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Guest: Dr. Kazunori Murata from the Department of Pathology and Laboratory Medicine at the University of Miami Miller School of Medicine.

Randy 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, Randy Kaye.

Serum protein electrophoresis is a technique used in the laboratory to diagnose and monitor several protein disorders, most notably monoclonal gammopathies. Monoclonal gammopathies occur when a group of plasma cells undergo excessive clonal expansion. These cells can produce monoclonal antibodies, also known as M-proteins.

After being detected by a serum protein electrophoresis, M-proteins can be further characterized by isotyping using a technique called immunofixation electrophoresis, or IFE. This technique uses antibodies against portions of the M-proteins known as heavy chains and light chains to classify them into different categories. However, one potential issue for this analysis is that some M-proteins can aggregate, leading to difficulties in interpretation. A commonly used method to break up aggregates is to use beta-mercaptoethanol but this can be cumbersome and can present additional health hazards to laboratory staff.

The March 2026 issue of *JALM* features a focused report that describes a protocol using capillary electrophoresis immunotyping as an alternative to beta-mercaptoethanol with IFE for isotyping serum samples with M-protein aggregates. Today, we're joined by the article's corresponding author, Dr. Kazunori Murata.

Dr. Murata obtained his Ph.D. in pathobiology focusing on transplant immunology at the Johns Hopkins University, followed by a fellowship in Clinical Chemistry at Mayo Clinic. He is currently a clinical professor in the Department of Pathology and Laboratory Medicine at the University of Miami Miller School of Medicine. He serves as the Medical Director for Clinical Chemistry and Co-Director for Point of Care Testing for Jackson Health System. He also serves as Section Head of Clinical Chemistry for DPLM.

Welcome, Dr. Murata. What are some of the most common reasons a physician would order serum protein electrophoresis testing for their patient?

Kazunori Murata: So, typically, serum protein electrophoresis, more commonly referred to by the acronym SPEP, is often ordered by physicians when a group of diseases referred to as monoclonal gammopathies are included in the differential. Symptoms and clinical events that can make a physician include myeloma in their differential when working up a patient will be things such as, say, lesions detected in the spine, skull or other bones, compression fractures, unexplained anemia, unexplained renal failure or unexplained hypercalcemia.

Secondly, an SPEP will typically be ordered by a hematologist, oncologist, or sometimes a more specialized cancer center's myelomatologist to monitor a patient's disease progression from multiple myeloma or monoclonal gammopathy of undetermined significance.

An M-spike is a quantitation of the patient's M-protein which is secreted by the cancer cells. As such, it's essentially a tumor marker generated by the patient's tumor. Typically, as the patient's myeloma or monoclonal gammopathy progresses, the tumor or tumors increase in size. Meaning, they will secrete more M-proteins. Conversely, as the tumor responds to therapy, the tumor shrinks generating less M-proteins, resulting in a lowered M-spike value.

Randy Kaye: So what information is obtained by isotyping the M-proteins by immunofixation or immunotyping? What do different isotypes mean for the patient's disease?

Kazunori Murata: So what an immunofixation or immunotyping tells you is the isotype. Namely whether an M-spike seen on an SPEP is an IgG, IgA, or IgM, along with its light chain whether it's an IgG-kappa or IgG-lambda, IgA-kappa or IgA-lambda, and so on.

Furthermore, immunofixation and immunotyping are both more sensitive compared to SPEPs and so are able to detect M-proteins that may not have been detected by SPEP. They can also tell us important information regarding the electrophoretic mobility of the monoclonal proteins detected in the serum. The isotype is important as different isotypes have different implications for both diagnosis and prognosis.

Typically, IgM monoclonal proteins are present in lymphoma or leukemia patients and if they meet certain criteria referred to as Waldenstrom macroglobulinemia.

IgG or IgA monoclonal gammopathies can either be monoclonal gammopathy of undetermined significance or myeloma.

Immunofixation/immunotyping can also identify patients with M-proteins that lack heavy chains such as light chain MGUS, light chain myeloma, or light chain amyloidosis. These are important because light chain myeloma can, over time, result in renal failure as the monoclonal light chains, which are typically cleared in the renal tubules, can be nephrotoxic and lead to renal failure.

In the case of light chain amyloidosis, deposition of light chains can also lead to failure of other organs such as the heart. In patients that have a monoclonal gammopathy but not myeloma such as MGUS, the isotype matters greatly as the risk of progression from MGUS to myeloma is greater in non-IgG MGUS. In patients that are diagnosed with myeloma or MGUS, immunofixation can be oftentimes only ordered initially as once you know what the patient's M-protein isotype is, you can simply monitor the patient with just an SPEP and track the patient's M-spike quantitation.

However, once the patient's M-spike becomes undetectable, an immunofixation or immunotyping may become necessary, particularly for patients on clinical trials because one of the ways in which a patient's response to treatment is determined is by the detection of M-protein by immunofixation or other equivalent means.

Under the International Myeloma Working Group, or IMWG's, response criteria, a patient who has no detectable M-spike may be classified as having achieved very good partial response, or VGPR, while a patient who is negative by immunofixation or other equivalent means can be classified as being in complete response, or CR. At many large academic medical centers where many clinical trials involving myeloma patients take place, an immunofixation or immunotyping may be just part of an order set that is placed every time a patient comes in for an outpatient visit.

So, it's hard to predict whether a patient will be negative for an M-spike prior to actually performing an SPEP. Patients often develop other bands during the course of their treatment, in particular after autologous or allogeneic bone marrow transplantation. An immunofixation or immunotyping may be necessary in order to differentiate the patient's original disease clone from other clones that may arise in the patient over the course, over the patient's treatment regimen.

Finally, another important reason for performing an immunofixation is that one of the most common ways in

which myeloma patients are treated is by monoclonal antibody drugs, which can appear on both SPEP and immunotyping and immunofixation as clear IgG kappa bands. Currently, the monoclonal antibody drug that is most commonly encountered in the laboratory is a drug called daratumumab but there are multiple monoclonal antibody drugs currently being used to treat myeloma patients such as volantumab, elotuzumab, teclistamab, and others.

Oftentimes, these drugs can even be used in combination with each other. Because the vast majority of patients with multiple myeloma have an M-protein of the same isotype, IgG kappa, this tends to create confusion as to whether an M-spike seen on SPEP is due to disease relapse or simply due to the appearance of daratumumab peaks on SPEP. An immunofixation or IT can often help differentiate the patient's original clone from daratumumab either because the patient's IgG kappa clone is of a different electrophoretic mobility as can be seen on an immunofixation or immunotyping or alternatively, if the patient's original clone was not an IgG kappa isotype.

Randy Kaye: How does beta-mercaptoethanol break up the M-protein aggregates allowing for further isotyping of the M-proteins by IFA? Is this a commonly used technique?

Kazunori Murata: So that's an excellent question. The way that beta-mercaptoethanol works is by reducing disulfide bridges present between cysteine residues and proteins to their corresponding free thiols. This disrupts the tertiary and quaternary structures of proteins and allows them to unfold into linear polypeptide chains. This essentially helps break up any aggregates of IgM, IgA, and sometimes even IgGs that form in the serum of some patients during gel electrophoresis. This is essentially the rationale behind how BME can help resolve some smears that are seen on immunofixation electrophoresis.

Now, in response to your question regarding whether it's commonly used, yes, it is commonly used in both research and clinical laboratories. However, it also happens to be one of the most notorious and despised procedures in laboratories because beta-mercaptoethanol has a very powerful, unpleasant, and lingering odor. Anybody who has worked with this can recognize its pungent aroma and it tends to linger in the room even when working with it under a chemical fume hood. It's so unpleasant that there are even some laboratory staff who refuse to work with it.

Randy Kaye: Wow, okay. I'm just imagining that. Your study compared immunotyping to immunofixation after the use of beta-mercaptoethanol. So how does immunotyping differ from immunofixation?

Kazunori Murata: So what we found is that in our small sampling of cases, immunotyping was able to identify the isotype of the monoclonal protein without having to treat the specimen with beta-mercaptoethanol which, as I mentioned previously, is something laboratory staff generally are not fans of. Although we don't know exactly why immunotyping appears to be less susceptible to the interference seen on immunofixation electrophoresis, it may have something to do with the fact that the proteins are still solubilized in a buffer as opposed to being forced and squeezed through a gel matrix like what occurs in an immunofixation electrophoresis.

It's important to note that in terms of functionality, immunotyping accomplishes the same thing as immunofixation. It identifies the isotype of the monoclonal protein, detects it at the same level of sensitivity as a traditional gel immunofixation and provides information on the electrophoretic mobility of the M-protein. However, when compared to immunofixation, immunotyping is more automated, less skill-dependent and less prone to misinterpretation due to artifactual bands that may occur during application of the serum to the gel.

Randy Kaye: Thank you. What are some of the practical applications of the findings of this study?

Kazunori Murata: So essentially what we're proposing is that at least for certain cases where gel immunofixation may not yield a clear-cut identification of the monoclonal protein, immunotyping using capillary electrophoresis may potentially be a solution. There are actually many laboratories that have moved toward capillary electrophoresis for their serum protein electrophoresis, but still perform immunofixation by gel electrophoresis.

So in these situations, laboratories already have all the equipment necessary to perform both immunotyping and immunofixation because immunotyping can be formed on the same instrument that performs protein electrophoresis.

Randy Kaye: So you said that some labs are moving toward using immunotyping but do you think this work will encourage laboratories to move toward using immunotyping to replace the gel-based immunofixation?

Kazunori Murata: I hope so. A lot of high-volume and even some low-volume laboratories have moved toward capillary immunotyping due to the benefits of this method. Capillary immunotyping is less manual and more automated compared to gel-based immunofixation methods as I previously mentioned. Where the difficulty lies is actually in the interpretive aspect.

Many pathologists and clinical chemists are wary of moving toward immunotyping. It was actually not that long ago, maybe about 10 to 15 years ago, and we started to see some initial small-scale and actually somewhat flawed studies that seem to point toward immunotyping having a lower sensitivity in detecting monoclonal bands relative to gel-based immunofixation. However, in a large-scale study that Dr. Katie Thorne and I published in 2021, we were actually able to definitively demonstrate in a large cancer center setting that immunotyping and immunofixation are largely equivalent if the people performing the immunotyping reviews were appropriately and extensively trained in the interpretation of immunotyping.

It is true that immunotyping does require some getting used to and that it is a big change from looking for bands in gels. However, some manufacturers do offer excellent virtual as well as self-directed online training to ensure that people responsible for interpreting these electropherograms are interpreting them correctly.

- Randy Kaye: What do you feel are some of the limitations of this study?
- Kazunori Murata: So, our publication being essentially a short communication, this was just a sampling of a few cases. Although we do know that immunotyping can be helpful in some cases, we have actually in our experience also encountered some cases where even immunotyping was not able to entirely eliminate the interferences caused by polymerized IgM and IgA. So, more study is definitely needed in order to clarify when and how often immunotyping can overcome these interferences.
- Randy Kaye: Final question. How do you hope to build on your findings?
- Kazunori Murata: So, as with any good research projects, findings should always lead to more questions and this project is definitely no exception. As I mentioned previously, we do know that immunotyping may not be able to replace beta-mercaptoethanol in all cases of polymerized IgM and IgA. It would actually be very helpful for the laboratory community to be able to get a sense of when immunotyping will be helpful and when it may not.
- A larger study looking at what types such as IgA or IgM or IgG, as well as the quantity, Kappa or Lambda, or other potential factors which may affect when immunotyping may or may not be useful would be something that I would be interested in conducting.
- Randy Kaye: All right. Well, thank you so much for the information and for joining us today.
- Kazunori Murata: Thank you.

Randy Kaye:

That was Dr. Kazunori Murata describing the *JALM* focused report, "Immunotyping as an Alternative to Reducing Agents for Resolving Monoclonal Immunoglobulin Aggregates Observed on Immunofixation."

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.