Utility of Antinuclear Antibody Screening by Various Methods in a Clinical Laboratory Patient Cohort

Article:
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Guest:
Dr. Melissa Snyder is a consultant in the Department of Laboratory Medicine and Pathology at the Mayo Clinic.

Randye Kaye: Hello and welcome to this edition of "JALM Talk." It's from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. My name is Randye Kaye and I will be your host.

Measurements of antinuclear antibodies, or ANA, is used to evaluate patients suspected of having a connective tissue disease. Historically, this measurement was carried out by immunofluorescence, but more recently, other more automated methods such as enzyme immunoassay and multiplex immunoassay have been introduced into clinical laboratories. In 2011, the American College of Rheumatology released a position statement indicating that the gold standard for ANA testing is immunofluorescence. This has prompted laboratories using the other methodologies to determine how results generated by them compare to the gold standard method.

In the July 2016 edition of JALM, an article entitled "Utility of Antinuclear Antibody (ANA) Screening by Various Methods in a Clinical Laboratory Patient Cohort," focused on a comparison between the method documented as the gold standard by the American College of Rheumatology and two other methodologies available to measure ANA. The senior author of this article is Dr. Melissa Snyder. She's a consultant in the Department of Laboratory Medicine and Pathology at the Mayo Clinic.

In this role, Dr. Snyder provides oversight to the antibody and protein immunology laboratories and further, she is an assistant professor of laboratory medicine. Dr. Snyder is our guest for today's podcast. Welcome, Dr. Snyder.

Dr. Snyder: Thank you very much.

Randye Kaye: So nice to have you here. So first, let me ask you. Why did you undertake this study in the first place?

Dr. Snyder: Well, for a number of different reasons and I'll kind of go through those individually briefly here. The first reason that we wanted to undertake this study was observations by

clinicians and basically phone calls that we were getting into the laboratory, where clinicians are being confused because they weren't sure what methodology was being used for their ANA testing, as well as not being clear because sometimes they would get a positive result on one method and a negative result on another method, and not being able to understand why they were observing some of these discordant results.

In addition to that, there was a paper that was published in the *New England Journal of Medicine*. It was a case report documenting a 47-year-old woman with ANA testing performed, and they highlighted that there were discrepancies between the results that they obtained using different methodologies. Because it was published in the *New England Journal*, it did receive a fair bit of attention and I think it began to cause more concern with clinicians about their ANA testing.

Around the same time, there started to be some unusual proficiency testing results that were being observed for ANA testing through the College of American Pathology. Again, when we see these kinds of unusual results, there were discrepancies across methodologies, laboratories become very concerned about how their method is performing compared to other methods, how their particular assay is comparing, and what it means when we find these kinds of discrepant results.

Then lastly, as you mentioned in the introduction, there was the release of the position statement from the American College of Rheumatology, which basically dealt with ANA testing methodologies. They highlighted one method as the gold standard method and made several suggestions to laboratories that basically highlighted how the American College of Rheumatology felt ANA testing should be performed as well as reported.

So, it was a combination really of all those kinds of things coming together that really prompted us to say, we really wanted to take a fairly broad view, a fairly global view of ANA testing and really start to see if we could understand how these different methods were performing clinically, and what it might mean for our laboratory.

Randye Kaye: Okay, I see. So, in this statement by the American College of Rheumatology, they said that immunofluorescence is the "gold standard," but in your opinion, what is the gold standard method for ANA detection? Did you find anything?

Dr. Snyder: Well, I believe we did. So, let me just take one step back before we discuss what the potential gold standard could be, but the three primary methodologies that we're focusing on

in this paper, as well as the three methods that are most commonly used within clinical laboratories for ANA testing, would be the indirect immunofluorescence testing using the HEp-2 cells. We have the enzyme immunoassay, and then we have the newest methodology available, which is the multiplex immunoassay, also referred to as bead-based assays.

So, as I said, all of these tests are available to the clinical laboratories. All of them have FDA approval, and so then it becomes the question, which is the "best method" or which is the gold standard methodology.

As you mentioned, the ACR position statement did say that they felt immunofluorescence testing was the gold standard, and they were basing that largely on the sensitivity of that testing, primarily for the diagnosis of lupus. When they went and did their literature search, they found that immunofluorescence testing for ANAs tended to have the highest sensitivity in most studies, often times dealing with lupus patients, but they were focusing on that high sensitivity by not dealing so much with specificity.

So, when we looked at the findings from our study where we compared immunofluorescence, where we looked at enzyme immunoassay, as well as the multiplex immunoassay, we not only looked at sensitivity for diagnosis of connective tissues diseases, but also looked at the specificity of the testing, specifically within our laboratory testing population. In reality, what we found is that globally speaking, over all three methodologies, there was very little difference in the overall diagnostic utility of the testing. Although you could force the immunofluorescence testing to have the highest sensitivity, the problem with that is that they have very low specificity. Whereas if you look at the enzyme immunoassay, it doesn't quite reach the sensitivity that the immunofluorescence testing does, but it has a much higher specificity.

So as I said, overall, the testing compared very well, but you could have -- the testing either have very high sensitivity or specificity, but in large part, that depends on what particular cutoff you decide to choose for that particular testing. So, I would have to say that I don't really think there is a true gold standard methodology for ANA testing. I think it's important to acknowledge that each testing methodology has different strengths, but they also each have limitations as well. We need to understand what both the strengths and the limitations are of each test, because for an individual laboratory, one test methodology for ANA testing may be optimal, but that might not be the same methodology that is optimal for another laboratory.

Randye Kaye: Okay, that makes a lot of sense, so different testing methods for perhaps different purposes. Looking at these findings of the study; has it or might it change the practice in your laboratory the way you do things?

Dr. Snyder: To be honest, at this point, it's not really changing our practice, but what it did allow us to do is really to begin to understand -- because I should back up and say, our primary method is the enzyme immunoassay for our screening ANA test. That hasn't changed and that was one, again, one reason why we wanted to do this study because we needed to convince ourselves that our enzyme immunoassay truly is performing diagnostically how we want it to be performing and optimally how it should be performing. So, we're going to maintain the enzyme immunoassay testing methodology for our screening ANA.

However, the results from the study really give us a lot more information about how our method compares to other methodologies, so that when we get calls from clinicians or other laboratories where they're having discrepant results, or they're struggling with their particular method, we can at least have some data to help us understand what the nature of this discordant results might be. That will then allow us to provide a better interpretation and hopefully aid the clinician more when they're evaluating these patients with the suspected connective tissues diseases.

Randye Kaye: Looking at these aspects or findings from the study, in addition to what you're going to continue doing in your lab, are there any findings that might be relevant to other laboratories as well?

Dr. Snyder: So, I think there are data in the study that could help other laboratories. I think one of the strengths of the paper I believe, is the fact that we use a cohort of samples that were from the testing population from our laboratory. In other words, we didn't preselect a connective tissue disease group and we didn't preselect the healthy control group. We actually used, for the study, samples that were actually submitted to our laboratory for clinical testing. I think using that type of cohort makes this study more relevant to the clinical laboratory because you start to understand how these methodologies perform in the samples you're actually going to be receiving in the laboratory, and not well-selected predefined population.

Now, one thing I will say though too, is that we say within the study that we have looked at immunofluorescence, multiplex immunoassay, and enzyme immunoassay in terms of methodologies for ANA testing, but you have to remember that we used one particular assay for the immunofluorescence. We had one particular assay for

multiplex and one assay for the enzyme immunoassay. It's difficult to say that the findings from the study would be able to be translated onto other enzyme immunoassays from other manufacturers or other immunofluorescence tests.

So, we do have to keep in mind that there can still be differences even if the methodology is the same. Different kits or different assays may perform slightly differently, so I think this provides for other laboratories, I think there are some good data in here to help guide laboratories as they may be selecting an ANA method. However, we do need to keep in mind that as they go and then select the specific test, the specific assay that they want to use in their laboratory, the performance of that assay may not be exactly the same as the assay that was used in this particular study.

So, as I said, I think there's good information here, but laboratories do need to take into account, they have different testing populations as well as possibly using other methodologies.

Randye Kaye: Okay, thank you. There are always a lot of variables. It sounds like this is important information to throw into the mix of things you consider when you do this testing. Any last minute things you wanted to add? Anything in particular that we haven't spoken about that you think is important to mention before we close today?

Dr. Snyder: I would like to add that this study, it did come from the laboratory perspective. As I said, the cohorts of samples that we collected were part of our testing population. However, it is important to realize that this was a collaboration across different laboratories, as well as the collaboration with our rheumatologists and our biostatisticians. I think we all brought something to the table for this study that was important and it was a nice collaborative effort. So, I do want to acknowledge all the co-authors on this study for all the work that they contributed.

Randye Kaye: Thank you so much, Dr. Snyder. It's been a pleasure talking with you.

Dr. Snyder: Well, thank you very much. It's been fun to do this.

Randye Kaye: That was Dr. Melissa Snyder from the Mayo Clinic, talking about the JALM article, "Utility of Antinuclear Antibody Screening by Various Methods in a Clinical Laboratory Patient Cohort" for this podcast. Thanks for tuning in for "JALM Talk." See you next time and don't forget to submit something for us to talk about!