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Clinical Evaluation of a Blood Assay to Diagnose Paucibacillary Tuberculosis via Bacterial Antigens

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Guest: Dr. Tony Hu is an associate professor at the Bio Design Institute at Arizona State University's Virginia G. Piper Center for Personalized Diagnostics, and at ASU's School of Biological and Health Systems Engineering in Tempe.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Tuberculosis is a prevalent and potentially deadly infectious disease with a worldwide incidence of more than 10 million new cases each year, and in 2015 alone, 1.4 million deaths were attributed to TB. Much of the world relies on microbiologic techniques to detect tuberculosis including smear microscopy and mycobacterial culture which has a very long sample to answer timeframe.

Both methods also have low clinical sensitivity for paucibacillary TB cases which account for more than 60% of new TB cases each year in emerging endemic areas such as China. PCR-based methods are faster but also have limitations.

In the May 2018 issue of *Clinical Chemistry*, Dr. Tony Hu and his colleagues described a new peptide-based approach to detecting TB that overcomes those issues by using antibody conjugated nanoparticles, or nanodiscs, to bind specific peptides present in digested serum samples from cases of tuberculosis. These are then detected by benchtop matrix-assisted laser desorption ionization-time-of-flight mass spectrometry, or MALDI-TOF MS.

Dr. Hu is an associate professor at the Bio Design Institute at Arizona State University's Virginia G. Piper Center for Personalized Diagnostics, and at ASU's School of Biological and Health Systems Engineering in Tempe. He's our guest in this podcast. Dr. Hu, why is a new blood test so critical for TB diagnosis?

Tony Hu: Yeah. The active mycobacterium tuberculosis infection was called the MTB Infections represent a significant global health strike but it can be very difficult to diagnose and manage owning to the quantitative nature and the relatively poor performance of current diagnosis. And most of the TB now is diagnosed using the bacteria culture which is very

time-consuming and not very sensitive to probably 40% of the cases seen in developing countries.

And the PCR-based gene expert introduced to improve speed and specificity but also have a poor sensitivity when the patient secretes very low bacteria. And it's very common for the HIV and TB co-infected patients and also the pediatric patients. And instead of using the sputum as the substrate so that blood could be highly dissert to identify some biomarkers and for the TB diagnosis.

Yeah, if we can actively capture some indicator from the blood and we could mostly cover all the subtypes of the TB forms.

Bob Barrett: So, compared to the many different current methods, what impact could your new assay have for laboratories?

Tony Hu: Yes, and all assays actually are drafts of the sensitivity and the speed shortcoming associated with the active TB diagnosis and also meet the several criteria for the WHO requirement. And first, we can use a very small and noninvasive specimen like serum or plasma. And the second one is it doesn't require the bacteria isolation, so that means we don't need the sputum from the patients. We know that there are a lot of the patients, they don't produce the sputum or it's really hard to collect the sputum.

And the figure number three, it has very high sensitivity and specificity for active TB cases in the extrapulmonary TB culture and active, and also HIV infected TB patients. And on four, is we can directly quantify the MTB antigen secreted from the bacteria for rapid monitoring of MTTB therapy effect, which is very important for the TB management because we know there's a lot of multiple drug TB resistant to patients.

And number five is, we can use a streamlined to high throughput operation in the clinic and the research finding because we were actually integrated with a nanotechnology with MALDI-TOF Mass Spec. So, the MALDI is a highest throughput reading, can offer high throughput reading out. And the sixth, the last one can be performed by using the equipment which already approved by the FDA for other diagnostic assays. So, it's very easy to translate.

Bob Barrett: As I understand it, there had been previous efforts made in capturing mycobacterium tuberculosis antigens from blood. How does the assay described in *Clinical Chemistry* differ from the others?

Tony Hu: Yes, that's a good question. And over the last decade, we have been seeing a lot of effort in capturing the MTB

antigens directly from the blood. So, the difference of our assay from others is we actually -- we're not only looking at the antigen itself, we actually analyze the peptide fragment derived from this antigen. Because if you would only look at the antigen, there are three difficulties, the number one, most of the cases, bacteria secrete very low amount of antigens. So, it's really hard to detect them using the conventional measures. And reason number two is once the antigen secrete to the host, they could be immediately encapsulate by host of proteins. So, they block the detection site when they use the immunoassay to try to profile them.

And number three is even if you can profile the such antigen, and you're not sure if it actually really comes from the MTB or other non-tuberculosis mycobacteria because there's several major mycobacterium and they secrete the homogeneous antigen. So, use the traditional immunoassay, there's no way to differentiate them.

And so, why we want to use the peptide and a peptide as the target? Because number one, we can dissociate when you do the digestion, and we can dissociate the bonding between the antigen with the other host of proteins or antibody and they can release more peptide from this complex.

And function number two is, by analyzing the peptide fragment and based on the difference of teeny-tiny amino acid sequence, and then we can profile this peptide that really come from antigen secreted from the MTB, but not from the other NTM which is non-tuberculosis mycobacteria.

Bob Barrett: Doctor, what is the special function of the nanotechnology that your group incorporated in this new assay?

Tony Hu: Yeah, we actually introduced nanoporous particles in this and there are also two functions. And that number one, this nanoporous particle can offer to enlarge the surface area, and then you can conjugate the hundreds of times of antibody on the particles and the efficiency reach the peptide you like to target. And that's the first step.

And then, without the illusion, you can directly spot the particles on a mass spec template. Then, when you insert this template into the MALDI-TOF mass spec and turn on the machine, the laser will be shooting on the particle. And in this phase, the particle will apply the second function, which is called energy transfer. They will absorb efficiently all the energy from the laser and they pass this energy to the analyte which is the peptide you like to profile and it makes them analyzed easily. So, eventually, those peptides that

can be captured by the detector of MALDI-TOF mass spec offered a very sensitive signal on the mass spec profiling.

Bob Barrett: This technique appears to use specialized equipment that may not be readily available in some countries where the test is needed most. Do you see that changing or how can that issue be addressed?

Tony Hu: Yeah, we have to understand the need of a TB diagnosis from the two different aspects for this. And at first, we really need marker with strong clinical utility. We have been focused on the antigen protein itself for type, but always filled. And as I mentioned, when we profile the peptide and direct it from antigen, we can address both sensitivity and the specificity issue. So, to profile those peptide, the mass spec seems the only option for us now because there is no other immunoassay can efficiently profile the peptide and also, from the need of a clinic. Right now, another blood test is a T-SPOT. So, the T-SPOT needs fresh blood. So, even though they don't need the mass spec, that's large equipment, the operation could be very labor-intensive. And it's really difficult to conduct such a procedure in the resource limited area.

So this test, our assay, needs serum or plasma. It could be the frozen samples. So, whenever they collected a sample in any type of area and that they froze them and they ship to the central lab, and they can still receive the report. So, that's one option.

And the other one, we also realized the need of, one, of a care, the wise. So, in our group, we're developing the different type of reading out tools such as the mobile phone-based detector and also the nano pyrosequencing detector. So, those studies are in the development.

Bob Barrett: Well, doctor, let's look ahead. What are the future prospects for this assay?

Tony Hu: Yeah, good question. This assay can also open up the new possibilities for records of diagnosis of a wide range of other infectious disease. And actually, we are also in the sites of TB and we're also targeting on several other non-tuberculosis mycobacteria and HIV and the Ebola. And many of them have been sponsored by a federal agent grant. They should be relatively simple to generate the similar assay to quantify the low-abundance disease associated to antigen in the blood or other bodily fluid. I believe it could have the broader application to serve the infectious disease.

Bob Barrett: Are there any plans for eventual commercialization?

Tony Hu: Yes, we do. Actually, we established a startup company last year, which is called, NanoPing Technologies, and just received the first round of investment. And now, the company is fully focusing on establishing the pipeline and the production standardization. And near soon, I think, they will prepare the FDA finding.

Bob Barrett: That was Dr. Tony Hu from the Biodesign Institute at Arizona State University's Virginia G. Piper Center for Personalized Diagnostics and at ASU's School of Biological and Health Systems Engineering in Tempe. He's been our guest in this podcast from *Clinical Chemistry* on a new assay to aid in the diagnosis of tuberculosis. I'm Bob Barrett. Thanks for listening.