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Lin Li, Christopher J Lyon, Sylvia M LaCourse, Wenshu Zheng, Joshua Stern, Jaclyn N Escudero, Wilfred Bundi Murithi, Lilian Njagi, Grace John-Stewart, Thomas R Hawn, Videlis Nduba, Wael Abdelgaliel, Thomas Tombler, David Horne, Li Jiang, and Tony Y Hu.

Sensitive Blood-Based Detection of HIV-1 and Mycobacterium tuberculosis Peptides for Disease Diagnosis by Immuno-Affinity Liquid Chromatography–Tandem Mass Spectrometry: A Method Development and Proof-of-Concept Study

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Guest: Dr. Tony Hu is a Professor in Biochemistry and Molecular Biology, Biomedical Engineering, and Microbiology at Tulane University.

Bob Barrett:

This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Tuberculosis is a common cause of death in people living with HIV, in part because of the substantially increased risk of developing TB infection in this patient population. As a public health measure to facilitate early diagnosis, the WHO recommends that people living with HIV undergo screening for active TB as part of their regular care visits. Similarly, the CDC recommends that patients with TB undergo HIV screening. Simultaneous testing of HIV and TB would help healthcare providers comply with these recommendations, but the limitations of currently available diagnostic tests make this difficult. To support an integrated care delivery pathway for patients with HIV/TB coinfection, there is an urgent need for new tools that overcome the shortcomings of current test methods.

A research article appearing in the December 2023 issue of *Clinical Chemistry* describes a new diagnostic test for the simultaneous detection of HIV and TB and compares its performance to currently available assays. Why is there a need for such an assay and how would it improve the care of patients with HIV and TB coinfection? Does it show substantially better performance than other tests that are currently used in clinical laboratories?

In this podcast we're pleased to welcome the article's senior author. Dr. Tony Hu is a professor of Biochemistry and Molecular Biology, Biomedical Engineering, and Microbiology at Tulane University in New Orleans. His research focuses on engineered multiomics, nanomedicine, mechanism-driven biomarker discovery, and assay development. Dr. Hu, let's start with the basics. Why is a blood-based test so important for patients with HIV/TB coinfection?

Tony Hu:

So this is a very serious problem for the HIV and the TB coinfect patient got diagnosed, especially for the TB pathogen. And right now the current gold standard for TB

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diagnosis still relying on the sputum and either culture or GeneXpert. But as for HIV negative patient but for TB positive patients, and because their immune system got crashed, and they cannot produce a lot of sputum. So in the clinic, it's difficult to collect a sputum sample from the HIV positive patients and a lot of patients got false negative result. But a blood-based test is urgent[ly] needed for those patients and because no matter where the bacteria located or if they can generate the sputum or not, and as long as the TB bacteria is active and they will secrete the antigen into the blood, and we can detect them. So that's the reason we develop this blood-based test.

Bob Barrett: Let's go to that target you have. Why does this new assay target antigen peptides?

Tony Hu: Oh, great question. And we ever make a lot of effort on the antigen detection. So at first we're targeting on the whole length protein and for HIV and the p24 is a well-known antigen and many companies developed the ELISA based detection I say. But there's an issue if we only target on the whole length of a protein first and there's only a very short time, like about two weeks and you can detect the antigen p24 in the blood.

But afterwards, your body will generate the antibody and bonding with those antigens, and then block the detection site to the ELISA and make the dose of p24 really difficult to detect it. So when you select the HIV specific peptide you need to use the trypsin to digest this full length protein and they release the peptide we want and simultaneously they also break the bonding between the antigen and the antibody. That's actually give you the much higher signal representing the p24. So at a TB site, and we're targeting on the CFP10, that's also the well-known antigen for tuberculosis, but it only belong to the TB and the TB is a member of a large family which is called mycobacteria and the TB has 190 brother and sister, and many of them also secrete the homogeneous CFP10, but a peptide can carry the specific amino acid sequencing. So the same thing we just using the trypsin to digest the CFP10 and release the peptide carrying the sequence so that can show the TB result more specifically.

Bob Barrett: Now other than diagnosing HIV and TB, can this assay be used to answer other clinical questions?

Tony Hu: Well, still in the TB and the HIV site and not only for diagnosis, they can also follow up the treatment. So as we show in this paper, and we captured some patients with IRIS, and IRIS is spelling as immune reconstitution inflammatory syndrome. Okay, so in other word, the patient with the coinfections which pathogen you want to treat, if you want to treat the HIV and you may boost the bacterial load, but in another side

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if you want to treat the TB and you may boost the viral load. So how to capture the best time point to detect this change is very important during the treatment course.

And also definitely there's other need for the different pathogens to detect the antigen peptide instead of the antigen themselves. For example, like several non-tuberculosis mycobacterial like *M. avium* and *M. kansasii* and the current gold standard for those pathogen detections still rely on the culture. So it's time consuming and very expensive and the most important thing is very poor sensitive. But this method, especially using the blood, can really address the current issue.

Bob Barrett: So how do you plan to commercialize this assay?

Tony Hu: Yeah, we have the startup company called the NanoPin right now has been working on the TB assay and recently included the HIV part for the couple of years. So they're already communicating with the FDA and also get feedback and in 2021 the assay was recognized by FDA as a breakthrough technology. And 2022 the company also got small business grant phase two from the NIH. So they're working hard on translating this assay into the practice.

Bob Barrett: Well finally Dr. Hu, what challenges do you foresee in transitioning this from research and development to routine clinical use?

Tony Hu: Yeah, so this is several challenging. First technically, and this assay actually rely on the use of mass spec and the concept of using mass spec. Many people think the mass spec is a big instrument, hard to operate and they may block some interest, but actually it's not. And the mass spec right now has been popularized in the hospital and also the CLIA lab and like a Quest, LabCorp, and they already have a thousand of mass spec throughout the nation. It's easy to test and also very easy to incorporate with other exam. We have to change people's concept so make them to more accept more the use of a mass spec.

But in another hand, our research group also developing several portable device to profile the peptide, like a nanopore sequencing method, and we published this work last year on the *Nano Today*. So this is the effort to make the whole platform more affordable and portable. And another challenging is for validation because now everybody knows the gold standard has a limitation in identifying nearly half of active TB population. But we don't have a very good cohort with good reference. So that's actually really needed the effort from the clinicians, epidemiologists, and clinical staff for the better organize the cohort for the validation.

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Bob Barrett:

That was Dr. Tony Hu from Tulane University in New Orleans. He served as the senior author of a research article describing a new mass spectrometry assay for the simultaneous detection of HIV and TB in the December 2023 issue of *Clinical Chemistry* and he's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.