is not statistically significantly different but clearly there is a tendency towards higher amounts of these natural IgM in women from the evolutionary point of view, you can imagine this possible because the requirement for cleansing of dead cells, of dead tissues in the body probably is more required in female women than male. So then if you look into the Covid infected patients, you'll see that actually trend is opposite, the women have comparatively less amount. Again, it's not statistically significant comparatively, then there is a trend towards less amount of these natural IgM after the SARS-CoV-2 infection than men. Then you have under normal conditions, you have higher amount and after the SARS-CoV-2 infection you have lower amount. So the difference between being a normal person and then after SARS-CoV-2 infection seems to be quite large in comparison to the male, which is pretty interesting. Again, it is not a statistically significant point, but it is a trend probably should be studied in more detail in the future in long term patients up to three years after SARS-CoV-2 infection.

Noreen Jameel 00:54:11
So could this possibly be looked at later as a signal of why women get more autoimmune diseases than men?

Bhupesh Prusty 00:54:19
I would like to do this and I would encourage immunologist, autoimmune specialists who are doing these studies probably doing it and know it. I'm not very sure that this is something which should be tested.

Emily Kate Stephens 00:54:31
This conversation is basically a call to immunologists out there to now take this and develop further studies from which we might possibly be able to establish more than just Long Covid ME CFS pathophysiology

Bhupesh Prusty 00:54:49
I'm not an immunologist at all, I started working on IgM IgG and natural IgM's during the course of this study so my my knowledge on immunological aspect is very limited, I'm a biologist. My expertise is virus infection. So definitely this is something which should be studied in depth in future, I try to convince some of the immunologist in my surrounding if we can look into this at least in mice, because it is probably easy to do this and hopefully we can write some funding applications because this requires a lot of involvement and we probably can do it in collaboration with hardcore immunologist who work with B cells in mice. Particular with plasma B cells.

Noreen Jameel 00:55:30

We started the conversation talking about reactivation of Herpes viruses EBV causing problems with the mitochondria and the cell function. Then we moved on to the increase fibronectin and the decrease in natural IgM and with the proposition I think you were saying that that's caused by the SARS-CoV virus, right or is it caused by the reactivation of these other viruses ?

Bhupesh Prusty 00:56:07
So in the Long Covid group of patients after the first SARS-CoV-2 infection, the decrease in natural IgM is so clear, so pronounced in all the patients that we have tested this tells us in this moment that it is very hard to link directly to herpes virus reactivation because if I see in 60% of patients then I can say that okay, I can go back and check whether the herpes virus reactivation. But this has been in 100% of the patients we tested so it is safer for me at this moment to link it to the SARS-CoV-2 infection than herpes virus reactivation, but I am betting on myself here is that there is something going on with the herpes virus reactivation because it has to do with very specific tissues and we need to understand if the herpes virus reactivation, and we have to see here that we found more than 50% of HSV1 dUTPase antibody in severe Long Covid patients. We have a strong reactivation of EBV and HSV1 in these patients in all the Long Covid patients so we are looking into only the dUTPase antibody so we don't know exactly what other antibodies are there and things like that. And as these viruses are reactivating in very specific localised tissues, there are no ways that we can clearly tell that these viruses are reactivating throughout the body. When we have a SARS-CoV-2 infection you see the effect immediately. You don't see the effect in case of herpes virus that is the problem over here. So, it is safer at this moment looking into the data looking into the statistical correlation of data it is safer to say that effect in Long Covid patients is only because of the SARS-CoV-2 infection but if you go back to the ME CFS story and see that the severe ME CFS patients have the same feature. Then you tell yourself they don't have the SARS-CoV-2 infection. Here they have the virus reactivation again this tells us that there is a second player in this story at this moment we cannot put them together in one bin. So let's keep it separately. Let's tell that it's a post viral illness starts with the SARS-CoV-2 infection in Long Covid patients starts with something else like Lupus virus, reactivation in ME CFS patients, but the end product is same - the loss of the natural IgM and increase in the circulating fibronectin amounts .

Noreen Jameel 00:58:09
What's scary about this is that the SARS-CoV-2 seems to have an almost immediate effect, you're talking six to 12 months, right? Whereas ME CFS is much longer, slower burn with the loss of natural IgM, because their symptoms get worse over a longer period of time, whereas Long Covid patients almost in two months are suffering.

Bhupesh Prusty 00:58:32
So this is interesting here because you can see different viruses have different effects. We always say that a person has Parkinson's, Alzheimer's, you can actually detect the market 10 years or 15 years before that and developing Parkinson and Alzheimer doesn't mean that you develop yesterday. We developed over a period of years, the same with ME CFS patients, but depending on the virus and how fast and how severe the virus infection is, you can get instant result and it is in case of the Long Covid patients who have a very strong effect. This is very interesting here There seems to be that No Long Covid group in our study the IgM against the fibronectin and other natural IgM is seems to be higher than the severe and the mild patients they are seeing the signs of recovery of this natural IgM's that brings us to the story why some people after four, five, six or eight months tells that okay, we are now cured of Long Covid because of some or other reason whether it's a feel good factor yoga or music, listening, whatever is happening today is a biological process going on and how fast your body is capable of recovering and this is a natural process. Again, you do not need probably the medications completely to do this, but the bodies start to heal and the healing process takes time. And over the period of time some of them managed to come back to the level which is no more harmful.

Some of them they cannot manage to come back to that level rather goes on decreasing and they crossed the boundary of conversion which is very much similar to the ME CFS we do not have the possibility to study ME CFS patients while they're developing the ME CFS. In the Long Covid are in the presence of the SARS-CoV-2 infection have the possibility to do that.

Noreen Jameel
So, it's a violent assault from SARS-CoV-2.

Emily Kate Stephens 01:00:17
So, you're a biologist, you are not here to treat people, but what do we need to explore here for patients to get better? Who do we need to now take this research to and I know that you have spoken to various people, what are your thoughts on the direction we go in for potential treatments?

Bhupesh Prusty 01:00:41
It's a very interesting area of research at this moment and I feel that a lot of potential treatment strategies which can be followed over here most interesting and most relevant at this moment is the IVIG. I spoke to clinicians and some of the clinicians have the opinion or the idea that IVIG has a lot of side effects. I am not in a position to talk about it at this moment because I don't know. So, the intravenous immunoglobulin therapy has been tried, probably without knowing why it is being tried, but it has been tried quite successfully by some clinicians in the field of ME CFS this is something we should think about, at least together with the immune absorption, which is quite popular in Germany and other places. Immune absorption so basically you try to pull out all the auto antibodies from your plasma from your serum using columns. The serum and plasma passes through the column where the IgG against autoantigens auto antibodies are absorbed. So, you basically cleanse your plasma serum or body fluid from these antibodies.

Noreen Jameel 01:01:52
That sounds similar to what a lot of people were doing initially with apheresis where they were cleaning out the fibrin.

Bhupesh Prusty 01:01:57
So similar thing. So this has been tried in a similar way Øystein Fluge and colleagues in Norway have been trying the rituximab trial which is again targeting the B cells producing these antibodies. I think there is a great potential here. The idea of replacing the natural ICM at the same time of removing the auto antibody producing B cells is something that we should think about.

Emily Kate Stephens 01:02:22
I like the way that you say that like it's just as in one way it's easy, straightforward,

Bhupesh Prusty 01:02:29
A non-clinical person I have to be careful what I'm telling you because patients are desperate but I can understand the frustrations in the field. Anything which is being told here can be taken immediately into action which is something that should not be done because there are pros and cons here and clinical advice on all these topics has to be taken into consideration. So IVIG is one potential option that should be tried and tested, or other things in the literature that I read about for example, the plasma B1 cell therapy, bone marrow derived cell transfusion, then we have peritoneal cell transfusion. These are some of the discussed therapy options, which is available which can target replenishing the natural IgM. So what I'm trying to tell you is that those experts who have the ability to think about it and develop treatment strategy, I would advise or recommend them to think about replacing the natural IgM and think about changing the plasma B1 cell repertoire inside this body so that we can produce more amount of this

natural IgM what is also possible that if we can produce this recombinant proteins so IgMs like any other recombinant antibody therapy which has been developed, and we can put them back into the body. This is also another way to tackle the situation whether that will be successful, how successful whether there will be side effect or not. This is something which is beyond my capacity to tell.

Noreen Jameel 01:04:04
You basically have said in this paper and all this work that you've done with all your colleagues that you possibly have found a mechanism for the Long Covid.

Bhupesh Prusty 01:04:13
This is possibly the key state, the initial state to link the SARS-CoV-2 infection to the recovery state why some people recover, some people doesn't recover and go into the mild and severe Long Covid and why autoimmunity potentially develops in these patients. Of course, we cannot explain other clinical features that we see in the Long Covid patients directly in the form of this natural IgM. It's always a chain reaction... It's a vicious cycle. More things are changing. Of course, there is mitochondrial dysfunction happening because of these auto antibodies are producing and to mitochondrial dysfunction, the changes in the cellular physiology itself will have an effect there is a combined effect of more than one factor. So, I would not claim that we solved everything. But this is a very important thing which is I guess in my opinion it has never been done before. That you can link biologically ME CFS with Long Covid in the form of a protein biomarker and how it is being changed over a period of time. This is the most beautiful part of the story. Whether it is solving the whole dogma of Long Covid development or not I don't claim that yeah,

Noreen Jameel
We have a biomarker ?

Bhupesh Prusty
In my opinion, yes, we have a biomarker which some of the colleagues or some of the patients will not agree with me that it is a biomarker because it is not 99% accuracy but if we claim that combination of 10 or 15 or 20 Proteins can give me the accuracy of 95%. And I say one or two proteins in our case gives me 85% of accuracy and keeping the wide variety of clinical features that we see in these patients 85% of accuracy with two proteins of one protein is a very strong potential to be a biomarker.

Emily Kate Stephens 01:06:02
And are those tests that we could have done at a normal phlebotomy service somewhere that does blood test, or are they tests that require very specific equipment and will be only available at very specific places like coming to you?

Bhupesh Prusty 01:06:21
No. This is the beauty of the story that majority of the concluding experiments that we did with a large number of patients are based on Elisa assays and Elisa assays are frequently done in any clinical pathological lab equipped to do this type of assays. So the fibronectin Elisa that we did is actually commercially available to anyone any lab in the world can do it and this is actually cheap. The cost of these experiments are extremely low, the IgM and IgG that we measured against natural IgM is something is not commercially available at this moment. So we have to develop it. The technology we have not patented the technology or we are not claiming to patent this technology's it is truly available any lab can actually adopt it and they can develop it easily within weeks' time. It can be done in any pathology lab which is equipped to do Elisa assays which is the case in almost everywhere in the world means that all these tests are easily

reproducible, easily doable in any part of the world. Whether it's a rich country or poor country. It doesn't matter.

Noreen Jameel 01:07:24
So, what's your next step now, what is the next phase of your amazing work?

Bhupesh Prusty 01:07:29
This is an interesting question and the next phase...

Emily Kate Stephens 01:07:31
You're gonna have a little sleep now?

Bhupesh Prusty 01:07:36
No. I have a big thing to do. I have to find a job because I don't have a clear job possibility in my current place after April next year. So now I will focus more on searching for a new place for my lab where I can go and continue working and then we will have a new home from 2024 where we would like to focus on post viral illness in more detail looking into virus reactivation and natural IgM's, which is a very interesting topic. As we discussed we do not have a clear link between these two things at least in Long Covid patients, so we would like to develop an animal model where we would can have herpes virus reactivation SARS-CoV-2 infection and then looking into the plasma B1 cells natural IgM's in the mice model, we have plenty of movies available. The other aspect would be to look into the prospect of developing more complex autoimmune diseases like multiple sclerosis or lupus in long term Long Covid patients if the auto antibody levels are high, which are the auto antibodies are going to contribute to the development process and if there is any potential of any specific drugs targeting specific antibodies again, not all auto antibodies can be combined with natural IgM therapies to look if that can be tackled in other chronic neurodegenerative and autoimmune diseases like multiple sclerosis.

Noreen Jameel 01:09:01
One last point I just want to clear up if replenishing our natural IgM and people with Long Covid like Emily and I helps us get better. Would the same be true for ME CFS patients?

Bhupesh Prusty 01:09:13
That's very interesting as we discussed the ME CFS patients particularly the severe ME CFS patients, and what we see is being sick for a very long time. So they have a lot of secondary comorbidities. They have developed different clinical features over the period of time, which is a consequence of the ME CFS but the instant recovery process would not be very positive for them.

Emily Kate Stephens 01:09:34
So that might be the same as you know people like me who have had it for three years.

Bhupesh Prusty 01:09:39
That's what I try to model in my paper that we can probably divide the development of ME CFS as well as the Long Covid in two phases. One is acute phase one is chronic phase. The acute phase is what is going on with the Long Covid patients now this gives us a learning possibility to how the acute phase developed to chronic phases. The chronic phase is the one which we see in ME CFS patients, we see so much of difference between mild moderate ME CFS patient versus severe. The recovery probably is more complicated than that in the Long Covid patients but we are just beginning and we are learning one part of the story. If we have a biomarker we have the potential to change the story. I somehow feel that fibronectin circulating fibronectin is playing more important role in ME CFS patients than in Long Covid patients that's the chronic part of

the story because fibronectin can bind to TLR2 TLR4 receptors and can induce strong innate immune response that is the antiviral response that we normally see in ME CFS patients always feeling the sickness even if you do not have a viral infection going on, you feel the viral infection like condition because your body is producing constantly innate immune response, thinking that there is virus infection is going on there. fibronectin can create a false virus like condition then the high amount of ross that can be produced by the immunoglobulins affecting the endothelial cells can also contribute to this and then the fibronectin can actually cause hyper activation of the platelets and Mast Cell Activation and things like that. So these are all chain reaction has already gone through ME CFS patients probably not that strong in Long Covid patients I think the chain reaction probably will decrease once we tackle the cause. Again, it probably needs complex multiple treatment strategies at any point of time.

Noreen Jameel 01:11:33
That sounds like the Long Covid story, doesn't it? A complex multiple strategy?

Bhupesh Prusty 01:11:37
Yeah, I think the first Podcast was very wildly successful, but it was also a tiny bit controversial. There is a group of individuals who always say that this paper is not peer reviewed why to discuss it or to make it available for public and creating hopes or false hopes. I always a say paper written is a paper published. Today or tomorrow it will be published and the data will be validated, the sooner it can be validated and the sooner it reaches to the public you have the possibility to either reject it or accept it. In both cases I'm happy with it. As a scientist we always do research something stand out something doesn't stand out. Not that we are trying to give you false information. It is always there are certain parameters of every experiment that we always don't know and human body is so complex. You might get completely unusable unexpected things later on. The sooner you can test it and find positive or negative evaluation for this work the better is the prospect of finding a treatment. So that is the only reason why we are coming here. The paper will be published whether it is a big journal or small journal only on the basis of the content and the peer review process. This has nothing to do with making a podcast or going to the patients and telling them what we are doing because patients information is not going to influence the evaluation of the paper. It is a completely different process. The only reason why we are here is to make the data available open to everyone as soon as possible. It's up to you to take it or reject it. I'm happy with it either way.

Emily Kate Stephens 01:13:19
Join us next week as we hear others experiences have Long Covid. Share your stories and questions at TLCsessions.net. Follow us on Twitter and Instagram for the latest updates and if you found this interesting please do subscribe.