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Guest: Dr. Shaun Yang from the Department of Pathology and Laboratory Medicine at the UCLA David Geffen School of Medicine.

Randye Kaye:

Hello, and welcome to this edition of *JALM* Talk, from *The Journal of Applied Laboratory Medicine*, a publication of the Association for Diagnostics & Laboratory Medicine. I’m your host, Randye Kaye.

Identification and characterization of pathogens is the cornerstone of infectious disease diagnostics. While traditional bacterial or fungal cultures from blood or tissue specimens remain the standard, their sensitivity is limited by many factors, such as specimen adequacy, use of antibiotics, and the inability to grow certain pathogens.

Nucleic acid amplification-based tests such as PCR are increasingly used, but are inherently limited by their targeted design. Plasma cell-free DNA metagenomic next-generation sequencing is becoming more widely available and has the potential to overcome the limitations of traditional methods. This technology sequences millions of cell-free DNA fragments from a single sample in parallel via high throughput sequencing with subsequent processing through a bioinformatics database. However, these tests are costly and often complex to interpret.

The January 2024 special issue of *JALM*, entitled “Molecular Testing in Medical Practice: Challenges and Triumphs of the Genomic Age,” features an article that evaluated the clinical interpretation and impact of positive results from the Karius test, a cell-free DNA metagenomic next-generation sequencing assay at a single institution.

Today, we are joined by the senior author of the article, Dr. Shaun Yang. Dr. Yang is an associate professor in the Department of Pathology and Laboratory Medicine at the UCLA David Geffen School of Medicine, where he also directs the Molecular Microbiology and Pathogen Genomics Laboratory. Dr. Yang is a molecular biologist and ABMM certified clinical microbiologist with extensive experience in molecular diagnostics, microbial genomics, genomic epidemiology, antimicrobial resistance, and clinical virology. He is also a pioneer in the clinical applications of next generation sequencing in infectious diseases. Welcome, Dr. Yang.

Firstly, why did you decide to conduct this evaluation of positive metagenomics test results for infectious diseases?

Shaun Yang:

Yes, so, I had to maybe extend it, going back a little bit the history of metagenomics. I think this went back all the way to 2013, about eleven years ago, when Dr. Charles Chiu from UCSF first published a case report on *New England Journal of Medicine*. And then in that study, he successfully, for the first time, utilized metagenomics for pathogen detection, which really revolutionized how this disease diagnosis can be done, moving us into the genomic medicine.

Obviously, this still new in the medical field once you have something that with ten-year history, people are still learning about it and obviously, there are a lot of controversies and questions still around it. But one thing is clear is that metagenomics is just a tool. It's a new technology, it's not coming to replace a lot of the conventional methods, but rather it's an addition. But how to use this tool becomes essential question about this. People are getting better of utilizing this test, but we still don't really know exactly the best circumstance to order this test.

So, even though most infectious disease clinicians understand the value and they know it's useful, but the practice really varies dramatically. You have a wider spectrum of some ID doctor would really love this test and they order this really on a regular basis. And to a certain degree, they probably over order it and certain infectious disease doctor are very cautious and they don't really use it that much.

So, this question about how to order? What's the best patient population, the right timing, what's the right condition to order? This is still unclear and everybody would give you their own opinion. On the other hand is there are a lot of result patterns that some of them we know they are probably questionable. They are not useful.

We just never had a very definitive data to really confirm that. So, that's another reason we want to do this study.

Now, the third reason is really just the quality improvement in terms of how can we regulate this test? How to maximize that benefit of a cost ratio? I think it's a very critical question.

Randy Kaye:

Dr. Yang, what were the primary aims of your study?

Shaun Yang:

Yes, so, there are actually three primary aims of the study. First, we want to understand what type of results have the highest clinical impact, or what kind of result have the lowest clinical impacts, and how we select those were actually somewhat based on our clinical experience for many years.

The second aim is trying to find out what type of patient population can benefit from this test the most. What kind of clinical conditions correlate with the higher positive impact of this test?

And then the third goal is as a pilot study. Remember what we eventually want to do is to analyze all more than 1,000 cases of all the carriers tests we send out over the past 6 to 7 years. And we need to find out a good way to analyze this data. As a pilot study, we try to find a good algorithm, for the workflow, set up criteria, and set up statistical methods to analyze this data. So, these are the aims of the study.

Randye Kaye: Can you tell us what were the main findings of your study, and were any of these findings surprising to you?

Shaun Yang: So, yeah, there are basically three main findings. The first, on a high level is that, out of the positive results, what we found was about 30% useful, meaning that they led to positive clinical impact.

Obviously, this is a very selective sample size. If we assume that all the negative results, which accounts for about half of them, were having no clinical impact. And then, we can really just divide this by half, meaning that we estimate the positive clinical impact to be 15%, which is roughly our clinical experience.

Now, this is really compared with the positive impact, which is about 7% to 8% back in 2018, and we know that the reason really why this percentage doubled was really because due to several reasons. One is that we regulate this test. This test is now being strictly only ordered by infectious disease providers.

And the second reason is that people have learned a lot. Even though there's not really a very clear formula about how to audit this, but people develop a clinical experience and they are able to use that in the daily clinical practice. And as a result, we see a dramatic doubling of the clinical impact of this same tool about three years later. That shows you, yes, it's a tool. How good it is? It depends on how well you use it.

So the second important finding is that, kind of, confirming our clinical experience is, in the beginning, we find that there were a lot of these polybacterial results that if you have a result that has three or more bacteria present, and a lot of these are really representative of what we call the normal flora, or human microbiome. And particularly, it's oral microbiome, which you have high frequency of *Prevotella Veillonella*, *Rothia*, oral *Neisseria* species. We know they are

all from your oral cavity. Everybody has them. And detecting them when they have three or more is something that we always feel it's useless. And our study actually confirmed that. We found that if it's oral microbiome detected in this test is zero positive impact. It's kind of confirming, as we use statistical analysis to prove that.

So, the third one was somewhat surprising is we tried to correlate the clinical impact with patient population. We didn't get any significant correlation. And I think the main reason was because these patients were already very, very sick with a lot of comorbidities, and the noise is so high that most of them are immunocompromised. Even though we can break them down into several categories, including transplant patient, including cancer patient, including patient who is immunosuppressed, still we didn't really see a very strong correlation, with only one exception, which we see a borderline significance.

The finding of molds in the results seems to correlate better with higher clinical impact in cancer patients. And that's something, I think also, is consistent with our clinical experience because these cancer patient, they tend to get mold, invasive fungal infection, particularly invasive mold infection, more frequently and finding them by carriers.

Based on our clinical experience, usually lead to early diagnosis and intervention, and that's something was consistent with our experience. The only thing is our sample size is so small that we didn't get the real clinical significance, which is a P value less than 0.05. So that was like somewhere between 0.05 to 0.1. This is another reason why we need larger study, which is already being partly done, and we need to expand the sample size to really show that statistical significance. So, these are some of the major findings.

Randye Kaye: You've already answered what was going to be my final question, which was what additional work remains to be done, and if any follow-up research needs to happen. I will end with this, though. Your article provides a table of preliminary suggestions on appropriate clinical use of plasma cell-free metagenomics assays. Can you summarize what are some of your main take-home messages to support other laboratorians and clinicians in using these tests?

Shaun Yang: Absolutely, I think the number one thing is you need to get clinical microbiologists involved, whether it's in terms of evaluation, implementation of the workflow, or the interpretation of the test results. I think there's so much clinical microbiologists can contribute to and guide clinician.

The second is about the restriction of this test to only infectious disease doctors. I think that also, as we've shown

that the clinical utility can improve, and diagnostic stewardship, if there is one in your institution, they should be involved in helping, implementing some basic policies and rules to prevent people from overusing or abusing this test.

Lastly, we really want to highlight. There are a lot of questions that are still not clear, especially in terms of what clinical conditions, what patient population you should use, and when to use. I think what we found was that, for cancer patients, if you have high clinical suspicion for invasive fungal infection, I think this test tend to have higher usefulness. And for other conditions that are being reported by literature, things like endocarditis, things like pneumonia in immunocompromised patients, I think they found higher utility. But still a lot of questions are unanswered and these all prompt the needs for more studies using larger sample size, using very unbiased approach, statistical analysis to really provide high quality evidence to guide the policy implementation. I think that's the message.

But again, ultimately, I would say this is a tool and we need to learn how to use it right. And only when we use it right, you can get the best benefit of this tool.

Randye Kaye: Okay. Thank you. And certainly, studies and any follow-up research will help inform us how to use this tool. Dr. Yang, thank you so much for joining us today.

Shaun Yang: Yes, absolutely. My pleasure.

Randye Kaye: That was Dr. Shaun Yang, discussing the *JALM* article "Elucidating the Clinical Interpretation and Impact of a Positive Plasma Cell-Free DNA Metagenomics Test Result—A Single Center Retrospective Study." This article is from the January 2024 special issue of *JALM*, entitled "Molecular Testing in Medical Practice: Challenges and Triumphs of the Genomic Age."

Thanks for tuning into this episode of *JALM* Talk. See you next time and don't forget to submit something for us to talk about.