Isotyping of Paraprotein Irresolvable by Routine Immunofixation Electrophoresis

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Guest: Dr. Xin Yi is Co-Director of Clinical Chemistry at Houston Methodist Hospital in Texas and an Assistant Professor of Clinical Pathology and Laboratory Medicine at Weill Cornell Medical College.

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.

Serum protein electrophoresis and immunofixation electrophoresis are critical laboratory assays in the identification of monoclonal proteins. Monoclonal proteins, also called "M spikes" or "paraproteins," may be due to monoclonal gammopathy of undetermined significance, but they may also indicate malignant diagnoses such as multiple myeloma or lymphoplasmacytic lymphoma.

Protein electrophoresis separates the proteins in a patient sample by their size and charge and allows for the detection of abnormal monoclonal proteins. Immunofixation electrophoresis is used to determine the type of monoclonal protein present. Both methods are technically challenging and require interpretation by a qualified laboratory professional.

Occasionally, anomaly such as immunoglobulin complexes or temperature sensitive cryoglobulins distort the protein separation and prevent accurate interpretation of test results.

A case study published in the July 2019 issue of the *Journal of Applied Laboratory Medicine* describes a patient whose monoclonal protein was not identifiable by routine protein electrophoresis and immunofixation methods. The Case Report suggests steps that laboratories may take to reduce artifacts and identify monoclonal proteins accurately.

The corresponding author of the study is Dr. Xin Yi, a board-certified clinical chemist. Dr. Yi is currently serving as the Co-Director of Clinical Chemistry at Houston Methodist Hospital in Houston, Texas, and as an Assistant Professor of Clinical Pathology and Laboratory Medicine at Weill Cornell Medical College. Welcome Dr. Yi, and thanks for joining us.

Dr. Xin Yi: Thank you.

Randye Kaye: Can you briefly describe your case and the challenges that you faced while performing the electrophoresis and immunofixation assays?

Dr. Xin Yi: Sure. This case is a 76-year-old man found to have increased serum protein concentration during workup for his severe anemia. And serum protein electrophoresis is an assay normally used to assess if patients have monoclonal gammopathy. And on this test, this patient has a smearing band extending from beta to the beginning of gamma region. And the rest of the gamma region staining was strikingly decreased.

So, after observing that, we know that this is an artifact. This could also be seen on serum protein electrophoresis when large amount of cryoglobulins are present. So, this band-like artifact present at or near the point of application, means that where the sample is applied on the gel.

The difference of our case is that the common solutions to resolve the cryoglobulin artifact did not work for our case. And also, on immunofixation, we saw one band seam across all the lanes with equal intensity.

So, each lane on the immunofixation represents one subtype of immunoglobulins including IgG, IgA, IgM, kappa, and lambda. Therefore, the artefactual band in this case prevented proper typing of the immunoglobulin process.

Randye Kaye: All right, thank you. Now, you mentioned cryoglobulins and why do cryoglobulins cause interference in electrophoresis and are there ways to avoid the interference?

Dr. Xin Yi: First of all, cryoglobulins are circulating proteins specifically immunoglobulins that clump together or precipitate when exposed to cold and redissolve when warmed up.

On the protein electrophoresis, cryoglobulin may precipitate on the media because its concentration on the media may be marginally greater than what's in the serum.

Also, the instrument we use to run electrophoresis controls the temperature at 20 degrees during the migration. So, cryoprecipitation on the gel may occur resulting in a band that may even persist after washing. So, this will show as an artefactual band on the electrophoresis.

Similarly, on immunofixation, the cryoprecipitation could happen before antibody added in and persist after extensive washing, yielding ambiguous result like what we saw in our case, a band across all the lanes on the immunofixation.

Also, what we found in our case is that, this patient's IgM, immunoglobulin, and the serum viscosity were both elevated.

IgM pentamer is very large in size. An increased amount of it caused the serum to become viscous and difficult to move through the lab work in the agarose porous gel resulting a band-like artifact at or near the point of application. So, that's the effect we see on the electrophoresis.

In addition, cryoglobulin, especially IgM, may present with rheumatoid factor activity or there could be non-specific immunoglobulin anti-immunoglobulin effect, which both cause other immunoglobulin bind with the IgM complex and interact with antibodies for other immunoglobulin resulting in one single band across all the lanes on the immunofixation.

So, there is published a method to resolve the artifact issue including using a reducing agent that comes within the test kit called, "Fluidil" or a stronger reducing agent called, "β-mercaptoethanol," which both could breakdown the disulfide band between non-specific protein banding, therefore, to resolve the cryoglobulin precipitation and non-specific immunoglobulin banding problem.

Randye Kaye: Okay. So, the solutions that you described in your patient's case didn't really seem to resolve the monoclonal band. Why is that and how did you ultimately resolve it?

Dr. Xin Yi: In this case, we did use both Fluidil and β-mercaptoethanol and also those two reducing agents together. We found that those treatments did break down some protein banding but not completely. So, it still failed to fully resolve the artefactual banding in our case.

In fact, after the combined treatment, we did see a faint area of IgM and lambda staining close to the single band across all lanes, but the resolution was really poor.

So, to achieve better and definitive isotype identification, we used another harsh protein denaturing detergent, SDS, which eventually provided a better isotype resolution, and we were able to report the immunofixation result as monoclonal IgM lambda.

This result was found afterwards to be consistent with patient's bone marrow biopsy result and also patient's urine immunofixation showed a monoclonal lambda light chain band.

Randye Kaye: Were there any limitations to your workup in this case or can you propose any additional experiments that could have been performed?

Dr. Xin Yi: Yes, there are definitely limitations. So, in this case, we can only hypothesize that this effect on both electrophoresis and immunofixation are due to either cryoglobulins or non-specific immunoglobulin anti-immunoglobulin banding. But unfortunately, we did not have enough samples to test our hypothesis and to determine what exactly caused the effect in this case.

So, this patient did have a rheumatoid factor lab result which was negative and it would be good if we can perform the cryoglobulin test as well. But we suggested to the physician to monitor this patient's monoclonal gamma globulin level by quantitative method and not by protein electrophoresis due to this artifact. So, we did not have any additional sample from this patient to do other proposed study.

Randye Kaye: Thank you, Dr. Yi. I have one last question and that's just, do you have any suggestions for other laboratorians who may face similar challenges in performing electrophoresis and immunofixation?

Dr. Xin Yi: I think from this case and our workup, we could provide experience to our colleagues that on electrophoresis and immunofixation gel, an artefactual band across all lanes could occur as a result of cryoglobulins with or without rheumatoid factor activity, or due to immunoglobulins that could non-specifically bind to other immunoglobulins.

Previously published methods to resolve this problem include using a reducing agent, Fluidil or β-mercaptoethanol, to break down disulfide band between proteins.

Our study provided another solution, especially when the first two mentioned agents cannot fully resolve the artifact. We found that the stronger protein denaturing detergent, SDS, could be used to achieve better disassociation between immunoglobulins and therefore better resolution on the gel.

Also, our study determined that sample pre-treatment at a higher temperature, 56 degrees, also improves the resolution of the result while maintaining the gel staining quality.

Randye Kaye: That was Dr. Xin Yi from Houston Methodist Hospital describing the Case Report "Isotyping of Paraprotein Irresolvable by Routine Immunofixation Electrophoresis" from the July 2019 issue of JALM. 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.