Diagnosis Old and New Tools and Challenges, sponsored by the Southeastern National Tuberculosis Center. I'm Karen Simpson, co-executive director of the SNTC.

Before we start today's event, I have a few housekeeping items to go over. Today's event is being recorded. The webinar is scheduled for one hour, including the question-and-answer period at the end of the presentation. To verify your participation in this event (audio skip) questions for the speaker at any time during the presentation by typing in your question in the Q&A pod. Questions will be addressed at the end of the presentation.

Welcome to the third of eight sessions on Advanced Concepts in Pediatric Tuberculosis sponsored by the Southeastern National Tuberculosis Center. Today we present Diagnosis Old and New Tools and challenges. Today's presenter is Dr. Entesar Husain, joining us from Kuwait. She trained in pediatrics and infectious diseases at British Columbia's Children's Hospital, Vancouver, Canada. She is currently [audio skip ] Medicine at Kuwait University. She is also a consultant of pediatric infectious diseases at MBK hospital for pediatric hematology and oncology in Kuwait. Finally, Dr. Husain is a consultant at the National Advisory Board of Vaccination in Kuwait. Now I'll turn it over to Dr. Husain.

Can you hear me?

Yes, we can.

Okay. It's great to be with you guys today and to (inaudible), and I will be talked about Advanced Concepts in Pediatric Tuberculosis, Diagnosis Old and New Tools and Challenges, and this is the third in the series of the concepts, basic concepts in pediatric tuberculosis. At the end of this presentation, the attendees should be able to describe old and new tools used in the diagnosis of tuberculosis in children. Also, they should be able to understand advantages and limitations of each tool in detection on diagnosis of tuberculosis, know the different samples that can be used [indiscernible] for diagnosis of TB; be able to identify the indications for examination of cerebral spinal fluid in a patient with symptomatic tuberculosis, and finally, be able to evaluate a patient with suspected TB infection.

In general, majority, three quarters of infection of TB infections in children manifest as pulmonary manifestation, while 25 to 30% have extra-pulmonary manifestations. Among the most common presentations of the extra-pulmonary TB in children are based on lymphatic presentation, followed by meningeal presentations.

What are the challenges that face us as doctors to diagnose tuberculosis in children? TB is not considered in the diagnosis, in the differential diagnosis of children, especially in areas with low endemicity. Tuberculosis can mimic many common childhood diseases, and infections, including pneumonia, viral infections, malnutrition, and HIV.

The physical manifestations of tuberculosis vary and differ with age of onset, while younger children can manifest with any manifestation and look sick and very ill, older children tend to have pulmonary TB and look totally asymptomatic. Finally, one of the major challenges that TB in children has paucibacillary nature. One milliliter of sputum in children has been reported to have ten to the power of four of colony forming units of acid-fast bacilli, which is under threshold of detection, which makes detecting tuberculosis in children very difficult.

Before I go into the presentation, I would like to present three cases that I would like the audience to keep in their minds before we answer them toward the end of the presentation, and hopefully my presentation throughout will help them to figure out how to diagnose these cases.

The first case is a five year old girl from Egypt, who has been longstanding failure to thrive anorexia.. She has persistent cough associated with wheeze, and she partially has responded to bronco dilators. She had half recurrent febrile illnesses, for which she has received various courses of antibiotics. She has been treated in the past six months as a bronchial asthma where prophylaxis has been prescribed for

her, but still she had no improvement. This is her chest x-ray. As you can see here, this is her chest ray. The question here, think about how would you manage this girl. So this is the first case.

The second case is a two-year-old boy from the Philippines who had presented with right cervical lymphadenopathy measuring two-by-three centimeters for the last one month. He had received two course of antibiotics, the first one is oral Clindamycin for one week, and then when he did not improve, he was admitted to the hospital and received intravenous Cefuroxime, without much improvement. He is thriving well. As you can see, his weight and height are within appropriate centiles. And one week before presentation, he developed stridor. This boy was vaccinated with BCG, when he was in the Philippines, at birth. Again the question is, what should be done next for this child?

The last case is of a one-year-old girl who presented -- or who had been vomiting in the last 24 hours and presenting with deteriorating level of consciousness. In the past one month she has unexplained febrile illnesses. For the last three months, she's been taken care from a nanny from Ethiopia in the last three months. So what will be the best diagnosis test to perform on this girl?

I think now we can move on to talk about diagnosis of TB. Of course in terms of diagnostic priority, diagnosis of active TB remains of highest priority: On the other end, diagnosis of latent TB infection is also a priority, because all these cases, if they were not detected adequately treated, would end up with active TB.

So we have two extremes on the scale, latent TB infection and active TB infection. Latent TB infection has been covered thoroughly with Dr. Maraqa in the last session, and basically talked about TST skin testing and (indiscernible). What I will do today is talking about is how to diagnosis active TB. I will be presenting four different approaches to diagnose active TB. The first approach is symptom-based; the second is radiological-based, the third is immunological-based, and finally, organism-based approach.

First, symptom-based approach. Now this can be used thoroughly in endemic countries where there are limited resources to diagnose TB, and also can be used in low-risk children who are immuno competent, who are in the older age group above three years of age, because these are the categories where TB will present or show in a slowly progressive fashion.

The main disadvantage of this clinical scoring system is it is severely limited by the absence of standard symptom definitions, and it's inadequately validated. The clinical scoring system consists of well-defined symptoms, which are persistent and have non-remitting nature or character. The most helpful symptoms include persistent non-remitting cough or wheeze, history or documentation of failure to thrive despite food supplementation and adequate nutrition, if the child presents with fatigue or reduced playfulness. This can be an entry to diagnose or think about tuberculosis.

In terms of diagnosing TB cervical lymphadenitis, patients who are presented with persistent, more than a month, of lymph node enlargement can be thought of as having TB. The size matters, if it is more than two-by-two centimeter, and if there is no visible local or surrounding cause, and if the lymphadenitis or lymphadenopathy did not respond to first-line antibiotics.

What about radiologically-based approaches? Chest x-ray remains the most practical and helpful test in everyday practice. It's usually provides accurate diagnosis with suspicious symptoms if it evaluated by an experienced clinician. The only limitation of chest x-rays is that it is subjective -- it has subjective interpretation. It's a great test. Sensitivity can reach up to 70 or 80%. Specificity is less, 60 to 70%. Despite these adequate sensitivities and specificities, there is a huge inter-reader availability, and interpretation is highly variable and depending on the radiologist's experience.

Now I'm going to go through a series of x-rays to show you how children with tuberculosis, pulmonary tuberculosis, present with their variation of chest x-rays. This is a child who had TB, but he has a normal chest x-ray. You can see here a little bit of an infiltrate, but usually, because of the small lymph nodes and adequate airways the chest x-ray as a presentation can be normal. Compared to this chest x-ray where the child has primary tuberculosis, enlarged lymphadenopathy and a mild infiltration here.

This is also a presentation of a child who has TB, where the hilar lymph nodes have enlarged and resulted in enlarged mediastinum, as you can see here. So this is hilar and mediastinal lymphadenopathy, which is highly suggests of tuberculosis.

Lymph node enlargement can result in caseation and obstruction of one of the major airways, which results in secondary atelectasis, and this is working similar to a foreign body obstruction. Another presentation, again, where the lymph nodes can obstruct an airway and result in compensatory emphysema, as it happening here, where the midline mediastinal shifts to the right. Another presentation is miliary TB. I think everybody is familiar. This happens in malnourished children and children with TB, where there is disseminated tuberculosis. And finally, cavitating lung disease, this is a patient who has tuberculosis and here is presenting with a cavity, and is this also a manifestation of pulmonary TB. Chest CT scan, high-resolution CTs are mostly sensitive tool, which are available to detect hilar lymphadenopathy or early cavitation, and also, they can differentiate between caseation and normal or homogenous lymph nodes.

We move on to talk about immune-based approaches. The problem with these immune-based approaches is that tuberculosis has wide clinical spectrum of diseases. It's varies from LTBI to different forms of active diseases, which can be mild or severe. There are other factors that influence in your response to these tests, such as BCG vaccination. In addition, there are exposures to environmental mycobacteria, which can make these immune tests positive in a situation where it is not mycobacteria TB. And lastly, HIV co-infection, which affects immune system, and makes these test likely to be misleading.

TST test has been talked about thoroughly in the last presentation. It is a skin test, beautiful, but it lacks both sensitivity and specificity, because it can detect other than mycobacteria TB. T-cell assays like T-spot test and quantiferon-TB Gold are more specific than T-test, but they do have their own limitation. They fail to differentiate the LTBI from active diseases. They require large amount of blood from a child for examination. In addition, there is a limitation of data in children. The test, there are a lot of reservations about using T-cell assays in very young children. And lastly but not least, it is expensive, so it cannot be processed or handled in countries or places with limited resources.

Now, there are new diagnostic approaches, immune-based, including antibody detection, antibody assays, and the use of a transdermal test, which specifically is called MPB-64 skin test, and both of them are aimed to diagnose active TB, or probable active TB rather than LTBI. The only problem with the two tests, that they have not been validated or with limited studies in the pediatric age group.

Finally, let's talk about the organism-based approaches. Microscopic examination remains the cornerstone of diagnosing TB and controlling TB. As you might be aware, the standard is to use the Ziehl-Neelsen stain, which requires light microscopy or bright-field microscopy. In addition, there is another stain, which is Auramine stain, which requires fluorescent microscopy. In most of the developed countries or high-income countries, they use fluorescent microscopy as the standard practice instead of the Ziehl-Neelsen stain, and I will show you the difference. So this is a Ziehl-Neelsen stain here, where you can see here the acid-fast bacilli, compared to the fluorescence or to the Auramine stain, where you can see the acid-fast bacilli shining, fluorescing.

Now the advantages of microscopic examination, it's rapid, it is inexpensive, so it can be used in all the countries, even with limited resources, and it identifies the majority of infectious TB patients if acid-fast bacilli were present in the sample. The disadvantage is the sensitivity. It has a huge variable sensitivity. It can range between 20% and 80%. The sensitivity is basically determined by so many factors. It is dependent on the type of specimen. The specificity of microscopic examination is higher in respiratory samples. It is less in others; for example, respiratory fluid. It is also dependent on what type of population. And in areas where there is low TB incidence, it can be false read positive for non-tuberculosis mycobacteria.

Also, sensitivity is dependent on the stain used; for example, with Ziehl-Neelsen stain it can detect 100,000 bacteria per ML, while the detection rate is less, with less amount of bacteria for fluorescent, up

to 10,000 bacteria per ML. So it varies method used. Also, it depends on the experience of the microscopist, if they have been experienced with seeing acid-fast bacilli or not. And finally, sensitivity is less in children. It depends on the age of the patient. Older children tend to have more sensitivity or the sensitivity increases in older children, as compared to younger age group. And finally, with smear microscopy it can tell you if it's acid-fast bacilli, but it cannot determine the drug resistance, and this is one of the big faults.

Culture, positive culture remains the standard, the gold standard for diagnosis of TB in a child who is symptomatic. It can be done in solid or liquid broth media, and the advantage is, it's not only providing a positive or negative culture, but if it is positive, it can also provide susceptibility of the mycobacteria. The limitation, it has a slow turnaround time. Usually it takes between six to eight weeks to culture or to finalize the result of the culture of mycobacteria TB. It is expensive, it depends if it is automated liquid broth systems. It has poor sensitivity in children who have active TB. Cultures tend to be negative in 70% of children who have probable TB. And finally, it's expensive in poor resource countries.

There are new diagnostic approaches, again, to detect microorganisms, examples like caloric cultures, specific cultures like TK-medium, and phage-based tests, and microscopic observation in drug susceptibility or MODS assay, and all of them, the objective of all of them is to diagnose active cases of TB. The disadvantage, none of these three tests have been well validated or established in children.

PCR assays have been used to diagnose active cases of TB and resistance to Rifampin. They are rarely available in endemic areas, and there sensitivities are poor because of the paucibacillary nature in children. However, they can be of a better outcome if they were used in non-pulmonary samples, for example like TSF, though they have been extensively evaluated, but there is no evidence to favor the widespread use of PCR to diagnose TB.

Now we move on to talk about what samples should be used to evaluate patients with tuberculosis. Everybody by now knows that gastric aspirate is the main standard in pediatric population. It is indicated in children with possible TB who cannot expectorate sputum, so, probably, it's for children less than seven years of age. Usually it consists of collecting two or three fasting early morning samples as gastric aspirate. So a gastric tube or a nasogastric tube has to be inserted to collect the sample.

The advantages, microscopically it can detect between zero to 21%. Culture can detect, again variable number, between zero to 75% in children who have clinical diagnosis of TB. Disadvantages, it's an uncomfortable procedure. As you can imagine, inserting a gastric or nasogastric tube, it's unpleasant for a patient and it's difficult to implement. It has to be performed immediately upon the patient when the patient wakes up, so it cannot be done as an outpatient, the patient has to be hospitalized to do that test.

Induced sputum, the induction of sputum is through the use of 3% hypertonic saline. A single sample can have the same-year as the three gastric aspirates. Sensitivity is high. It can detect 75 to 100% of culture-positive TB case. And in addition, the yield is not only higher for gastric aspirate, but it's also higher for nasopharyngeal aspiration. The limitation, again, the patient needs to be hospitalized for induction of sputum, and also, induction of sputum pose nosocomial risk of transmission if no adequate infection control precautions have been taken into place.

Third is the sputum. A least sputum samples between of five to ten ML are needed to be collected to be tested for microscopy, as well as culture. The disadvantage, only 10 to 15% of the sputum samples can reveal acid-fast bacilli. The yield of the third sample or the third sputum smear is only 10 to 5%, and the yield of the third culture is 5 to 10%, especially in HIV-infected people. Bronchoalveolar lavage is another sample, a sample that can be used when spontaneous sputum or induced sputum are unavailable or they have been negative and you're still suspected that the patient might have TB.

Now the disadvantage, it poses a discomfort to the patient, it is expensive, and also it contributes to nosocomial spread of TB if it is performed without appropriate infection control measures. The yield, though, is very high, up to 77%. What about pleural fluid? If the patient had pleural effusion, usually the color can be yellow. It can be tinged with blood. The characteristic of the fluid obtained can have a

characteristic of mild exudate with a little bit of elevated protein, low sugar, a little bit high white blood cells. Acid-fast bacilli smear tends, usually, to be negative because there are very few organisms there. The cultures, however, can be positive in 30 to 70% of the cases. The thing which has really good yield is the biopsy of a pleura, because it is likely to yield acid-fast bacilli stain positive culture, and there has been evidence of granuloma formation.

Urine is non-invasive. There is excretion of mycobacteria tuberculosis in the urine, which has been well documented, and there are new sensitive bacteriological or antigen tests, which are being tested to detect tuberculosis. Bone marrow, again, they are very good samples. They are sources that can be considered in cases with probable, which has disseminated TB.

Cerebrospinal fluid, again, it's a fairly invasive procedure. The bacteriological yield is low, but the CSF analysis, which I'm going to show later, is important to know, and it can guide you towards a diagnosis of TB. It should be considered if there are signs suggestive of tuberculosis meningitis. And usually these signs are subtle in young children and you really need to have high index of suspicion, especially in endemic countries.

The characteristics of the cerebrospinal fluid include white blood cell count, between 10 to 500. Initially there can be increased lymphocyte -- increased neutrophil, but later there will be increase of lymphocytes. The glucose is characteristically very low, it's less than 40 milligram per deciliter, but it can be even as low as 20 or lower. Protein can be as high as 5000 milligram per deciliter. And 5 to 10 ML of CSF usually acid-fast bacilli is positive in 30% of the cases, but the culture can be positive in up to 70% of the cases.

Fine needle aspiration, it has an excellent bacteriological yield. It has minimal side effects, though it is invasive. And this is the procedure of choice in children who have superficial lymphadenopathy. So before we go to the cases, I would like to conclude that acid-fast bacilli microscopy and culture in pediatric population remain the gold standard for diagnosis of TB. So now we don't have a test with greater discrimination to diagnose TB. In general, if there is a child with a positive skin test and clinical or radiological findings that suggests tuberculosis and a history of contact with an adult who has TB, the child should be treated for tuberculosis. The drug susceptibility that should be included or used in the child is similar to the adult contact to that child. PCR might be an aid in the diagnosis of extra-pulmonary TB. Still, we're waiting for biomarkers, notable biomarkers either in the blood or in the urine, so we can reliably distinguish active from LTBI in children.

Now we go over the cases that I have presented earlier, and hope by now you have formulated a management plan to diagnose these cases. Let's move on to the first case, where, again, to remind you a five-year-old girl who is Egyptian, with failure to thrive anorexia, who had persistent cough associated with wheeze, partial response to bronchodilators, recurrent febrile illnesses, treated with bronchial asthma for six months without any improvement. And if you look at the chest x-ray, let me show you here, there is a little bit of infiltration despite several courses, despite she has been treated as bronchial asthma for a long time.

So this lesion was very suspicious. She was taken care of by the pulmonologist, who decided to look at the lesion further and did the CT scan of the chest. So this is what he found. He found here dilated airways, multiple dilated airways, not only where the lesion is but it's more bilateral, more on the left, so it is by lateral. So that's what raised the suspicion that this child had pulmonary TB. There is no history of TB contact for this particular child. So TST was done, which showed seven millimeter induration. She was admitted for early morning gastric aspirate for three days. That was taken. And unfortunately the acid-fast bacilli were negative, but subsequently, the TB cultures were positive. And let's look at the result of her aspirate, and you can see here.

Okay, so the culture has shown mycobacteria tuberculosis, which is time sensitive to all anti-TB medication. But look at this, the sample was sent on the 24th of December, 2005 and the results came back on the 5th of February, 2006, two months to diagnose that patient who has been around for one year or six months.

The second case, who is the two-year-old boy from the Philippines, who has lymphadenopathy not responding to either oral or IV antibiotics, and it is of a big size, two-by-three centimeters, though he's been thriving. But he developed strider, which made the doctor who was seeing him to do this test, chest x-ray. And you can see he had a mass, big mass over here. Now that was very suspicious that this could be lymphoma or not. So subsequently TST was done, which showed here the result. You can see here there is a huge induration, measured probably 22 millimeters in duration, and there is a central ulceration. So that was, in addition to the presentation of that lymph node, highly suspicious whether this lesion is TB or not.

The final confirmation came when -- sorry, again, the chest CT scan was done. And I apologize for the poor quality, but this is what I had on my file. As you can see here, this was a (indiscernible) lymphadenopathy, but I hope you can appreciate the difference between the two areas here, the white area and the darker area, which was described by the otologist as a central necrosis of that lymph node. And subsequently, there was a fine needle aspiration done on the cervical lymph node, which was three-by-two centimeter. The direct microscopy showed acid-fast bacilli. Pathology showed granuloma, and they were able to stain for acid-fast bacilli. And the culture showed mycobacteria TB, sensitive to all first-line anti-TB medication.

We move to the third case, an unfortunate one-year-old girl who had vomiting and deteriorating level of consciousness for one day, fever, febrile illnesses. She has been taken care of by a Ethiopian nanny for the last three months. Because of the presentation mainly was (indiscernible) deteriorating level of consciousness, this girl's immediate action was that she had a CT scan of the head, and this is the result. And as you can see, she has dilated ventricles, and she had loss of rugae in her brain, so almost she had a brain edema. And subsequently, the neurosurgeon was consulted for these people, and they decided that the best thing is to put a shunt. So she was admitted to the ICU, the neurosurgeon was consulted, and a shunt was inserted where we were able the get CSF.

Now these are the findings on the CSF: WBC were 548. The majority of them were lymphocytes. She had high protein in the CSF, very low glucose. The gram stain was negative. Acid-fast bacilli were positive. The clinical progress of this girl, because of the acid-fast bacilli stain and the CSF was positive, she was immediately started on anti-TB medication. The CSF for TB PCR came back in two days. It was positive. She remained in coma for one week, and, unfortunately, she passed away.

The nanny was investigated, and, actually, it was found that she was coughing for two months. She has been seen by several doctors, and none of them diagnosed that this nanny was probably an active case of TB. Her chest x-ray showed right middle lobe pneumonia, and her sputum tested positive for acid-fast bacilli.

Finally, the TB culture of the CSF of the girl came back after one month. It was positive. And that highlights -- this case highlights that it is important with nannies or caretakers who have been coughing for months, suspect TB, especially in countries which are low endemic just to avoid the passing or the transmission of infection from the nanny to younger children.

I think this is my last slide, and I thank you very much for listening.

Thank you very much, Dr. Husain, for that presentation. I do have some questions that some of the audience have asked. So let me start with more like a comment than a question, but I would like to hear your input on this. One of the attendants commented that they actually used induced sputum. They're able to use induced sputum in children, even in the clinic as outpatients, and they have been able to use that even in kids as young as three years of age. So any comments on that?

Yes, you can do it in children who are -- if that clinic is well equipped, it has good infection control precautions or measures, and if the patient can come, and if they have the standard, I think it can be done. Not necessarily patients need to be hospitalized. I think in my country this is what we are doing, we hospitalize patients. And in young children it can be induced, and the sputum can be induced with

hypertonic saline, even younger age group, two years old, if they can induce the sputum and these children can cough.

Yeah, that's right.

I think it can be done, yeah. If they have the setting. If they have the setting.

Exactly, I think that is really the key thing. Not everybody -- not every clinic has the setting to (inaudible) here in Jacksonville, who have had the luck with inducing sputum in the kids in the clinic, and that really helps to avoid the expenses of the admission to the hospital, is to obtain the sample. And so that has been helpful. And the other thing that I have to comment on that is that there is actually published literature, mostly from South Africa, where they actually use induced sputum, even in babies, but what they do -- that is in the hospital, but what they do is they actually induce the sputum through hypertonic saline. But instead of having -- obviously you can't ask the baby to cough, they actually do aspiration, granular aspiration after they induce the cough. And their yield was actually quite good. They then compared it to the yield of gastric aspirates, and the yield of the induced sputum, doing it that way was higher, much, much higher than the gastric aspirate. So that is a technique that in some places they're starting to use, which is, as I said, even in babies as young as a few months old. But they do need to be admitted, because they need to be watched for like --

Apneas, and -- yeah.

Exactly. Another question, can you comment on the use of stool to isolate mycobacterium tuberculosis, stool samples.

I didn't see anything while I was reviewing stool samples, and, actually, these days we have a child -- actually, it's not a child, it's a 14-year-old girl who presented with melena, not actually melena. She was actually passing the fresh blood. So the gastroenterologists had done a colonoscopy, and they were able just to get a sample or biopsy, which tested -- which showed granuloma acid-fast bacilli positive, so she had intestinal TB. Now it was diagnosed through a biopsy but not through the stool. And, actually, when I was reviewing the literature, I didn't find anything in the stool. And unless you've seen anything, Ana, I don't know. I cannot answer this back. I haven't seen anything on the stool.

Yeah, there is actually some literature. Hopefully there's literature, some in children, but I did find some literature on adults with HIV, and sometimes in settings where it's really hard, especially like in underdeveloped countries where it's really hard to obtain sometimes induced sputum. Actually, there's a study with patients with HIV that they were able to isolate, with a pretty good success rate, mycobacterium in the stool. Now it doesn't necessarily mean that they had gastrointestinal TB. But what the thought is that people, when they cough, they swallow their own sputum and then they can actually excrete it in the stool. So there is some literature that looks promising. I think it's just something that needs to be validated a little bit more. But I think in children it would be probably a good thing if we can prove that it helps, because that way we don't have to do the gastric aspirate.

Yeah. Of course, yeah.

So somebody asked, in the case of the child with meningitis, if the child was vaccinated with BCG, and if the BCG then failed, it wasn't protective?

Actually, this is a case, probably it's an old, old case, probably presented in 2002 and 2003, and unfortunately, at that time, our vaccination schedule included the BCG at the age of four years. So we did not have BCG at birth or an early intension the way we do it now. It was only in 2004 or 2005, that we switched from a four-year-old to a two- to three-month-old. We had a huge debate over the switching, but we finally were able to switch because of not only this case, other few cases who had TB disseminated TB earlier in life, and that's why we did the switch. It sounds awkward the way it was, but this is the way I found it when I cam back, so.

All right. No problem.

Yeah. So that girl -- to answer the question, that girl did not have BCG, unfortunately.

Yeah. Another person asked, is BCG protective?

BCG is not protective as compared to (indiscernible) analysis. It's not protective against pulmonary TB. However, it is protective, or it decreases dramatically the incidents of disseminated TB, TB meningitis in infants less than -- or in children less than four years of age. But, still, they can get pulmonary TB. So between mortality and morbidity affection in younger age group, dissemination in meningitis, but it's not an absolute protection.

Yeah. I think that's really important for the audience, especially in the United States, because since we don't use BCG here, we're not very familiar, or, in general, the people are not familiar with it. And when we evaluate children that are coming from countries where they get BCG, it's very important to understand that they can still develop pulmonary TB; that the BCG is mostly for protection of more severe disease like meningitis or miliary. But it is not that effective for pulmonary, so that kids with proven, even scars, that they have got a BCG, can develop TB, and it's not non-protective in that case. So that is a good question, and it's good to clarify that.

And, actually, sorry, can I just make a comment. And the interpretation TST as per the red book is not entirely dependent on BCG. So disregarding BCG, you should interpret TST. Got it?

Yes. Okay. Another question is, do you have any comments on the use of expert assays for the diagnosis of pulmonary TB in children? Are you familiar with that expert assay?

Gene expert -- gene expert, I think I came across when I was reviewing for this presentation. I'm not really -- I didn't find anything solid to use gene experts in pediatrics. In adults, yes, it is used. I don't know. Yeah, I think it's pretty much the same, depending on the sample, I guess. But, yeah, we've used it here in some cases, and it has been useful. So, yeah, you can use it. Obviously it's not available everywhere. But where it's available.

How about the comment on the PCR, the use of PCR, more broadly use of PCR. I don't know. That was just a question.

More broadly, like what, like for sputum?

Yes. Or for any sample in pediatrics.

Yeah, it's very good in fluid, in sterile fluid. CSF (indiscernible) pleural fluid, however, it's not of adequacy in the use of sputum, either use or gastric aspirate. So we basically, because of the limitation, we don't always have PCR available, but whenever we have PCR available, we use it for extra pulmonary TB-like fluids. Extra pulmonary fluids, yes.

Yeah, I agree. And we also use it in tissue. Sometimes when we have either lymph node biopsy, you can actually use it on the tissue, and that can distinguish more presently MTB from non-tuberculosis mycobacteria. So sometimes when it's important to try confirm the diagnosis quicker, we use it in tissue, and it has been good also.

Another question. This is not something that you covered in your lecture today, but maybe you want to comment on this. They ask about the age limits to use the interferon gamma release assays versus the tuberculin skin testing in children, like what would be the age limit for one of the other?

The majority, are they talking young children, the assays are not reliable. So probably something like five- or six-year-old is around the cutoff age, and this is what I discuss with my microbiologist here.

That is correct. I agree with you. I think we use five as the cutoff, and in younger than five, we definitely prefer to do TST and not the interferon gamma release assay. Above five, it's fine. It's basically use one or the other, not necessarily both. I think we only use them together when there is, for example, if the TST is positive and the parents are from countries where the kids have gotten BCG and they are not convinced that it's really latent tuberculosis infection, we sometimes use the igress [ph] just to convince the parents that it is true infection. But it's not something that we do routinely, like sending both tests. We usually do one or the other.

I think the use of both at the same time is only indicated for situations where it would basically benefit for the adherence to do the medications, and also when we have high-risk situations, if the suspicion is pretty high, sometimes we do both. It's because in those cases either one of them is positive. We take it as a positive.

Yeah.

Let me see if I have -- there is a question about treatment, but we're going to defer those questions for the talk. We have a special one for management and treatment, so I'm going to just defer those questions for that other lecture.

Okay.

Let me see. I think that summarizes the questions, so thank you. Thank you very much for your participation, and thank you for your lecture.

You're welcome.

And for the audience, just a reminder that the next webinar is on November -- the second Thursday in November. So it's les than a month away, just because this one was rescheduled because of ID week. So the next one is November 13th, and this one is about clinical manifestation. So I hope you join us there. Thank you.

Thank you very much. And I really enjoyed this experience.

Okay.