Concurrent Anti-PR3 Immunoassay and cANCA Indirect Immunofluorescence Testing Provide Complementary Information for Clinical Laboratory Detection of Antineutrophil Cytoplasmic Antibodies

Article:
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Guest: Reagan Badger is a third-year medical student at the University of Washington School of Medicine in Seattle, Washington.

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. Antineutrophil cytoplasmic antibodies, known as ANCA, are associated with a spectrum of systemic necrotizing small vessel vasculitides.

In the clinical laboratory, ANCAs are detected using indirect immunofluorescence and multiplex immunoassays. Typically, a cytoplasmic ANCA staining pattern on immunofluorescence corresponds with antiproteinase 3 antibodies and is associated with the diagnosis of granulomatosis with polyangiitis. When immunofluorescence and serologic testing results are complementary and consistent with the clinical presentation and other findings, a diagnosis can be made more confidently. However, occasionally, the two methods can yield discrepant results.

The January 2026 issue of *JALM* features an article that retrospectively investigated the agreement of cytoplasmic ANCA immunofluorescence and antiproteinase 3 immunoassay results at one institution over a four-year period. The authors conducted medical record reviews of patients with discordant test results and provided insights into the clinical significance of these findings and the implications for testing algorithms in clinical laboratories.

Today, we're joined by the article's first author, Reagan Badger. Reagan is a third-year medical student at the University of Washington School of Medicine. Over the past year, Reagan completed a post-sophomore fellowship within the University of Washington Department of Laboratory Medicine and Pathology, including the Clinical Immunology Laboratory, where the work for this article was conducted. Welcome, Reagan. First, can you describe the motivation for conducting this study at your institution?

Reagan Badger: Yes. So, at our laboratory, which is the Clinical Immunology Laboratory at University of Washington, immunofluorescence or IFA, and multiplex immunoassays on the BioPlex platform

are performed concurrently for ANCA detection. And as part of our laboratory quality assurance practices, we monitor both the IFA and BioPlex positivity rates, and we have long-noted occasional disagreement between these platforms. So, this study was really intended to further explore these discordant results over a span of four years of testing. And of note, our preliminary findings showed that more than 60% of specimens that were positive for anti-PR3 were actually negative for cANCA using the immunofluorescence. And of course, we would expect these two values to correlate with one another.

So, we focused our subsequent analyses on exploring potential causes of this disagreement with consideration of both analytical or assay-specific factors, as well as clinical or patient-specific factors.

Randye Kaye: All right. Thank you. So, you already described a couple of findings, but were there other major findings of the study? Particularly, I'd like to know, how often were there discrepant results between the two types of analytical methods, and what were some of the causes or the scenarios in which you found those discrepant results?

Reagan Badger: So, as I mentioned, we found that there was bidirectional disagreement between the IFA and the BioPlex for clinical ANCA detection. So, among those that were anti-PR3 positive, about 61% were negative for cANCA by IFA. And then conversely, among cANCA positive samples, 16% were negative by BioPlex testing. So, it was really these discordant subsets that directed our further analyses. And of note, the disagreement we observed was not just for those titers or antibody levels near the assay-specific thresholds or cutoffs that we use in our laboratory, in fact, there were often specimens with very high-titered cANCA, but a negative anti-PR3, or conversely, a very high anti-PR3 antibody level, but a negative cANCA.

And we wanted to determine whether this disagreement was potentially manufacturer- or instrument-specific, so we first tested a subset of our samples using the commercially available Phadia assay in order to compare this with the BioPlex. And we observed 100% concordance between the BioPlex and Phadia for ANCA detection, which told us that the disagreement we were observing is most likely intrinsic to the testing methods or may, in fact, represent an underlying clinical phenomenon. And then, given this, we wanted to further evaluate patients who had discordant cANCA and anti-PR3 results using chart review.

So, we looked at both patients who were cANCA positive and anti-PR3 negative, as well as patients who were cANCA negative and anti-PR3 positive, and that was around 20

individuals in each group that we looked at. And we found essentially two patient groups or cohorts that emerged from this analysis, which we think are potential causes or scenarios to explain the discrepant results.

First, there were patients who had other rheumatologic or autoimmune conditions, possibly suggesting a nonspecific autoimmune reactivity. And then, perhaps of greater interest, there were also patients who had prior diagnoses of ANCA-associated vasculitis who were on chronic immunosuppressive therapy, which suggested to us that there may be a treatment-related effect. And then the final piece of our analysis essentially focused on further exploring those treatment effects. And to do that, we were looking at changes in cANCA and anti-PR3 over time in patients who had serial laboratory testing, and that was predominantly patients with a prior diagnosis of ANCA-associated vasculitis who were being followed clinically in order to monitor their disease activity. And essentially, what we saw is that both laboratory values changed over time, but the changes were not always synchronous, and there was occasional disagreement between the two assays.

Randye Kaye: All right. Thank you. Can you say more about any diagnostic implications of this study?

Reagan Badger: Yeah. So, previous and really prevailing guidelines have deemed it appropriate to utilize multiplex immunoassays for primary ANCA detection, like the BioPlex, without necessarily having a categorical need for confirmatory IFA, although there does remain significant variation between institutions regarding this. At our institution, we perform these two tests concurrently for ANCA detection, and this provides what we think is a complementary approach by incorporating both of those methods.

So for primary ANCA detection, really the advantage is fewer misdiagnoses, and in the context of disease monitoring, we think that using both tests allows for a better ability to monitor treatment response over time, given that both the cANCA and the anti-PR3 can change over time in response to therapy, and they're not always in agreement with one another.

Randye Kaye: Okay. So, you conclude by recommending that complementary approach, incorporating both methods. Anything more to say on that recommendation, or have you pretty much covered it?

Reagan Badger: Yeah. I suppose to clarify, our recommended approach would be using the concurrent IFA and BioPlex methods as opposed to doing any reflux testing or relying on either method alone for both ANCA detection as well as monitoring disease

activity, though of course we do recognize that an isolated positive result on either test can occur for a number of different reasons, including possible nonspecific reactivity. So, of course, there are potential false positives to be aware of as well.

Randye Kaye: So, the paper also discusses continued testing over time to monitor your patients. Do cytoplasmic ANCA and antiproteinase 3 results trend with disease activity?

Reagan Badger: Yes. So, our examination of patients who had serial ANCA testing suggests that both cANCA titers and anti-PR3 antibody levels do trend with disease activity, as well as with the effects of immunomodulatory therapy. So, we looked at a subset of 22 patients who had serial ANCA testing, and of those, 13 had synchronous changes in the cANCA and anti-PR3, meaning that these 2 values trended together. And through chart review, we were able to show that the peak titer or antibody level correlated with periods of disease activity or flares. That is, there were active diagnosis-related symptoms at the time of testing. And both antibody levels, as well as titers, showed a response to immunomodulatory therapy, meaning that they decreased over time in response to initiation of treatment. But then 9 out of these 22 patients that we examined did have asynchronous results with either the cANCA or anti-PR3 level remaining persistently negative over time, while the other value changed significantly.

So, really, the takeaway was that neither test was more useful in monitoring disease activity over time within our study. It is worth noting, however, that in the literature and often in clinical practice, we do see anti-PR3 more typically used as a value that's monitored over time, and that's primarily because we know that anti-PR3 suppression is a documented effect of rituximab, and that's one of the mainstays of therapy for ANCA-associated vasculitis. But by performing serial monitoring of both of these values over time, we were able to see treatment effects reflected in one or both of the laboratory values, often with disagreement between them and this is not just true for rituximab, many other types of immunomodulatory therapy were represented in our patient cohort as well.

Randye Kaye: One more question. Were there any discordant cases or patient groups identified in the study that may require some further investigation?

Reagan Badger: Yes, there were several groups. First, among our patients who had discordant cANCA and anti-PR3 testing results, we had described, as I mentioned, a number of different autoimmune conditions other than ANCA-associated vasculitis, potentially introducing some nonspecific autoimmune reactivity. In particular, this cohort was actually

enriched for interstitial lung disease, and that was seen in about a quarter of the patients who had discrepant results that we examined on our initial chart review. And although interstitial lung disease is a recognized complication of ANCA-associated vasculitis, it is something that we usually consider in the context of pANCA, that is perinuclear ANCA, or anti-myeloperoxidase, anti-MPO-associated disease, and more rarely in those with isolated cANCA reactivity. I believe that has been described in the literature just briefly. But this, to us, represents a potentially unique study population, and further exploration really is needed to determine whether that ANCA reactivity has any effect on the ILD disease presentation, particularly in the absence of a concurrent ANCA-associated vasculitis diagnosis and just having this isolated reactivity.

And then the second thing is we specifically focused on cANCA and anti-PR3 in this study, but we also identified significant discordance between p-ANCA and anti-MPO, similar discordance, I would say, to the cohort examined here. And I do think that likewise warrants some further investigation. Isolated pANCA and anti-MPO reactivity is seen in a number of different autoimmune conditions, and this has been described in the literature. But similar to the present study, I think it would be interesting to evaluate both the pANCA and anti-MPO over time to determine whether they vary synchronously or asynchronously, and also in response to immunomodulatory therapies, so I think that would be a useful direction for further studies.

Randye Kaye: All right. Thank you so much for joining the podcast today.

Reagan Badger: Thank you.

Randye Kaye: That was Regan Badger from the University of Washington School of Medicine, describing the *JALM* article, "Concurrent Anti-PR3 Immunoassay and cANCA Indirect Immunofluorescence Testing Provide Complementary Information for Clinical Laboratory Detection of Antineutrophil Cytoplasmic Antibodies." 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.