A Case for Early Intervention in *P. aeruginosa* Eradication

TRANSCRIPT

Segments

Segment 1

DR. PETER MOGAYZEL: Welcome, I'm Peter Mogayzel, director of the Cystic Fibrosis Center at Johns Hopkins, and I want to welcome you to a program about pseudomonas eradication. We're going to introduce you to Daniel and talk about pseudomonas eradication in various ways, through videos and discussions from the panel, and also with questions and answers from you as well.

This program is accredited for CME for both physicians and nurses. There's an hour and a half of accreditation through Johns Hopkins Hospital and Johns Hopkins University.

The learning objectives for this course are to:

- Evaluate the pros and cons of P. aeruginosa eradication.
- Summarize the current evidence and expert opinion informing eradication best practices.
- Discuss key data from significant eradication trials including ELITE and EPIC, and ALPINE.
- Integrate evidence-based strategies to assess and improve eradication in the early stages of P. aeruginosa eradication.

This program complies with HIPAA regulations in the United States. The faculty disclosures for the program directors are here, Dr. Boyle's disclosures. And the program, as I said, is accredited by Johns Hopkins Medicine and Johns Hopkins Nursing, the logistics of the program were put together by DKBmed.

This is supported by an educational grant from Gilead, but Gilead had no input into the development of the program or the presentations.

So let's start off by meeting Daniel. You may recognize some of the other players here, but let me introduce you to Daniel and we can talk about him throughout the program.

DR. MOGAYZEL: This is Marianne and her three-year-old son, Daniel. Daniel has been my patient since he was an infant. His CF was diagnosed by newborn screening, he has two copies of the F508del CFTR mutations. Overall he's been doing well.

Today is not Daniel's regularly scheduled appointment. I've asked Marianne to bring him in sooner because his last airway culture grew Pseudomonas aeruginosa.

MARIANNE: How did he get this infection?

DR. MOGAYZEL: We don't know exactly where he got the pseudomonas. It's very common in the environment; about 80 percent of kids or adults with CF will eventually become colonized with pseudomonas. Often we find it on routine cultures when you come to clinic.

MARIANNE: How do we know Daniel has it?

DR. MOGAYZEL: As you know, we get a culture at every visit, and the last throat culture that he had grew pseudomonas.

MARIANNE: You said that it was in his throat, but isn't pseudomonas a lung infection?

DR. MOGAYZEL: You're right, we do a throat culture at every visit, and the culture that we did the last clinic visit grew pseudomonas. You can't be 100 percent sure that it's in his lungs because a throat culture is not a perfect representation of what's growing in his lungs, but it gives us a good idea of what's growing there.

MARIANNE: Should we do more testing?

DR. MOGAYZEL: You could do additional tests, we could do a bronchoscopy to look down in his lungs and get cultures of the mucus from his lungs and see if the pseudomonas is there, but that's an invasive procedure and since he's not really having any symptoms I'd prefer not to do that at this point.

MARIANNE: But he doesn't seem sick.

DR. MOGAYZEL: He doesn't, and that's a good thing. The fact that he doesn't have any symptoms means it's more likely that we can eradicate the bacteria. By treating the pseudomonas early we have a better chance of getting rid of it. As pseudomonas stays in the lung it develops a biofilm, a coating, so we'll try to get rid of it by using inhaled antibiotics.

MARIANNE: What do you mean try?

DR. MOGAYZEL: Pseudomonas is not always cleared when we do therapy so it's possible that we won't be able to get rid of it, but often we do. After eradication though the pseudomonas can come back again and often does.

MARIANNE: If it will come back, why begin treatment now?

DR. MOGAYZEL: It's important to treat early. There's a lot of research telling us that the longer we can put off chronic infection, the healthier his lungs will be. So it's important to try to treat now, get rid of the infection, and prevent it from coming back in the future. So the first question we need to ask is, should we do eradication therapy.

(End video)

DR. MOGAYZEL: I'd like to introduce Margaret Rosenfeld who is going to be our first speaker.

Segment 2

DR. MARGARET ROSENFELD: Good evening, thanks for coming. I'm Margaret Rosenfeld from the University of Washington School of Medicine and Seattle Children's Hospital in Seattle. I'm going to be speaking for the next 15 minutes or so on a clinical perspective on why eradication is important.

The learning objectives of my talk are:

- Describe the importance of early detection of P. aeruginosa infection.
- Describe the rationale for eradication therapy for newly acquired P. aeruginosa infection.
- Describe the accuracy of oropharyngeal cultures compared to cultures obtained by bronchoscopy for identifying P. aeruginosa infection.

Let’s start with some background about P. aeruginosa infection in cystic fibrosis. As all of us in this room are aware, it is the sentinel pathogen in cystic fibrosis, even though there are other important players and communities of organisms. About 80 percent of adults in the United States are chronically colonized with pseudomonas, and a particularly chronic infection is clearly associated with more rapid lung function decline and chest x-ray score decline, poor nutritional status, more frequent hospitalizations and ultimately poor survival.

Pseudomonas is, in general, initially acquired form the environment, as opposed to patient-to-patient transmission. The environmental strains presumably enter the host through the lower airways by inhalation or perhaps from upper airway reservoirs like the sciences. Initial pseudomonas isolates are typically nonmucoid, are present in relatively low density, and are highly antibiotic-sensitive which offers a window of opportunity to eradicate these infections before they become chronic. Therefore current guidelines of care emphasize early detection and antibiotic treatment of earlier initial pseudomonas infection.

Pseudomonas is acquired in childhood at a rate of about 16 percent per year. In other words, you have about a 16 percent risk of acquiring pseudomonas each year of life in the first six or so years of life. Relatively few risk factors have been identified, so high risk CFTR functional class puts you at a greater risk for earlier acquisition that includes F508del and living in warmer and wetter climates, as well; not a lot of other risk factors are known, though.

As opposed to chronic infection, initial infection is certainly not associated with overt changes in clinical status. There’s not a dramatic drop in lung function or a clear change in height or weight, or for that matter, generally symptoms when pseudomonas is initially acquired. However, after initial pseudomonas acquisition there is a greater likelihood of hospitalizations as compared to prior to acquisition. In the preeradication era, pseudomonas isolation before the age of five was associated with poor eight-year survival, but that study is from the 1990s and in the pseudomonas eradication era that has not been repeated.

As you’re probably well aware, there is a transition from initial to chronic infection that generally progresses over a period of years, and both host and pathogen characteristics promote the transition to chronic infection. In terms of host factors, the dehydrated airway surface liquid and abnormal mucociliary clearance that are the hallmarks of cystic fibrosis make it easier for the bacteria to persist in the airway. There is also impaired function of antimicrobial peptides, the innate immune system, and neutrophilic inflammation which try to clear the pathogen but also do some collateral damage to the airways, causing bronchiectasis and then setting up for poor mucociliary clearance in turn, so there’s a vicious cycle.

Pseudomonas has multiple mechanisms to adapt to the CF airway milieu and lead to chronic airway infection

including, as was already mentioned, biofilm formation, so the communities of pseudomonas, and in fact, other organisms as well, can begin existing in these structured communities; they're encased in an alginate matrix. There is also development of the mucoid phenotype, and both of these lead to increased antibiotic resistance. Therefore, chronic pseudomonas infection is extremely difficult to eradicate with antibiotics.

To review the stages of pseudomonas infection, at first acquisition we now recommend an early antipseudomonal eradication treatment regimen, and if the eradication is successful, the patient develops a pseudomonas-free state again, but as Dr. Mogayzel mentioned in the video, is likely at some point to become infected a second time and then more treatment is offered, hopefully leading to successful eradication again, but again the pseudomonas is likely to come back. And since the standard of care in the United States has been to offer aggressive antipseudomonal antibiotics at the time of isolation, we don't have a good idea of how long this pattern goes on before there may be chronic infection; that's a subject of some ongoing research. But unfortunately it can also lead to eradication failure at any point in time, and then the individual is at much higher risk for chronic infection, which again tends to happen over years.

Let's discuss how to detect pseudomonas infection, which Daniel's mother asks some very intelligent questions about, is not straightforward at all. Detection of infection in patients such as Daniel is quite challenging because, of course, these young patients typically do not expectorate sputum. So that we are relying on oropharyngeal swabs or else more invasive tests such as bronchoalveolar lavage, each of which has distinct advantages and distinct disadvantages.

In the United States, oropharyngeal swabs are the usual source of microbiologic specimens, and they're recommended at least quarterly. Although the diagnostic accuracy for oropharyngeal cultures compared to lower airway cultures is not great, there may be some importance of isolating pseudomonas from the upper airway in its own right, as it may serve as the reservoir for lower airway infection.

Our group and others have looked at the diagnostic accuracy of oropharyngeal cultures compared to bronchoalveolar lavage obtained at the same time. Dr. Wainwright's group has also done this. We've all found similar things, which is basically a higher specificity and a higher negative predictive value than sensitivity or positive predictive value.

To put this in plain English, if you do not isolate pseudomonas from the upper airway, it's relatively likely not to be in the lower airway, but if you do isolate it from the upper airway that's no guarantee that it is also in the lower airway.

Let's move to antibiotic treatment of early pseudomonas infection. The objective of such treatment is to eradicate pseudomonas while it's still antibiotic-susceptible and present at low density. We talked about the window of

opportunity to treat early because of characteristics of early pseudomonas isolates and once the infection becomes chronic it's difficult or impossible to eradicate.

The Copenhagen CF Clinic back in the 1980s originally proposed the strategy that's now become the standard of care in most countries. However, as we'll be discussing, there is not universal consensus on the most appropriate protocols to use.

Dr. Tiddens will be going into more detail on this, but approaches have definitely included inhaled, oral, and IV antibiotics alone or in combination and for various lengths of time as well. In general, these regimens have shown relatively similar and relatively high eradication rates, though again Dr. Tiddens will be going into more detail there.

The clinical efficacy is distinctly more difficult to evaluate than the microbiologic outcomes. Unfortunately it's somewhat difficult to compare results across studies because of different eligibility criteria, different endpoints, and in particular different definitions of eradication success or failure.

Two relatively recent consensus statements have been issued, one from the European group and one from the Cystic Fibrosis Foundation here in the United States. I won't read those to you, but both definitely recommend tobramycin solution for inhalation as first line therapy for initial or early pseudomonas infection.

In summary, tobramycin is the most widely recommended treatment, but the optimal regimen is still far from known. Eradication success is generally high, but there is about a 20 percent failure rate. We may need personalized approaches based on a risk factor profile. Despite eradication of pseudomonas in most cases, we still definitely see bronchiectasis, air trapping, and abnormal lung function in young children. So what's going on there if we're eradicating the bacteria? Is it due to inflammation associated with the bacteria that's not turned off, is there a role of this whole community of microorganisms that we're coming to call the microbiota, what are the other things that we're not successfully treating with antibiotics?

Thank you.

Segment 3

DR. MOGAYZEL: We've talked about the benefits of eradication therapy, but I want to make sure you understand what it entails. My plan would be to have Daniel get inhaled antibiotics. Inhaled antibiotics aren't approved for kids less than six, but we use them frequently even in little babies. The goal is to get as much antibiotic, as much medicine into the lungs as possible, and this is the best way to do that.

So there are two antibiotics that we could use that are inhaled. We use inhaled tobramycin, which is a medicine that's recommended by the Cystic Fibrosis Foundation guidelines.

MARIANNE: Are there side effects?

DR. MOGAYZEL: There can be. Some kids cough with the therapy and can have a hoarse voice. Children that tend to wheeze can wheeze with antibiotic therapy; however, many kids like Daniel tolerate the antibiotics without many problems.

MARIANNE: Is the nebulizer difficult to use?

DR. MOGAYZEL: It's not difficult to use. It may be a little bit different from one you've used in the past, but I'm going to have Cherie, our respiratory therapist, come in and go over it with you.

Can you take a big breath? Can you do it again? Good job. So can you do that when you're doing your treatment, I bet you can. That's very good.

CHERIE: The most important thing is that you keep everything clean. The treatment itself lasts about 15 minutes, but setting it up and cleaning it at the end will take a little bit more time. The first thing you're going to do is take the nebulizer cap off, take the ampoule of medication, make sure you squeeze all the medication out and the ampoule is completely empty. It is also important that you keep the lid very tight and secure before the treatment.

MARIANNE: Okay.

DR. MOGAYZEL: To get the most medicine into his lungs, Daniel needs to breathe through his mouth. He'll need to use a tight fitting mask and you'll need to get him to sit quietly and breathe deeply for about 15 minutes. The nebulizer will make a sputtering sound when the medicine's gone.

CHERIE: After the treatment is finished, you will need to clean all the parts. You can clean them with warm, soapy water — dishwashing soap is good. At the end of each treatment, you'll want to disinfect all the parts. The best way to do that would be to boil them for five minutes with distilled water, just make sure all the parts are dry before you put them away.

DR. MOGAYZEL: It's not all that complicated, but it is time-consuming. But inhaled antibiotics are the best therapy for both pseudomonas eradication and for chronic therapy.

MARIANNE: I think we can handle it. Right, Daniel?

(End video)

DR. HARM TIDDENS: So good evening, I'm Harm Tiddens, I'm from Rotterdam, Netherlands, and I thank the organizers for having me here. I'm going to address the treatment options. What are the approaches for treating Daniel? My learning objectives:

- Describe effective approaches to eradication therapy for newly acquired P. aeruginosa infection.
- Describe advantages and disadvantages of various P. aeruginosa eradication strategies.
- Describe the importance of adherence and proper administration

technique in the success of P. aeruginosa eradication therapy.

What do I consider when I see pseudomonas in my outpatient clinic and when a patient like Daniel comes in at age, three years? Cooperative or noncooperative? Socioeconomic factors, of course, will be of different importance in some countries. What is the history of pseudomonas infection in this boy? What is the phenotype of the pseudomonas? Is it mucoid, like what you see on this plate over here, and what is the evidence out there in the literature on the efficacy of various treatments?

I'm not so confident about this, I must say, because all those studies have different details. But I want to point out some what I think are relevant issues.

I was very skeptical that aerosol could eradicate pseudomonas, but then the study by Ron Gibson came, where young kids who had an infection had a lavage to prove that they had a lower airway infection with pseudomonas and then were treated for one month with twice daily inhaled tobramycin, 300 mg. He succeeded in eradicating 63 percent, and that was established at eight weeks after treatment was completed.

Next is the ELITE study, and again patients were randomized to 28 days or 56 days of tobramycin treatment. And what is important in understanding the study results is that patients who had positive antibodies were excluded from randomization. So one quarter of patients were not included in the randomization process.

But if you take those patients who were randomized at four weeks after treatment, 92 percent cleared. If you now take the patients who, and for the endpoint they included patients who four weeks after completion of treatment didn't have pseudomonas and those were followed up, and those were 65 patients, and they, of those patients, at 12 weeks after completion of treatment, 86 percent cleared pseudomonas.

Then there's the EPIC study. It's a complicated study, but what was important is that in two arms, tobramycin solution was compared to tobramycin plus ciprofloxacin. In that large study, at 10 weeks, 87 percent of patients cleared pseudomonas.

I think what is an important message of this summary is, first, how they determined successful eradication. Is it one month after completion of treatment, is it three months? What are your inclusion criteria, and what patients have we studied over there?

The next list shows three more studies by Taccetti comparing one month of tobramycin with ciprofloxacin versus colistin one month with ciprofloxacin. About half of the patients included were children below age six, in Daniel's age range. In this study there was 66 percent eradication.

There's the study from our Belgian colleagues, who compared tobramycin versus colistin plus ciprofloxacin and the latter was given for three months. In that study, depending on the duration at which you evaluated eradication, it varied between 44 and 65 percent.

The ALPINE study, which was recently published, investigated the efficacy of three times daily aztreonam nebulization. In that study 47 percent were children, and at 28 weeks, 56 or 58 percent cleared pseudomonas.

What's important in this study is that patients were included independent of their pseudomonas antibody status. If just focus on patients who had negative pseudomonas antibody, the clearance rate is in exactly the same as in the ELITE study. So I will say that the numbers are pretty equal between all those studies.

This is another study by Ron Gibson, where he did a lavage at baseline, which shows the discrepancy between what you culture in the oropharynx and what you culture at the lavage, because all patients had a positive culture result from the lavage, but the oropharyngeal culture was negative in two patients.

At day 84 this cohort again had a lavage, and at that time four patients didn't clear. Importantly, two patients have the mucoid strain and they didn't clear. And we know that the mucoid strains are difficult to eradicate.

In the next study, I tried to understand how to compare those studies. I think they should be depicted in the way shown here in the study from Belgium, to show a pseudomonas-free survival curve, and comparing the two regimens. From this curve you can understand that at time zero all patients had cleared, this is after treatment, patients who cleared the pseudomonas, and you see the survival curve for patients, how long they remained free of pseudomonas.

The big change is especially in the first months after you complete your treatment. I think this kind of analysis helps you to understand what happens in such a cohort.

This is some more detail about the ALPINE study. Let's see form this study what we can expect for Daniel. The ages studied are two to six years. At week 16, 62 percent cleared the pseudomonas. What's important here, there doesn't seem to be a very big difference between the age categories and your chances of success or failure.

This was his first pseudomonas infection, which means he has a 66 percent chance of clearing. If it were a recurrent infection, the chances of clearing are lower.

The baseline culture in patients who had in the study two consecutive positive cultures within a short time frame, the chance of clearing is lower. It might represent a higher bacterial load, so the chance of culturing positive twice is higher.

With nonmucoid strains, the chance of clearing is higher. What's surprising in the ALPINE study, it's only one observation of a patient who cleared a mucoid strain. And we don't know anything about Daniel's antibody status.

We know that a history of positive pseudomonas lowers the chances of clearing the pseudomonas. Elevated antibodies are the same: two positive cultures within a short time lower the chance of clearing. A mucoid strain also lowers the chance of clearing.

What we don't know, what has not been properly investigated, is what are the chances equal of clearing the pseudomonas, especially in very young kids? What about severe structural lung disease? What about the distribution of disease, central versus small airway disease? What about the concentration of the inhaled antibiotics in diseased areas? I'm going to explain this a little more. Here is the treatment for inhalation competence, uncooperative character, and poor socioeconomic conditions.

So in a nutshell, age. On the left you see the bronchial tree of an adult, and there are particles of 5, 3, and 1 microns. The large particle has the high probability of being deposited near the bifurcation of the central airways, where air flow is turbulent. The smaller particles have a higher probability of making it all the way down to the smaller airways.

On the right is the bronchial tree of a healthy child in the middle, and there also the 3 micron particle has a higher probability of being deposited, because the airways are narrower and the air flows are higher in children. Of course, we don't talk about treating healthy children; we talk about children who have inflamed airways and mucus obstructions in the airways. In these children the smaller particles have a higher probability of being deposited centrally.

This is important, because if you look at these two CTs, on the left is a child who is doing quite well. Here is quite a normal CT at age two and on the right side, a two-year-old child who has pseudomonas, severe bronchiectasis, mucus impaction, and large areas showing small airway disease.

So there is wide heterogeneity in structural abnormalities of the patients even at that age range. And that is shown in this next slide where we compute the lung volume, which shows abnormal features, in this case bronchiectasis, mucus plugging, and bronchial wall thickening.

On the X axis you see the percentage of the lung which has these abnormal features, and along the Y axis you see the number of patients who had that percentage, the frequency distribution.

So there are patients who hardly have any damage, where on the right side you have patients, 60 percent of the lung is already showing quite some damage. So heterogeneity.

Also, in the small airways, on the expiratory scan we compute the volume of what is called trapped air, representing small airway disease. Again, there is a wide distribution of severity, patients with 1 percent trapped air, while on the other hand there are patients with 60 percent trapped air.

So why is that important? If you inhale your medication and you're healthy you have a homogeneous distribution of your inhaled aerosol as you see on the left side, while if you have diseased lung there will be a preferential flow to the better persevered areas thanks to the increased airway resistance in diseased areas and this slower expansion of such

areas, and also increased deposition at the sites of obstructions.

If you want to read more about it, this paper is in press and will be available soon.

What about distribution of inhaled antibiotic? Well in the literature you read a lot about the high concentrations in sputum, but you have to remember that that represents central airway deposition.

The microorganisms are distributed homogeneously, and may be even move prevalent in the small airways. So there is a concentration gradient of the antibiotic going from high in the central airways to lower in the smaller airways. That leaves us with a number of questions: what are the concentrations in the small airways; what is the impact of breathing pattern on how much of the drug gets there; what is the influence of particle size; what is the influence of structural lung changes?

We investigated this in a study in our patients because we wanted to model what were the concentrations of, in this, case aztreonam. We wanted to investigate its relation to the concentrations throughout the bronchial tree to see whether it was affected by structural changes. We used computational fluid dynamics on the scans routinely made in our patients, inspiratory, expiratory; I will not go into details.

These are the results. On the left is a color-coded map of the concentrations throughout the reconstructed bronchial tree of the patient. Everything above yellow is adequate concentration.

In the central airways the concentrations are okay, while in the small airways are still in the yellow range, so it's still okay. For these patients with more structural disease there is a very high concentration in central airways and more in homogeneous pattern in the segmental bronchi, and the smaller airways have insufficient distribution of the drug.

From this study we learned that in most lobes the concentrations are okay, especially the lower lobes, but in the upper lobes the concentrations are lower. For a modeling study, if you model to worst case scenario, up to 28 percent of lobes would receive insufficient drug. So this seems to play a role that we have to keep in mind.

To summarize, the success rate of eradiation varies widely, 44 to 92 percent depending on the inclusion criteria and on the criteria for eradication. We know the success rates are lower when we have two or more positive cultures before starting therapy, positive antibodies, and mucoid phenotype, as well as other risks of failure like poor adherence, poor inhalation competence, severe structural lung disease, and an uncooperative child.

I hope I made clear that if a patient has already had one episode of pseudomonas, chance of eradication will be lower. Thank you very much.

Segment 4

DONNA PEELER: Hello, my name is Donna Peeler. I am the pediatric clinic nurse coordinator at Hopkins, and I have worked with patients for many years and I'm glad to see a lot of nurses here. Because I think what the physicians have been presenting, they often hand off that baton to us. They have given the families the information about pseudomonas and what the treatment plan is, and then we are to help them carry it out and figure out how that we're going to do this.

My learning objectives are:

- Explain the rationale for P. aeruginosa eradication protocol to patient and family.
- Explain medication side effects, order of mediations and equipment cleaning and disinfection with patient and family.

The ideal setting for communicating with patients about pseudomonas is in the clinic, but often they may be getting a phone call from the physician because the culture result has come in after they've left clinic. Then I will follow up with them, and usually, if there has been too much time in between conversations I get a lot of questions after they have investigated what pseudomonas is and have some concerns. Cultures are routine, but it is never a routine phone call when you have to call a family, whether it's a young infant, a toddler, or a teenager to tell them that this is their first positive culture for pseudomonas.

If I'm talking to a younger patient and their family and they have never done nebulizer treatments, we're starting from ground zero ordering equipment, making sure they have the proper compressor, nebulizer cub, proper fitting of the mask. If they have done albuterol treatments or something like that in the past then I ask them to show me how they've been doing it, because a lot of them do blow by. So we reteach them how to do proper nebulizer technique with inhaled antibiotics.

Our older patients may have already been on nebulizer treatments, so the emphasis is on their technique. We check the order of their medications and make sure they understand what each medication is for, in conjunction with their airway clearance. For them this is obviously an extra treatment burden.

I go through some of the medication side effects, what they can expect, what things they should alert us to, and what things we are not concerned about. Some of them may need help connecting with patient assistance programs, so we help with that and make sure no prior authorization is needed.

For some of the younger patients, as we saw in some of these videos, is you do not want to force a treatment. You don't want to do it during a time when they're tired or hungry. I know many parents have told me that sometimes they wait until the babies fall asleep, and that's not ideal either. Or for the ones that are running around, the parents will try to confine them to some kind of stationary

chair where they can entertain them and keep them still long enough to do treatments.

But the key element for all ages is supervision. Obviously the younger ones need hands on, but for younger teenagers I'm going to start recommending hidden cameras. While they're very autonomous doing their independent treatments, parents could find out a lot about how they're doing their treatment. It's been proven that if a parent is just in the next room, not even directly standing there watching them use the nebulizer, it makes a big difference in their technique and in their adherence.

Finally, and of utmost importance, we emphasize cleaning and disinfection and the difference between the two, replacement of nebulizer cups and filters, and use and care of the compressor.

We use a lot of handouts. There's some wonderful website guidelines to reinforce this, but there is also sometimes a gap between the time that we go over a lot of this and they get their medication. So sometimes they're coming in after they've gotten their medication and we can reinforce some of the teachings hands on.

Thank you.

Segment 5

DR. MOGAYZEL: We attempted pseudomonas eradication with Daniel using inhaled tobramycin. He tolerated the therapy well, and so did Marianne, his mother. She developed a system for treating him, cleaning, and disinfecting that worked for her, and after 28 days of treatment a follow-up throat culture did not grow Pseudomonas aeruginosa; however, his routine throat culture six months after that found that he was infected with pseudomonas again. I've asked Marianne to bring Daniel back in today to discuss our options for next steps.

MARIANNE: We did everything we were supposed to do. Why did the infection come back?

DR. MOGAYZEL: We're not entirely sure. It could be the eradication didn't work, or we failed to clear the bacteria the first time because of resistance, or it may be that the eradication did work, the pseudomonas went away, and now he's infected with a new strain of pseudomonas.

So we have a few choices at this point. We could do another treatment with inhaled tobramycin alone, or we could add a second antibiotic, an oral antibiotic like ciprofloxacin, so you do tobramycin and ciprofloxacin. It's also possible he's actually chronically infected with pseudomonas, and we should start chronic therapy where we alternate an inhaled antibiotic every other month.

MARIANNE: For how long?

DR. MOGAYZEL: If that's the same it would be indefinite. It might be a lifelong therapy. I'm not quite ready to commit him to that though, I think we should try eradication therapy again but this time use a different antibiotic. There's

another antibiotic we could use that's called inhaled aztreonam. It takes about two or three minutes to administer and it's done three times a day instead of twice a day the way tobramycin is. There's data showing that it's effective as well for eradication therapy.

The side effects are similar to what you see with inhaled tobramycin, things like coughing, sore throat, and occasionally wheezing. There are some more rare side effects that we could talk about as well.

MARIANNE: Is the same nebulizer used?

DR. MOGAYZEL: It uses a different nebulizer that's specially made for aztreonam. It takes about two to three minute to administer the medicine three times a day, and Cherie can come in and show you how to use it.

CHERIE: Now this nebulizer is a little different. When you take the lid off, make sure you don't touch any parts inside the reservoir, because it could clog up the head. The medication comes in two parts. The first part has the powdered medication down in the bottle, which has a metal tab you'll need to pull off, and then take off the rubber top. Then take one of the saline vials, squeeze all the saline down into the vial, put the lid back on, the cap back on, and gently swirl the medication. Once you see that all the powder's dissolved, then take the rubber cap off, open up the nebulizer lid, and pour the medication down in the reservoir. Once you get the medication into the reservoir, make sure the cap is back on tight.

After each treatment, clean all the parts of the nebulizer with some warm water and some dishwashing soap, and then disinfect all the parts. The best way to do that would be to boil them for five minutes with distilled water and then once they're dry then you can put them back on the container until the next use.

DR. MOGAYZEL: So are we in agreement that we should go forward with the aztreonam therapy for eradication?

MARIANNE: I'd like some time to think about it and talk to my husband.

DR. MOGAYZEL: Sure, that would be fine.

(End video)

DR. CLAIRE WAINWRIGHT: Well good evening, I'm Claire Wainwright and I'm from the Royal Children's Hospital in Brisbane and I'm going to talk to you today about what to do when reinfection occurs.

The learning objectives for this section are to:

- Identify risk factors for recurrent P. aeruginosa infection.
- Describe the approaches for treatment of recurrent P. aeruginosa infection
- Describe methods used to define chronic P. aeruginosa infection.

I'm going to start with this slide, which shows that the infection can clear and then start treatment again, but at each infection, there's a chance the infection

might not clear, in which case it's regarded as chronically infected. The problem we've all got is exactly how to define that.

Not many multisite, longitudinal studies have followed children from birth or looked at the prevalence of reinfection. The prevalence of reinfection is likely to depend on a number of things: the time you monitor; the treatment received for the initial infection; the timing of the treatment that was given; adherence, et cetera.

For example, as you saw in the previous presentations, if you don't take the treatment, it won't work because it's not a good luck charm. It depends on how you had it, and it may also depend on other treatments that you're getting. There's a very interesting abstract to this meeting, looking at inhaled corticosteroids, for example, and the risks of infection with pseudomonas. We think that staph prophylaxis may also increase risks. Geographical site may be important, particularly places with warmer climates.

And then there's the type of sample collections. There may be differences between upper airway collection and BAL. What about the frequency of sampling? If you only do a BAL once a year, you're clearly only going to find your pseudomonas once a year, so it's going to affect the frequency that you find it.

Then there are definitions. So if you are doing a BAL, how much pseudomonas is important, 1 pseudomonas, 10, 100, 1,000? where do you draw the line?

What about age? We talked a little bit about age, I suspect age is important, but we really don't know very much about it.

So let's look at the couple of studies that have looked over time at children in the same age group as Daniel. Let's look first of all at the EPIC protocol study, which compared historical data from the epidemiological study of cystic fibrosis — and this was large numbers here — and the EPIC trial. The mean age here was 5-1/2 years, so the average age was a little older than Daniel, but it did include infants right down to the first few months.

The length of follow-up of this study was 80 weeks, so around 1-1/2 years. And 35 percent of children in the EPIC study and 54 percent in the historical cohort had pseudomonas recurrence. There are differences between those two studies that may be related. In the historical cohort study the treatment protocols were less regimented and there was less early treatment compared to the EPIC study.

The problem here is that only OP cultures were collected and no genotype was collected on the samples. So we don't have a good idea whether the recurrence was caused by the same genotype. On top of that, there was some inconsistency in OP cultures in the historical group.

This study was a clinical trial across Australasia in which children were randomized to either have BAL or OP cultures. They were randomized in the first few months of life and they were managed right the way through to the

age of five. They all had OP cultures and half of the cohort were randomized to BAL and half to the OP cultures throughout the study, but at the end of the study at age five they all had a BAL.

In the study, 82 out of the 157 children who were followed to age five acquired pseudomonas in the first five years of life, and 44 percent or 36 children reacquired pseudomonas in that time. There was an average of 2.8 years of observation past the first acquisition.

Many single site, smaller studies have looked at the prevalence of reinfection. If you look through these studies, you've got smaller numbers, they're single site and various ages are included. In most of the studies the children are quite a bit older, so not quite so relevant to Daniel. But you'll see that the rates of reinfection vary from 100 percent from the middle study in this slide, the Munck study, to around 51 percent. So it's quite variable.

The best estimate of prevalence of reinfection with pseudomonas in young, preschool children, I think would be between 35 and 44 percent of children who receive prompt initial treatment over the next two to three years, and that's based on two of the largest studies, the EPIC study and the ACFBAL study.

Now we get to the next question, is it reinfection or is it treatment failure? The only way we'll know that is to genotype the samples, because if it's a different genotype then it's more likely to be a reinfection, and if it's the same genotype it doesn't exclude the fact that you could have been reinfected with the same genotype. But certainly you can exclude the persistence of infection if it is a different genotype.

What about the site of sample collection? Well let's look at site and genotype. In the Munck study they used either sputum or a catheter passed through the nose to the laryngeal aperture. There are two different sample types in this study. Fourteen of 19 acquired a new genotype. The other study looked at a smaller number of patients who had chronic infection. Ten of the 11 had identical pseudomonas genotypes, so almost all had the same genotype. But seven of the 14 who didn't become chronically infected had an identical genotype. So it increases the risk, but it's quite difficult to be sure.

Going back to the ACFBAL study, what about the BAL group? Let's look at these. Thirty-nine of 79 children who were randomized to this group had cultured pseudomonas in the BAL. We had a cutoff at what we thought was a significant amount of pseudomonas, and we decided that was 1,000 colony forming units/mL, but we may or may not have been right about that.

Now 11 percent, nine of the 79, had pseudomonas cultured in the OP but not in the BAL. So as was pointed out before, the OP culture may overrepresent what's going on in the tract. But at the end of the five years, only one child we thought had chronic pseudomonas infection on BAL cultured pseudomonas at the age of five. So in the BAL study we accurately predicted that child would have chronic infection.

In the standard group we did OP cultures and no BALs until the age of five. In this group, 43 of 76 children, 57 percent in the standard group, had pseudomonas cultured. Two of the 43 had pseudomonas cultured at age five, having cleared the infection previously on OP, and they had the same genotype in the BAL at age five as in the OP culture early on. Was that reinfection, was that persistent infection, were they chronically infected? We don't know. We'd called them not chronically infected because they didn't have pseudomonas on repeat cultures throughout. We also had four children who had chronically cultured pseudomonas on their OP cultures, but none of those children had it in the BAL at the end.

So there's an importance to the site of collection, serial pseudomonas BAL cultures mostly had different genotypes. Twelve of 14 children had different genotypes, however in the serial OP cultures, only three of 11 children had different genotypes. So genotype substitutions were more frequent among isolates from BAL than from OP cultures, and that was highly statistically significant.

But at age five there was no difference between the standard group or BAL groups. And the microbiology on BAL was exactly the same. Clinically there was absolutely no difference between the two, so from the clinical perspective, at age 5 there was no advantage to having had the BAL.

Let's get back to Daniel. It's unclear whether this is a reinfection or failure to clear. We've got no genotyping. It's also unclear whether the infection is only in the upper airway, or is it in the lower airway as he's well and he's had only OP cultures. Treatment, however, is likely to be successful for the lower respiratory tract, even if the infection has persisted in the upper airway. So treatment's going to work regardless. That's what I think.

So do we treat, and does it matter how we treat? Well, you've looked at these studies, and basically all show that eradication does work but there is some failure. The optimal therapy is still not known, but successful eradication does reduce chronic infection. We also think that the minimal therapy should be one month of inhaled tobramycin or colistin and ciprofloxacin.

Exactly what do we need to do once treatment is finished? We're going to check OP cultures once the treatment is completed. We're back here again, so we've got to decide whether this is chronic infection or whether it is an ongoing, intermittent infection.

If he's still positive for pseudomonas and now we're in a nearly evidence-free zone, what are we going to do? Are we going to say this is chronic infection, we're going to give up on this young child? Remember, he's under age five and we do know there is an increased risk with chronic infection, particularly in younger children. So are we going to try another treatment, are we going to give it another go, are we going to switch therapies?

If the OP culture remains pseudomonas positive, then do we think he will have chronic infection after that and would we consider a BAL to determine whether he has chronic infection? Exactly what do we need to do once the treatment's finished — if he's negative for pseudomonas after treatment is completed do we think he's intermittently infected? So we keep culturing OP cultures and if he becomes positive for pseudomonas again, do we start again?

But what if within 12 months you've got three positive cultures? He might have cleared each time but if you look back, over half his cultures in that 12 months are positive. Again, I'm afraid we're in an evidence-free zone in deciding what to do. Do we change tack with treatment again, do we admit him to hospital, do we use a different eradication regimen, do we consider BAL?

I think this brings us very nicely to the discussion.

Segment 6

DR. PETER MOGAYZEL: One of the things that came up is culturing and reculturing, when would you think it's a good time to reculture after you're treating somebody? Margaret, do you want to start, right when you finish or…

DR. ROSENFELD: I'd be happy to start, I think there's a lack of standardization or evidence base there. I think one critical message that Ron Gibson found in his study that Harm Tiddens alluded to is that it's very important not to obtain an OP culture while the patient is actively still on an inhaled antibiotic because oral contamination with that antibiotic can inhibit growth of pseudomonas that might be there. So in our clinic we typically wait one to two weeks after completion of therapy.

Our protocol is typically 28 days of therapy, obtain a culture a week or two later, and if still positive then a second month of therapy.

DR. MOGAYZEL: Harm, would you do it that way?

DR. TIDDENS: As a routine, we try to get six cultures within a short time frame after completing eradication therapy. We give the patients cotton wool swabs to take home, with envelopes to send the swabs to us. I would say within two months we get six cultures.

DR. MOGAYZEL: Claire?

DR. WAINWRIGHT: Our treatment protocol is a little bit more like Margaret's, but we also quite often admit our children to hospital for two weeks of IV antibiotics, and then we give the inhaled tobi. For children who are completely asymptomatic we usually just use inhaled, but many kids have a cough and those kids who have a wet cough we'll often bring into hospital.

DR. MOGAYZEL: I had several people ask questions about IV antibiotics, is that the breakpoint if there are symptoms, that's when you go with IV?

DR. WAINWRIGHT: We don't know yet, we have no evidence on that, and it's going to be very important to look at the results of the TORPEDO study when that comes out. We go with the two

weeks of IV antibiotics because the BAL study had remarkably low prevalence of chronic pseudomonas at the end of five years, and we use two weeks of IV antibiotics with two months of inhaled tobi and a month of cipro as a very, very aggressive treatment protocol. But at the end of the day we had very little chronic infection, so that's why we're doing it, but it's not good evidence.

DR. MOGAYZEL: No. And you bring up a good point, because we were talking about a month of tobi or a month of aztreonam or a month of colistin and ciprofloxacin, but other places use longer courses of therapy. Are we going about it the wrong way, or are we just looking for the minimal approach? Margaret, what do you think?

DR. ROSENFELD: I love Claire's idea of an evidence-free zone, because that's definitely what we're in. Some very interesting data was presented today by Nicole Hamblett from the EPIC clinical trial, which looked at the duration of the remaining pseudomonas-free. If you remain pseudomonas free for a year after receiving 28 or 56 days of inhaled tobramycin, that rate was pretty high. We tend to be less aggressive, but again, it's just we're working with the evidence we have.

DR. MOGAYZEL: The EPIC trial showed that adding ciprofloxacin didn't add a whole lot to the therapy with inhaled antibiotic, but someone asked a question about Harm's data which is, with all this diseased lung, wouldn't an oral antibiotic be helpful?

DR. TIDDENS: Yes, I think especially for failed eradication you have to phenotype your patients properly, and the clinical trial networks have to compare various strategies, and then try to predict the results from the phenotyping. I think the CT scan gives important information on let's say the volume of lung you cannot reach with an aerosol. In the Review of Microbiology there is a large review on aerosolized antibiotics. They explain the complexity of an antibiotic: it has to be soluble and not bind to sputum, and all those factors play a role. Certain IV antibiotics can go into the lumen and maybe you can get some of the antibiotic from the lumen into the airway wall. Those things all might play a role.

I think we should phenotype our patients, and we have several protocols, so let's see what comes from that.

DR. MOGAYZEL: So I asked Marianne about treating and she said she was going to think about it. So Donna, what do you do if she says no, I don't want to do therapy?

DONNA PEELER: I'm sure will discuss what her concerns are, because she may have some misconceptions about side effects or other things, so it's important to clarify her concerns. We'll talk about the pros and cons of waiting. Typically a lot of the families, if they are concerned about it, ask me to me spell pseudomonas, so I know that they're going onto the internet. Then I get all these questions about the damage and prognosis and all that.

I would hope that we could give her some information that would just help her understand pros and cons of waiting.

DR. MOGAYZEL: We've talked about what kind of questions we were going to get and various things, but the question that came up that I hadn't thought of at all but several people asked is, what about other kinds of pseudomonas, is this just for Pseudomonas aeruginosa, what do you do about fluorescens and putida and all those other things, or other? Margaret, what do you do?

DR. ROSENFELD: That's a great question. Again, it comes down to evidence. We have no evidence that the nonaeruginosa pseudomonads are of concern or are pathogens, so in our clinic we typically do not treat them.

DR. WAINWRIGHT: We do treat them sometimes, so if we picked it up on a BAL and there's quite a lot of it there, we do treat it. And we've sometimes had children where it's the only positive culture in a sputum and you ask, what are we going to do, the child's coughing, he's got a wet cough? That's what we're culturing, so we treat it.

DR. ROSENFELD: But I think to be fair that's slightly different than just this asymptomatic kid who shows up with…

DR. WAINWRIGHT: That I wouldn't treat, if the child is asymptomatic and it's just there, I wouldn't treat it; but it's completely logical really, isn't it? I mean we're treating Pseudomonas aeruginosa when the child's got no symptoms, I mean it doesn't really…

DR. MOGAYZEL: Well this is the moment for an audience poll. So for Pseudomonas fluorescens, how many people would treat that in an asymptomatic child, hands up? How many people wouldn't treat it, leave it alone? I think there is more leave it alone than treat it out there in the group. Okay.

Segment 7

DR. PETER MOGAYZEL: I think we have a few more minutes to talk, a couple of more questions, because we have a unique opportunity to have people from around the world talk about pseudomonas eradication. One of the things that came up is bronchoscopy, and talking about that, when do you factor that into your practice, is that a routine thing every time, or something that you do when people fail therapy, when they're sick? Margaret, do you want to start that one?

DR. ROSENFELD :I think that's one area where practices around the world do vary quite a bit, because of lack of standardized recommendations or protocols. We typically have a fairly high threshold for bronchoscopy, maybe more than some other centers even in the United States, so we've come to think that having pseudomonas in the upper airway itself may be important, and so we'll treat there. We typically reserve bronchoscopy for failure of antimicrobial treatment directed toward the pathogen that was isolated from the upper airway.

DR. MOGAYZEL: Harm?

DR. TIDDENS: Yes, our routine is biannual CT scan and if we cannot explain the progression on the CT scan through cultures, then we do a bronchoscopy, going to the sites where we see the structural abnormalities. We often find pseudomonas, but it might also be Aspergillus or atypical mycobacteria.

DR. MOGAYZEL: You bring up a wonderful point It is a challenge in this age group to get objective measures of lung function. There are some preliminary functioning tests you can do in little kids. LCI, which someone asked about, is an up-and-coming therapy perhaps, although still in the research stages, and CT scan which is available widely, but I don't think widely used on a routine basis.

DR. TIDDENS: In Europe now, I think about 60 to 70 percent of the clinical trial network centers use CT routinely now.

DR. MOGAYZEL: Claire, what are your thoughts?

DR. WAINWRIGHT: We probably have the same sort of use of the BAL as Margaret does, so having used BAL a lot, we wound that right back when the BAL study was finished because the evidence didn't show any clinical benefit, so we stopped doing it. And we did pick Aspergillus and steno and all sorts of other things, but unfortunately, despite aiming treatment at what we found, at the end of five years there did not appear to be any difference in the microbiology, regardless of what we had done, and no difference clinically. So we stopped doing it.

I have wondered though whether we should look at things like LCI more aggressively in young children, and in particular for monitoring children, looking at how we target in the health therapies, because you do wonder whether if you have in homogeneity you might have worse deposition. And I had wondered whether anybody started to look at that.

DR. MOGAYZEL: I think you're absolutely right. As Harm points out, this is a very important issue. Are there downsides to doing therapy, we're talking about doing throat cultures and doing therapy, we talked about the pros and good things, but are there downsides to this do you think, Harm?

DR. TIDDENS: There is probably some overtreatment. A study from Utrecht showed that you can have temporary colonization. So every time we jump on it, we for sure over treat somewhat, and of course, any antibiotic will be a disturbance of the microbiota. But the curves show that overall it's worth it.

DR. MOGAYZEL: What about Daniel or other people? When do we say they're colonized and stop eradication therapy, is there a point where you say enough is enough and we're going to be on chronic therapy?

DR. ROSENFELD: I think Claire gets to take that one.

DR. MOGAYZEL: Claire's going to take that one, she brought it up. Three cultures in a year, three cultures in six months?

DR. WAINWRIGHT: I used to say yes to that but I've stopped, and the reason for that is that we've found that families hate it once a young child is regarded as chronically infected. The families have often asked, can we just try one more time, and we've often given in to that and said, let's try one more time but let's do something different. We'll often completely switch around. The first two times we'll usually use tobi, the last time we'll probably just switch to another inhaled antibiotic. We have colistin, but we don't have aztreonam available. So we usually go for colistin and ciprofloxacin, and if we do that, we usually bring them into hospital as well. We just give it an all out go and occasionally we get lucky. But it's quite difficult when you've got a family in front of you saying I don't want to be chronically infected, can we try this again.

We desperately need some studies. We need to know how best to treat reinfection and whether and how often we can treat reinfection before we call it chronically infected. And maybe we should all be doing antibody tests to predict the ones that are going to fail. But I'm not 100 percent sure even that's going to work.

DR. MOGAYZEL: Margaret, your thought on antibodies?

DR. ROSENFELD: On a population basis in early infection some antibodies are weakly predictive of success or failure of eradication. But the area under the curve is not great, so I think with an individual patient they're not particularly helpful.

DR. MOGAYZEL: I echo what you say about labeling somebody as chronic, and we've often taken the approach of let's do three cycles and see where we are at the end of three cycles.

Someone asked a question about azithromycin. We didn't talk about azithromycin, and is there a role for azithromycin in all this? Margaret?

DR. ROSENFELD: That's the OPTIMIZE study that's just being started now, so stay tuned and we'll know in about five years.

DR. MOGAYZEL: For the group that doesn't know, what's that study?

DR. ROSENFELD: The OPTIMIZE study is a randomized, controlled trial of tobramycin solution for inhalation at first isolation of pseudomonas in kids 0 to 15 years of age, and then again whenever pseudomonas is r-isolated from an OP culture. In addition, they're randomized to receive either azithromycin or placebo thrice weekly for the 18 month trial duration. The primary endpoints are microbiologic as well as pulmonary exacerbations.

DR. MOGAYZEL: And Claire mentioned the TORPEDO trial, which is a trial of IV antibiotics that's being done in the UK to look at the addition of IV antibiotics on top of colistin and ciprofloxacin as an approach to therapy. We don't know the optimal approach. We recommend particular courses of antibiotics, aztreonam or tobramycin or colistin, but there isn't an absolutely best therapy for a particular child. All these may be effective.

The last question I want to end with and people have asked is, does this signal that you should do additional therapy in some way? With pseudomonas, should you do something different with mucolytics or something that you weren't doing before? Or can you just do your tobi therapy or your aztreonam therapy and that's good? Does this signal something changed for you?

DR. TIDDENS: Clean your nebulizer.

DR. MOGAYZEL: Clean your nebulizer.

DR. TIDDENS: For sure. It might be the source.

DR. MOGAYZEL: But is this a change, do you change your practice otherwise other than doing the therapy for the infection?

DR. ROSENFELD: Since abnormal mucociliary clearance is at the root of why these patients are becoming infected with pseudomonas to begin with, we definitely reinforce airway clearance, and we might decide to start another therapy like Pulmozyme or hypertonic saline in the age range in which there is no evidence base to do that if kids are getting recurrently infected with pseudomonas.

DR. MOGAYZEL: Claire?

DR. WAINWRIGHT: We do the same. We would consider using Pulmozyme early. An interesting symposium this morning looked at the effect of Pulmozyme on releasing pseudomonas that might be stuck in a biofilm, and then with that release you think you might be able to kill off the bugs with the antibiotics. But whether that works is a whole other issue and we just don't know. But yes, we would think about starting Pulmozyme in patients with recurrent pseudomonas infection.

DR. MOGAYZEL: Okay, so I fibbed, I am going to ask another question. If someone is on chronic therapy, we're moving away from eradication, but you're on chronic therapy and they're no longer culturing pseudomonas, do you stop? Harm?

DR. TIDDENS: We stop after two years negative.

DR. MOGAYZEL: Two years?

DR. TIDDENS: Yes, and then we try to stop. I have not looked into it systematically, but I would say nine out of ten times when you culture within weeks, again you have pseudomonas. So suppressive therapy is very effective, but many of those patients relapse after stopping.

DR. MOGAYZEL: Margaret?

DR. ROSENFELD: Similar approach, though we don't have a defined period of two years and probably similar findings. The only thing I would say is in these very young kids where it's not exactly clear how to define chronic pseudomonas, I think we have a higher success rate in being able to take them off because maybe they were just intermittently colonized instead of chronically colonized.

DR. MOGAYZEL: Claire?

DR. WAINWRIGHT: I agree, we would probably be more aligned with Margaret's plan in that we don't have a set time of two years. But each of the

physicians does something slightly different.

DR. MOGAYZEL: I'd like to thank our panel. The presentations have been very good. I want to thank Daniel and his mother for being part of all this, and I want to thank you for coming and being part of this presentation.