Anti-Nuclear Antibody Quantitation: Calibration and Harmonization Adjustment via Population Interrogation

Article:
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Guest: Dr. Mark Wener is a professor and the Director of the Immunology Division in the Department of Laboratory Medicine and Pathology at the University of Washington. He is also a practicing academic rheumatologist at the University of Washington Medical Center.

Randye Kaye: Hello and welcome to this edition of JALM Talk from the *Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye. Autoimmune rheumatic diseases such as systemic lupus erythematosus are commonly associated with anti-nuclear antibodies or ANAs detected in a patient's blood. The gold standard reference method for ANA testing involves incubating the patient's serum with a human epithelial tumor cell line called HEp-2 followed by immunofluorescence staining to visualize the antibodies. When antibodies are detected, the sample is serially diluted to express the result as a titer. Unfortunately, there can be variability in technique and reagents which can lead to inconsistency is in test performance and result interpretation from lab to lab.

2019 Clinical Guidelines from the American College of Rheumatology indicate that serum demonstrating a positive ANA with a titer greater than or equal to 1:80 on HEp-2 cells represents an entry criterion for systemic lupus erythematosus classification. This criterion underscores the need for harmonization of ANA titer results between laboratories. An original article in the January 2022 JALM special issue on autoimmune diagnostics describes a meta-analysis to determine the expected frequency of positive ANA tests in healthy control populations at different titers. The authors proposed an approach by which laboratories can adjust their ANA assays to a standard healthy control population positivity rate.

On today's podcast, we are joined by the first author of this article, Dr. Mark Wener. Dr. Wener is a professor and the Director of the Immunology Division in the Department of Laboratory Medicine and Pathology at the University of Washington. He is also a practicing academic rheumatologist at the University of Washington Medical Center. Welcome, Dr. Wener. So, let's start with the title of your paper, "Anti-nuclear Antibody Quantitation, Calibration, and Harmonization Adjustment via Population Interrogation." It's quite a mouthful. What does that mean? What was the main objective here?

Mark Wener: You are right. It is quite a long title and quite a mouthful. And actually, in the paper, we abbreviate this method as CHAPI, C-H-A-P-I, since it really is very wordy, but the idea is really fairly simple. Individual laboratories running ANAs can calibrate their assays and aim to harmonize their assays with each other and with the expectation of commissions ordering the ANA test by adjusting their results according to the ANA testing in their local population, and we use the term population interrogation. So, calibrate, harmonize, adjustment, population interrogation, CHAPI. That's the idea.

Randye Kaye: Thank you. So, harmonization of ANA assays would be very beneficial. Can you explain a little bit more about how clinical laboratories would approach this?

Mark Wener: Sure. So, clinical laboratories are really accustomed to setting or confirming reference ranges of the test that they run by testing local generally healthy populations. That's how we set a reference range, typically. When this is done for a chemistry test, the lab would typically establish the technique and then afterward test the local population. What we are proposing here is that the testing and the evaluation of the local population is really used to adjust the method used in the laboratory. So, we call this population interrogation, and it's really a way to achieve calibration and harmonization of lab tests in a novel way.

Randye Kaye: All right. Thank you. So yeah, that does sound like it certainly would be novel. But why would a lab want to do this?

Mark Wener: Yeah, a really good question. You know, currently, when ANA testing, anti-nuclear antibody testing is done using essentially the gold standard method with immunofluorescence microscopy on HEp-2 cells -- really considered the standard method -- but the results can be extremely variable between laboratories, and this is a major concern for clinicians and laboratorians. And there really has not been an easy, well-established, and accessible method to standardized ANA tests.

Our proposal provides an approach for individual laboratories to achieve this.

Randye Kaye: So, what exactly would a lab needs to do to carry out this approach?

Mark Wener: Okay. Well, the adjustment that needs to be made to the local ANA method would be that about 20% of a healthy control population would have a positive ANA when screened at a 1:40 serum dilution and about 10% of a healthy control population would be positive at 1:80. serum dilution. If a lab tested a healthy population, the local reference population,

and their current method was in that range, then no adjustment would be necessary. However, if the healthy controls in their local population did not meet those expectations the lab would adjust the details of reading immunofluorescence intensity. This could be done, for example, by adjusting light filters, changing the light source and the light intensity, certainly training of laboratory staff, and number of approaches that could be made to make this adjustment.

Randye Kaye: I see. So, to repeat what you said in the article, it proposes that for ANA testing, a lab should aim to have 20% positivity rate at a 1:40 dilution and a 10% positivity rate at 1:80 for a healthy controlled population. How did you derive those recommendations?

Mark Wener: Yes. So, it's really based on the meta-analysis that was done in our paper. We re-examined the studies that were used to establish the criteria for lupus classification used by the American College of Rheumatology (ACR) and the European equivalent, EULAR. They looked at ANAs from a variety of studies in order to establish their criteria for lupus classification. From those studies, we selected only those studies that allowed us to determine the frequency of positive ANAs in healthy control populations, and that led to our meta-analysis which involved 14 studies totaling over 3,400 healthy controls and 1,700 patients with lupus. This approach and meta-analysis allowed us to establish the expected prevalence of positive ANAs in the healthy population and that leads to those numbers, 1:40 serum dilution testing is expected to have a 20% positive rate in healthy controls and 1:80 serum dilution is expected to have a 10% positive rate among healthy control populations.

Randye Kaye: All right. Thank you. Now, your article also describes this approach as reverse engineering. Can you explain what's meant by this?

Mark Wener: Yes, we use the ACR/EULAR lupus criteria that provides a description of the specificity of ANA testing in lupus, and we essentially reversed that analysis to give us a prescription for setting the specificity of ANAs in healthy populations. So, the original studies were descriptive to allow the ACR and EULAR to aggregate all of these studies and give us a description of ANA specificity and sensitivity. We are reversing that to say, okay, that's the target for specificity. That's how we're going to adjust our assay.

Randye Kaye: All right. And finally, what do you think is the clinical value of your findings?

Mark Wener: So, our data in this approach really empowers individual laboratories in a very pragmatic way to align their lab

performance with the expectations of clinicians using their assays and really with the field of ANA testing overall. That's the idea of harmonization. So, the individual laboratory can adjust their lab ANA performance to a targeted specificity and a healthy control population, in fact their own local population, and that really holds promise for improving consistency and the predictive value of ANA testing, and that's the idea of both calibration and harmonization.

Randye Kaye: All right. Thank you. That's always a good thing. Thank you so much for joining us today, Dr. Wener.

Mark Wener: And thank you for inviting me. It's really good to be here with you.

Randye Kaye: That was Dr. Mark Wener from the University of Washington discussing the JALM original article entitled, "Anti-nuclear Antibody Quantitation, Calibration, and Harmonization Adjustment via Population Interrogation." This article is part of JALM's January 2022 special issue entitled, "Autoimmune Diagnostics Fundamentals to Cutting Edge." Thanks for tuning in to this episode of JALM Talk. See you next time and don't forget to submit something for us to talk about.