CDC AFM Biorepository and Moderated Roundtable on AFM Research with Q&A Session

You can view this presentation at: youtu.be/3s16Lj-8NTI

Roberta Pesce: [00:00:00] Welcome to our first talk of day three, where we'll be learning more about the Centers for Disease Control and Prevention AFM Biorepository followed by a moderated round table on AFM research with Q and A session.

[00:00:18] Joining us are Eileen Yee, Medical Epidemiologist of Acute Flaccid Myelitis, a Domestic Polio Team Division of Viral Diseases at the National Center for Immunization and Respiratory Disease Centers at CDC, Dr. Amy Rosenfeld, Assistant Professor of the Department of Microbiology and Immunology at Columbia University, Dr. Nischay Mishra, Assistant Professor at the Center for Infection and Immunity at Columbia University, and Dr. Janell Routh, Medical Officer, Lead, AFM and Domestic Poliovirus Team at CDC.

[00:00:47] This panel will be led by Dr. Carlos Pardo, Professor of Neurology and Pathology Division of Neuroimmunology and Neuroinfectious Disorders and Director of the Johns Hopkins Myelitis and Myelopathy Center at Johns Hopkins University School of Medicine in Baltimore, who will start off the conversation by giving a brief overview on AFM research and highlight the importance of these research studies. Dr. Pardo, hello. Over to you.

Dr. Carlos Pardo: [00:01:13] Thank you so much. Thank you so much, Roberta. It is great to be here this morning. Thank you everybody for being present for this round table about acute flaccid myelitis. As you know, acute flaccid myelitis has been a major challenge for many patients and families since 2012, but very likely has been a challenge for other families even before that because it's very possible that acute flaccid myelitis is one of those disorders that were frequently ignored, misdiagnosed, or basically not very well recognized previous to 2012, when acute flaccid myelitis emerged as a major disorder affecting children in the United States.

[00:02:05] Acute flaccid myelitis has been recognized around the world as a problem that affects predominantly children and produce a quite significant amount of neurological disability and a very important amount of stress, not only for patients, but also for the families. Acute flaccid myelitis is a very difficult disease. It appears to be an infectious mediated disorder. Many members of the families are affected by respiratory problems and despite many members of the family or the household are affected by respiratory problems, only young children are affected by acute flaccid myelitis.

[00:02:50] And even only few of, only one of the children in the family may be hit by acute flaccid myelitis. Next. So, what we understand of acute flaccid myelitis is it's a very aggressive, very acute evolving problem. And you can see in this slide that is based on the characterization that was produced by the CDC in the patient population that was evaluated during the outbreak of 2018, that the majority of patients, 94% of the patients have a preceding illness characterized mostly by fever and respiratory disease. But over the course of few hours, up to 48 hours, many children actually develop a very aggressive neurological problem, particularly because patients get paralyzed and patients, many of them, almost 50% of them, actually require intensive care unit management because the patients develop respiratory failure.

[00:03:54] And, unfortunately, a very large population of patients that are affected by acute flaccid myelitis are left with significant neurological disability. And almost a third of, a quarter of them, actually, are left with paralysis in both upper and lower extremities. And almost 40% of the patients are left with significant impairment in mobility, particularly in upper extremities.

[00:04:20] So, this is a very important problem that we may need to pay attention because it's producing a significant amount of disability and long-term neurological problems. If we take a look of the next slide, what we understand is that in front of us, there is a very long road for understanding and taking care of acute flaccid myelitis.

[00:04:47] Around the country, there have been different initiatives that are centered in improving diagnosis, improving surveillance. There has been a very important campaign for education of clinician pediatricians, neurologists for understanding acute flaccid myelitis. And there has been a very important effort, effort that has been centered in supporting families because the long-term problems, neurological problems that families are experiencing with these patients is overwhelming and obviously family support is critical.

[00:05:25] However, one thing that is extremely important is research, is what we are doing for understanding acute flaccid myelitis. And here is, we need to outline the different items that are in that long list for understanding acute flaccid myelitis. The first one is perhaps understanding the role of viral diseases and specific viruses like enteroviruses as a causal agent of this disease. We need to understand the immune system. We need to understand how the immune system is participating either as a defensive, defensive mechanism in the body or is even activating mechanism that may produce some injury.

[00:06:11] We need to improve diagnosis and we need to understand better how are the different factors associated with the prognosis and outcome. And a most important aspect is improve diagnosis, improve treatment and prevention for avoiding these difficult diseases. Next one. There are different efforts at the national levels and all of the efforts are related with understanding the disease by collection of clinical data, collection of biological samples, and understanding better the disease.

[00:06:44] One of those efforts, next, is the study that was launched by the National Institute of Health, the Natural History Study of acute flaccid myelitis that is coordinated by Dr. David Kimberlin at University of Alabama at Birmingham. The National Institute of Health basically is funding this prospective study to define the natural history, the risk factors, and understand better the pathogenic mechanism of the disease.

[00:07:11] Next one. So, when we talk about these studies, we talk about the studies that involve a lot of people around the country. And, at the moment, there are almost 35 centers around the country involved in the NIH Natural History Study. It's, again, collecting biological samples and centered in understanding the disease. Next one.

[00:07:35] And one thing that we are doing is a case control study. Case control study is the only way to understand risk factors associated with a disease. In that way, there is emphasis in including patients with acute flaccid myelitis that have developed the disease in which we are able to characterize the clinical features and we are able to acquire also biological samples. Next.

[00:08:02] We may need to get a control group, because the control group is the one that is going to help to understand as if, what we are seeing in our patient population is associated really as a causal factor or is not necessarily a causal factor. And we are basically involving relatives or household residents and the child that has been affected by acute flaccid myelitis to be participants as a group control. And again, it's extremely important to acquire this control group, because it's the only way to acquire also biological samples that may be used to facilitate a better understanding of all of those immunological and virological factors. Next.

[00:08:52] There are other studies like the genetic study that is led by Dr. Priya Duggal, who is a Professor of Epidemiology at the John Hopkins School of Public Health. And many families in the past eight years have been participating in this study that basically is directed to understand if there are genetic factors that are influencing the presence of acute flaccid myelitis. And at this moment, there are more than 150 patients and families that have been enrolled in this study.

[00:09:22] But the most important is that we need to come and basically continue our effort for collecting this data for understanding acute flaccid myelitis. And the effort by the CDC is very important and complementary to the effort that the NIH is doing at this moment. So, I will pass my microphone to Dr. Eileen Yee, who is at the CDC and is going to help us to understand what is the CDC doing to participate in these biorepository collections of clinical data and biological samples. Eileen, thank you so much for being present here this morning.

Dr. Eileen Yee: [00:10:03] Thank you so much, Dr. Pardo, for the introduction. And right now, I would like to just go over a little bit about the CDC AFM Biorepository. And again, I just want to say thank you so much for Rebecca, GG, Roberta, and Dr. Pardo, and SRNA for inviting me to this research round table today. And to share with you how CDC is involved in this research initiative. Next slide.

[00:10:29] As Dr. Pardo stated, there're many unanswered questions about AFM. And some of these were also discussed in February of 2020 at a workshop with the National Institute of Allergy Infectious Disease.

[00:10:41] And there was a great paper summarized by Lerner et al that, that listed some of the questions and the things that we're doing at CDC and also around the research community. And however, as Dr. Pardo said, that to answer some of these questions, it requires testing and analyzing clinicals specimens from patients with AFM. Thus, it was recognized quickly that we need to obtain and collect these biological samples prospectively from patients with AFM right now. And so, next slide.

[00:11:15] So, in general, a biorepository is a collection of biological samples, such as blood, stool, CSF, and other clinical specimens, that is kept in a facility capable of securing, handling, and storing biological materials for future use.

[00:11:29] Next slide. In late 2019, CDC awarded a contract to the General Dynamics Information Technology and McKing Consulting Corporation to develop and maintain an AFM Biorepository. McKing already had successfully designed and implemented another biorepository for CDC focusing on ALS. And so, they were a welcome colleague to assist CDC in this endeavor.

[00:11:56] The AFM Biorepository... the slide before. Thank you. The objective is to build a bank of specimens matched with epidemiological and clinical data that can be used by researchers to determine the cause and risk factors of AFM, as well as better treatment options for individuals affected by this condition.

[00:12:16] And this was again, as Dr. Pardo said, it was designed to align with the NIH and AFM Natural History Biorepository as much as possible and just be a complementary research project. You can keep this slide.

[00:12:31] This is the inclusion and exclusion criteria. The inclusion criteria is any patient that is age three months or older. So, we do include adults with onset of limb weakness involving one or more extremities suggestive of AFM within the previous 30 days, and for which a physician provided diagnosis of suspected AFM to participants and participants' family members, and that they agree to use their specimens for future use. An exclusion is obviously anybody who is at the NIH hospital and, or with a known condition other than AFM causing their limb weakness, or if they're a ward of the state or prisoner and, or they refuse to consent for participation. Next slide.

[00:13:13] Eligible participants can be identified through three mechanisms. They can actually be identified through the clinician or site coordinator at one of the partnering hospitals, when a patient is identified and admitted to the hospital. And secondly, they can be identified through the state or local health departments when a suspected AFM case is reported to CDC. And lastly, patients and their families can directly self-identify themselves and contact the AFM Biorepository directly to participate. In those instances, though, the participants will be asked to have their physician verify that they are a suspected AFM case. Next slide.

[00:13:54] Overall, patients are identified, and then informational materials are shared. Then the hospital or clinician, or even the health department, will contact McKing directly to make them aware of the potential participant. If the patient is willing to participate, then enrollment in informed consent will be conducted and coordinated by McKing.

[00:14:12] And if a collections kit is not already available, then a collection kit would then be shipped to the hospital or to the patient's home, if they've been discharged. Collection of either the acute or convalescent specimen will be done and shipped overnight to Fisher BioServices, where the specimens are then further processed for long-term storage. Next slide.

[00:14:34] The acute specimens are collected at the hospital anywhere from seven to nine days after a limb weakness onset. And this would include a blood sample, CSF, stool, or an NP/OP swab, and convalescent specimens would be collected at a patient's house or an area that's more convenient for them through a national phlebotomy agency, four to eight weeks after. And they're just collecting blood samples. And the plan is to merge all of these specimens from both NIH and CDC biorepositories into a common biorepository. Next slide.

[00:15:13] Currently the study is actively enrolling participants year-round since October of 2020. And they'll continue to do so until the end of 2022. Some highlights, quickly, is that there has been active engagement with key hospitals who are partnering with McKing. So, these hospitals are prepositioned and ready to report any case to McKing and collect specimens. So, there's been a hospital network that's been established by McKing to regularly remind them of the AFM Biorepository.

[00:15:41] And lastly, there has been a big campaign to increase awareness of this biorepository, through many different venues such as this and information can be easily accessible through the CDC and SRNA research website, and regular communication is sent out to members of multiple professional, medical, and neurologic associations. Last slide, please.

[00:16:04] For more information, you can go to the CDC AFM website, and you can find links to a table that describes both the AFM Biorepository and the NIH Natural History Study. And you can also contact McKing directly through their hotline and email address to ask any questions or to let them know of any potential participants with suspected AFM.

[00:16:24] Finally, I just want to acknowledge the work that has been done by McKing and, to the patients who've already and will be contributing to these biorepositories, we really thank you. We know that you may not be directly benefited right now, participating in the study, but we know that your contributions are greatly needed and appreciated by the AFM community. So, together we can answer more of these questions. Thank you. Back over to you, Dr. Pardo.

Dr. Carlos Pardo: [00:16:48] Thank you so much, Eileen. It's great to hear about the effort by the CDC that is in many ways overlapping very well with, for the NIH and the natural history study is doing as well.
[00:17:03] So, there are two members of the research community in our audience here and as presenters. I would like to hear from Dr. Nischay Mishra, who is at Columbia University. He's a researcher and also a very dedicated scientist to understand the immunology of infectious disorders. So, Nischay, we are collecting biological samples. We are very interested in diagnosis. Do you mind giving a brief view as a scientist, what you do in the lab for understanding AFM?

Dr. Nischay Mishra: [00:17:44] Thank you, Pardo, first for inviting me and SRNA for such a good initiative and thank you, Roberta, for taking me through the whole process of Hopin the other day. And so, I have a couple of slides prepared I will just share with you guys so we can talk about what I've been doing. I work at the Center for Infection and Immunity at Columbia University, known as Lipkin Lab.
[00:18:07] So, basically, we use molecular, virological, and serological, and immunological plates like assays and knowledge to build diagnostic and characterize genomic assays. Not only for a diagnostic in the lab, we take it further for approval processes also that can be useful and available for all.

Roberta Pesce: [00:18:30] Nischay, very quickly, I don't think your slides are being shared at the moment.

Dr. Nischay Mishra: [00:18:34] I'm sharing right now. Yeah.

Roberta Pesce: [00:18:36] Okay. Perfect. Thanks.

Dr. Nischay Mishra: [00:18:42] Can you guys see my screen?

Roberta Pesce: [00:18:47] Yes, we can.

[00:18:53] Yeah, it looks perfect. Thank you.

Dr. Nischay Mishra: [00:18:55] Yeah. So, briefing back about the work we have been doing. So, in 2019, we collaborated with CDC Respiratory Virus Group, Dr. Oberste's group, and then we, that time we were trying to investigate the association of EV-D68 directly or indirectly with acute flaccid myelitis. So, for that I built an array that is peptide array, covering whole capsid protein of all enteroviruses, some other pathogens also that were suspected back then - West Nile virus, Lyme disease, et cetera. And then we took the CSF sample from the AFM children, and then also controls we collected from Columbia University and elsewhere from the other diseases.

[00:19:36] So, how does it work? So, this is a glass slide. This can contain 180 to 200,000 peptides, peptides are printed on the glass array. We use the sample which contained the primary antibody. All the peptides are

covering the whole capsid protein with the 12 and 11 amino acid overlap, so we do not have any artifact, or we do not miss out any epitope binding sites. And it's very highly sensitive.

[00:19:58] The sample volume being used is less than five microliters. So, it can be like even the smallest volume sample available, we can do that. We did use the serum and CSF sample of the AFM patient and control. So, basically, we load the samples overnight, incubate the peptide, which may contain epitope binding site, binds with antibodies present in the sample. Second morning, we wash it and then use the secondary antibody which could have different labels. It could be Cy3, Alexa Fluor and Cy5, and we can do a at least two antibodies detection together.

[00:20:30] Then we take the virtual design, which is the coordinates of all 180,000, in total 3 million peptides printed on the glass array, and we overlap. And then after any signal comes up, we change the arbitrary unit, and we get to know if this peptide is positive or not.

[00:20:45] After that, all the continuous peptides, they are not printed together. So, to avoid any kind of artifact, they are all randomly placed. And then we do the regression analysis and data merging using some modeling tools. And then we identify which is the overlapping peptide sequence, which could be the epitope (that) is reactive to that sample.

[00:21:04] Using the sampler approach, we identified again, the consensus enterovirus motif, and we also identified a specific EV-D68 peptide only present in AFM patient. You see the lower panel. And this we published in 2019 with our colleagues at CDC.

[00:21:23] This is, you can see the CSF sample. They are all enterovirus peptide, which is the conserved motif, because they are, they share much homology. And we always predict, like, one of the regions, like, how the enterovirus sero diagnostic is difficult because they are very closely associated, and they can cross-react with each other or can enhance antibody for each other as well. We made the peptide ELISA also based on the EV-D68 peptide and then we tested the sample with ELISA as well, and we could find a very good correlation with the peptide array data, so that's telling us this can be used for diagnostic, like sero diagnostic, and this peptide is sensitive and specific to the EV-D68 detection in AFM patients. Based on that ideology, recently, like, last year or before, I collaborated with Amy Rosenfeld. She's also on this call and we wanted to expand this peptide array.

[00:22:17] So, I expanded the peptide array with the technology improvement. I can print now 390,000 peptides per sub micro array, total 6 million peptides. We expanded from capsid to the complete polyproteins. Also, we added the rhinoviruses because they are very closely associated with EV-D68. And we also had a random, a scramble peptide for the background collection.

[00:22:39] So, this peptide array is in use, I received a sample recently, like, last year, from Kevin Messacar and Ben Greenberg from Texas. We used a couple of their experiments last year. It wasn't very productive for the EV-D68 experiments because mostly it was involving the COVID research, but we did some experiments.

[00:22:57] We identified an additional peptide, which I will be analyzing more and then publishing on or, like, sharing with you all in the near future. I will take a pause for this, like, at this moment, what are, what else we are trying to do. So, since we have already identified these peptides that are EV-D68, I collaborated with the Amy, and then we wanted to see the utility of this peptide, how we can use this peptide for rapid diagnostics.

[00:23:27] So, we, we thought about it, like, because sometimes it's very difficult for the patient and clinician to know if a patient is positive for the EV-D68 in the respiratory acute phase. So, we wanted to make acute assay. We are looking at that and like a peptide to raise the antibodies.

[00:23:45] Those would be specific for enterovirus D68 detection. And we did some experiments with the mice model. We designed some peptides, and we did some ELISA, those look promising. So, we're going to move forward for antigen capture reduction assay, which we are working on. And then after, we will look for other enterovirus peptides as well that are specific.

[00:24:06] We are trying to expand this antigen-based assay or like some Luminex assay, which we can rapidly detect, and not only detect, we can differentiate which enterovirus it is within a couple of hours. So, then a patient that is detected with EV-D68 can be monitored at the time of respiratory illness itself, if they develop any kind of acute flaccid myelitis symptoms in coming weeks or something, and the clinician and researchers can be more specific about treatment as well as the diagnostics. Thank you.

Dr. Carlos Pardo: [00:24:38] Thank you, Nischay. This is very helpful. And, as the audience was able to see, there has been a very important effort from scientists like Nischay understanding the immunology of acute flaccid myelitis. And Nischay mentioned enteroviruses, but no better than Dr. Amy Rosenfeld to explain to us why we are concerned about viruses and particularly enteroviruses as a potential factor associated with AFM and, for the audience, there are a lot of scientists - immunologists, neurophysiologists, but I know, very important scientists here that play a critical role for understanding infectious diseases are virology.

[00:25:25] So, Amy, do you mind explaining to us why we are concerned about enterovirus in AFM and what is the work that you do for understanding the disease?

Dr. Amy Rosenfeld: [00:25:34] Thank you, Carlos and SRNA, for inviting me to talk to you today. So, I'm a basic scientist. I am a traditional, fundamental basic virologist, and I have taken over in my position, an extension of work that was done by Vincent Racaniello and others who studied polio. And polio is the traditional etiologic agent that we associate with acute flaccid myelitis or poliomyelitis. And actually, acute flaccid myelitis is a term that was used to go into the field and look at children and survey for paralysis due to poliovirus infection. And now we recognize that the entire family of enteroviruses can lead to AFM or acute flaccid myelitis, they are neuropathic pathogens.

[00:26:29] And so I'm not just interested in EV-D68, which is the most notorious agent of AFM today. But I'm interested in the entire family of viruses that associate or whose infection associates with AFM. And these include related viruses such as coxsackie A16 and enterovirus 71. And we do an entire panel of different viruses.

[00:26:57] We are attempting to make animal models to understand how the primary infection disseminates and enters the CNS, and causes neuronal death, and the immunology, and what the role of the immune system in the host is in that developmental process.

[00:27:16] In addition, we are also interested in understanding how the virus is transmitted, shedded, and understanding, and trying to understand where we can design interventions. Because, once the paralysis has been identified, it's really too late to design interventions and they must be done much earlier.

[00:27:40] So, when the child comes in with a respiratory infection or something or other, usually, unfortunately, many of these symptoms overlap with other respiratory pathogens. And so, we're interested with work with Nischay that we've been able to design a test that will distinguish and discriminate between these pathogens.

[00:28:04] And the family of enteroviruses has a lot of conservation. And we traditionally thought that, if you had the presence of antibodies against the virus, we could detect them, it was used as a tool to make a history of what diseases or what viruses one has been exposed to. And today we know that that is not as easy as

what we thought. There is a lot of similarity between these viruses and, therefore, a lot of the antibodies that are elicited during viral infection actually recognize the whole panel of viruses.

[00:28:39] And so we're trying to understand how that works and whether or not there's something about what you've been exposed to or the absence of development of antibodies that subjects you more to these secondary devastating consequences of the viral infection. And I'm happy to answer any questions and help you understand what it means to be a basic scientist in virology or just in general.

Dr. Carlos Pardo: [00:29:11] Thank you, Amy. That's very helpful. Roberta, if we can bring the entire group to the round table and I'd like to ask Dr. Janell Routh from the CDC, who is the leader in the surveillance effort for acute flaccid myelitis. Janell, do you mind giving us just an update about what we are seeing about AFM and enteroviral circulation in the past several months, in the setting of the current pandemic with COVID-19? Just give us, what is going on and what we are expecting in the future.

Dr. Janell Routh: [00:29:55] Carlos, thanks very much. And again, I wanted to thank SRNA and everybody for hosting this panel today. You know, it's been very interesting to track surveillance for AFM over the past few years. I think as everybody's aware, our last sharp increase in cases in AFM happened in 2018.

[00:30:18] We know that we've seen increases in cases since 2014, really when AFM emerged on the national scene. And we've had an every other year periodicity to that increase in AFM cases, in 2014, 2016, and then 2018. So, we were very much anticipating another increase in cases in 2020. However, that coincided with the COVID pandemic and, interestingly, I think some of the efforts to prevent the spread of COVID actually prevented the spread of other respiratory viruses, including enterovirus D68, as both Amy and Nischay mentioned.

[00:31:01] And so we did not see an increase in AFM cases in 2020. So, I think we were all thinking what was going to happen again in 2021. But as in 2020, we have not seen a sharp increase in cases in AFM, fortunately this fall either.

[00:31:22] We know that generally AFM tends to be seen in the late summer, early fall months of September and October. But, so far, we have not been tracking an increase in cases this year. Coinciding with that, we've not seen an increase in the circulation of enterovirus D68 either. Again, as Amy mentioned, we think that is the primary cause of AFM and these peak years are the years where we see an increase in AFM.

[00:31:53] So, neither an increase in AFM nor an increase in EV-D68 circulation, at least so far this year. It will remain to be seen what is happening. I know we are seeing an increase in enterovirus and rhinovirus circulation overall. But so far, not that particular virus that we do tend to associate with AFM in these peak years.

[00:32:17] We continue to be vigilant. We have a network of vaccine preventable disease coordinators in every state across the country where clinicians would report a suspected case of AFM. We're in touch with them constantly and monitoring the situation. So, keeping fingers crossed that we don't see an increase as we move into the late Fall now.

[00:32:41] And then all eyes will be on 2022. Again, it's very difficult to predict what we might see in 2022, but, you know, one of the main objectives of our AFM team here at CDC is to raise awareness and promote education so that physicians across the country understand what AFM is and can be vigilant for the symptoms. So, we will continue to do that into 2022 to make sure that we would catch any suspected case or increase in cases. Over to you.

Dr. Carlos Pardo: [00:33:16] Thank you, Janell. That's very helpful. So, I have a question first for Amy and later for Nischay. So, Amy, in the clinical setting, we collect nasal swabs, just to demonstrate there is virus or there is no virus. In a clinical setting, we collect blood samples to see if there are any markers to help us to identify AFM. As a researcher, do you mind explaining to the audience, how do you use those in terms of trying to identify the virus and how helpful those samples are and, and, and, particularly to emphasize why those samples are basically, extremely important for the future of understanding AFM?

Dr. Amy Rosenfeld: [00:34:06] So, we are, we use these samples to enhance our modeling, first of all. So, it's very, generally these samples are very, it's very hard to culture infectious virus from them. But we use them to determine, to identify the genomes or the RNA that is present there. And that gives us some indication of what pathogen is present.

[00:34:35] And then we try to model this using animals in the lab to mimic the human disease. And we use these samples to track to make sure our models are on track of what we think the disease actually looks like. And then to understand how the mechanistic, to do mechanistics studies to understand what makes AFM or what causes virus dissemination from the respiratory tract into the CNS.

[00:35:01] And we really like samples to understand also virus evolution, so whether or not the virus has changed over time and whether or not these changes associate with more or less severe disease. And so, this has really played out well in the public to some degree in the discussion of SARS-CoV-2 infections, vaccine resistance, or enhancement, or escape, and what we see as variants.

[00:35:31] So, whether or not you, we all agree about the interpretation of the data, we can see that the virus has changed from when it was first isolated to today, and whether or not those changes cause any phenotypic changes. And that's pretty much what we use these samples for, from any virus. So, EV-D68, entero 71, coxsackie A16, anything we want to track whether or not these changes really are helping understand whether or not the pathologies are also changing.

Dr. Carlos Pardo: [00:36:08] Thank you, Amy. So, Nischay, you were talking in your experiments about immunology for detection of enteroviruses. You were talking about peptides and all those aspects of, of your immunology research. But can you explain to us the value of understanding the immune system acutely and what you or Eileen mentioned in her presentation, the convalescent serum? So, what is the value when we are asking families to get, allow us to get biological samples acutely and also a few weeks after, or even months after? Do you mind explaining that briefly?

Dr. Nischay Mishra: [00:36:53] Of course, Dr. Pardo, like, so there's a couple of things. Like, mostly these children are at a very early age in their life cycle and then it's still important to know if they are exposed to this virus or any of those viruses, EV-D68 or any enterovirus, is it the first infection of their lifetime? Also, it's important to know because, we have talked about it several times, about ADE, antibody dependent enhancement, as well.

[00:37:20] So we need to know which virus, like enterovirus D68, the first enterovirus that's being exposed to the children that lead to AFM or there were previous enterovirus infections happened and in reverse order that can lead to. So, so far, we know that there are hundreds and thousands or more enterovirus infections compared to AFM cases. But knowing that is very important to know case and control.

[00:37:43] So, we do unbiased approach. We are splitting the viral protein in very, very small part. That's 12 amino acids. So, reduce the cross reactivity and antibody detection. And if we detect something in the AFM cases or not detecting the control cases or vice versa, we want to see after one month or two months, if those antibodies are still remaining in the patient, or they are depleting in that patient, or increasing in the

patient, and what are their roles - increase, decrease, or disappearance in the patient's outcome. So, if we do such a kind of, like, plus minus study, we will need to identify the answers and then we can take that lead and move on for therapy and the early diagnosis as well.

Dr. Carlos Pardo: [00:38:32] Thank you, Nischay. Eileen, one of the frequent problems that we have in the clinical setting is that many patients, when they arrive to tertiary hospitals, university hospitals, have been already treated with either steroids, or even IV immunoglobulins, IV or IVIG, or even plasma exchange. What should the clinicians do with those samples? Are they able to share those samples with the CDC Repository?

Dr. Eileen Yee: [00:39:03] Yes, they are. Especially as some children have been seen in outpatient where they received an antibiotic or a steroid. We still would like for them to submit a sample. And it's also the timeliness of the sample is really important, in terms of the success of finding a pathogen. So, we still would like to collect that and, at some point, be able to gather some more information about them.

Dr. Carlos Pardo: [00:39:30] Thank you, Eileen. Amy, one of the questions that families frequently asked to us in the clinical setting, is this a new virus or is this a virus that mutated because viruses have been circulating for centuries? So, is this a new virus or is this something that mutated from, and since 2012, for example?

Dr. Amy Rosenfeld: [00:39:51] So, it is not a new virus. It has not been mutating. It is a virus that was initially isolated in 1962, but that does not mean that it was not present beforehand. That's just when it was isolated. And, we have demonstrated, my work has demonstrated that the virus has always been able to cause or replicate within cells within the nervous system.

[00:40:17] So, it is not a new property of the virus. What we hypothesize is actually the fact that there is a baseline of AFM. And most children were, most cases of AFM, until the administration of the polio vaccine, were associated with polio virus infection because that is a more, what's the word, pervasive infection. So, there are more cases of polio. It is clearly a virus that is more robust than EV-D68 and probably more easily transmitted because it's via the oral fecal route and stuff. But with reduction of polio transmission, with the two very effective vaccines, an oral polio vaccine and IPV, it has opened us up to seeing things that cause AFM with reduced frequency.

[00:41:20] And so that's what our hypothesis is. And that actually, probably the administration of, or the immunization of children with different forms of the polio vaccine could also impair our ability to detect EV-D68, as we have, we believe that we have antibodies that are able to be induced by the polio vaccine bind to EV-D68 and prevent EV-D68 from disseminating into secondary organ sites, which is what the CNS is.

[00:41:53] And with the change of OPV to IPV, that process may have been interrupted. And so, it's not a new virus. It's not altering. It is something that was much lower, at a rate of one out of every hundred thousand patients who developed AFM due to the EV-D68 infection, versus polio, which is more on the par with one in every thousand infections.

[00:42:19] So, you can see when you're tenfold, a hundred logs, two logs off, why when you take a large population, you might not detect EV-D68 or automatically associate EV-D68 infection with the development of paralysis.

Dr. Carlos Pardo: [00:42:36] Thank you, Amy. This is a question for Janell. Janell, in the past, I need to confess that I was sometimes afraid of sending samples to the CDC for diagnosis, but I have learned in the past several years, the spirit of collaboration and sharing samples that the CDC as institution is starting to develop with researchers. How are those samples that we are sending to the CDC are going to be shared with Nischay, Amy, or other laboratories? Would you mind expanding on that?

Dr. Janell Routh: [00:43:11] Sure, Carlos. Yes. So, all the specimens that are sent to CDC from clinicians through the State Health Department arrive in our lab. We do, Carlos, as you mentioned, conduct diagnostic studies for entero, rhinoviruses and then, if those are positive, we move forward with typing of those enteroviruses to determine which types are present in the specimens of our patients with AFM or suspected AFM.

[00:43:41] The specimens then are stored in our laboratory and are available. We have had two great collaborations, one with Nischay's lab and then another out in the University of California at San Francisco to provide specimens, including CSF specimens, which are sort of the more valuable of the specimens that come in to us to use in their assays. And are happy to discuss additional collaborations with those specimens moving forward.

[00:44:16] So, it's a matter of getting in touch with us here at CDC and discussing that collaboration. As Dr. Yee mentioned, we are moving forward with the AFM Biorepository that is going to collect additional specimens to be banked. And those specimens then will be combined with the NIH Biorepository into a common biorepository that will be available to researchers moving forward.

Dr. Carlos Pardo: [00:44:47] Thank you, Janell. And there is one question from the audience about what we are learning about the prognosis for children who are diagnosed with AFM and if there is hope for improvement at 2, 5, 10 years out of the diagnosis? And actually, I will take the lead for answering that question. Our experience is there is very good evidence that the rate of recovery, it is much better in children when they are experiencing myelitis as compared with adults.

[00:45:22] There is one thing in children that is called synaptic plasticity and neurological plasticity that allow children to have a better recovery after injury of the central nervous system, either in the brain or either in the spinal cord. And AFM is another example of that. We have observed in the past several years, in our rehabilitation centers, that many kids affected by AFM are left with significant disability.

[00:45:50] But that doesn't mean that there is no hope for recovery. And we recently learned from one of our patients, for example, that was ventilator dependent, that, after almost two years, to be ventilator dependent with the intervention, rehabilitation, and effort, actually this patient is having a better ability to breathe himself.

[00:46:12] So, it's extremely important to understand that the process of rehabilitation is not only the medical intervention, but it's also intervention of the rehabilitation team and the physical therapist, occupational therapist, and the whole team that is participating in the recovery of these patients. So, this is a very important question because there is always hope if there is resilience and there is effort in improving all our patients with AFM, we will have much better outcomes in the future.

[00:46:43] I would like to ask our four participants, just briefly, is what is your wish and your main message for our audience, parents, particularly, and family members about the research on AFM? Amy, let me start with you. What is your main message?

Dr. Amy Rosenfeld: [00:47:06] My main message is that basic science is going to lead us out of this. It's about understanding how the body works, how the virus interacts with the body, and one should never under appreciate basic science, because this is going to tell us what we need to do.

[00:47:28] And it's going to reveal interactions that we did not know about or underappreciated. So, how the respiratory system is innervated and how that can lead to motor neuron disease, where upper extremities are impaired, and how the immune system responds to that and interplays with dissemination or intervention of the virus.

[00:47:53] And so it's, research is difficult. You're going to encounter a lot of failures, but you cannot give up. And so, I'm not giving up, so they shouldn't give up in the basic science because that's what, those risky experiments are going to lead us out of this.

Dr. Carlos Pardo: [00:48:13] So, Amy is telling us that scientists have resilience as well. Thank you. And Nischay, what is your main message?

Dr. Nischay Mishra: [00:48:22] My first main message is, like, I'm happy and I think we are fortunate we didn't see any cases of AFM last two years, and I hope it remains continuing, like, that way. But even that case, if anything happened then, like, all the efforts, like, all the parents and guardians who are listening to us, if they feel, and they get to know about any case like that, either from the community or anybody else, they should spread the message.

[00:48:48] People should reach out to us or Dr. Pardo in some other way so we can detect the problem properly. And like Amy said, yes, of course, the basic science has always been the backbone of doing things and using these, like, new modern methods, high throughput data, and machine learning, we have been doing things which were not possible maybe five, ten years ago.

[00:49:11] We have seen last year how quickly we could lead to the diagnostic as well as, like, therapy, as well as the vaccine for COVID-19. And I think that's a template for us, for other emerging diseases and infectious diseases, just we can work together, clinicians, parents, scientists, and the manufacturers, and the biotechnology, and the pharmaceutical group. We can resolve any issue if we work together and look at that and in a timely manner.

Dr. Carlos Pardo: [00:49:41] Thank you, Nischay. Eileen, what is your main message?

Dr. Eileen Yee: [00:49:44] Sure. I would just go along with, in terms of, for the parents and clinicians, to recognize AFM. And once you do to please hospitalize and report. And, because it's uncommon, that this is why these biorepositories are so invaluable and that we would encourage everyone to be able to participate, if possible.

Dr. Carlos Pardo: [00:50:08] Thank you, Eileen. Janell, what is your main message and final message for the audience?

Dr. Janell Routh: [00:50:14] Thanks, Carlos. I feel like it's echoing a lot of what Nischay and Amy just mentioned. I look around this panel today and I see representation from basic science, from you as a clinician, from Eileen and I here at CDC. And it reminds me that answers to this very complex illness are going to come from all of us, that it really is going to take each one of us playing our role to find the answers to some of the puzzling questions that are still out there about AFM.

[00:50:50] And it makes me realize how far we've come since 2018, our last big surge in AFM cases. I feel like we've really come together as a community, including SRNA and the parents, to really form a tight community that is working across all aspects of AFM, raising awareness, as Eileen mentioned, and then certainly the basic science research that Amy and Nischay are conducting. I'm really looking forward to seeing what we can find out in the next couple of years, because we certainly have come a long way.

Dr. Carlos Pardo: [00:51:25] Thank you, Janell. And my final message for all of you is the only way to go and take a very deep understanding of AFM is the spirit of collaboration. We are not able to tackle AFM if we don't have a good partnership with families, with the patients, with the healthcare providers, the scientists that are participating in the research, basic science research in the lab, and the epidemiologists that are looking

for the risk factors associated with these, the clinicians that are taking care of the patient, the therapists. So, this is a team effort.

[00:52:01] So, the main message is we need to keep working together with the help of the SRNA, we have a good group, the Acute Flaccid Myelitis Association, the parents that are part of the Acute Flaccid Myelitis Association, the scientists that are part of the Acute Flaccid Myelitis Working Group, and many other people are working together just trying to understand AFM.

[00:52:25] So, the main message is let's keep working together and participating in this important effort to help our patients and families. Thank you so much for being available this morning and thank you, Roberta, for allowing us to discuss about this important issue.

Roberta Pesce: [00:52:40] Well, yeah. Thank you all so much. We really appreciate your time. It's great to see so many experts getting together and discussing such an important topic. So, thank you very much, all.