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Clonality Determination by Detecting Unmodified Monoclonal Serum Free Light Chains Using On-Probe Extraction Coupled with Liquid Chromatography-High-Resolution Mass Spectrometry

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Guest: Dr. Priscilla S.W. Yeung is a Fellow in the joint Stanford-UCSF clinical chemistry fellowship and an Instructor in the Department of Pathology at Stanford University.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Serum free light chain measurement are a mainstay in the diagnosis and subsequent monitoring of patients with plasma cell disorders, including Waldenström macroglobulinemia and multiple myeloma. An increased free kappa, free lambda, or kappa lambda ratio outside the reference intervals suggests the presence of a clonal plasma cell population and merits further investigation. Immunoassays used in typical high-volume hospital labs measure the total free light chain concentration, which provides accurate and actionable information in most patients. However, confusion arises in patients with inflammatory disorders or impaired renal function, who have a high polyclonal free light chain concentration.

Does the elevated free light chain indicate myeloma or is it simply an artifact of the patient's chronic disease? Is a low-concentration monoclonal antibody hiding within the larger polyclonal background? To answer these questions, we needed a test method that can assess free light chain clonality. A new research article appearing in the December 2024 issue of *Clinical Chemistry* introduces a new test method for exactly this purpose, the measurement of monoclonal free light chains. How does it work? What unique information does it provide, and which patients could benefit from its use?

In this podcast, we have the pleasure of speaking with the article's lead author. Dr. Priscilla S.-W. Yeung is a Fellow in the Joint Stanford-UCSF Clinical Chemistry Fellowship and an Instructor in the Department of Pathology at Stanford University. Her current research is focused on applying top-down mass spectrometry and cell surface proteomics to discover improved biomarkers from monoclonal gammopathies and other disorders.

So, Dr. Yeung, let's get basic first. Can you tell us a bit about why we measure serum-free light chains in a clinical lab?

Priscilla Yeung: Sure. The main purpose of testing for serum free light chains is to help diagnose and monitor monoclonal gammopathies, which as the name suggests are a group of diseases that are characterized by clones of antibodies found in the blood. Although there are a few types of B-cell lymphomas that also secrete antibodies, monoclonal gammopathies are mostly caused by plasma cells.

In healthy people, the bone marrow contains a large heterogeneous population of plasma cells that secrete lots of different antibodies that help us fight infections. But when one of these cells replicates in an unregulated manner, that creates a clonal population. The most severe form of this is multiple myeloma, which is a cancer of the plasma cells, and it's the second most common hematologic malignancy, with a median survival of only five to six years.

There are also other plasma cell disorders that manifest outside of the bone marrow, like extramedullary plasmacytoma, which is a plasma cell tumor in the peripheral tissues and AL amyloidosis, which is when clonal antibodies deposit into organs and cause damage. All these diagnoses are made using a combination of clinical history, imaging findings, tissue biopsies, and laboratory data, which includes serum free light chains.

Serum free light chains are circulating antibody light chains that are unbound to the heavy chains. It is currently one of the diagnostic criteria for multiple myeloma, and an important prognostic marker for the progression of smoldering myeloma to fulminant myeloma. It is also especially useful for identifying patients with oligosaccharide myeloma and monitoring patients who have been treated, because the assays used to measure free light chains are often more sensitive than traditional electrophoresis-based methods.

Bob Barrett: So, how are serum free light chains typically measured?

Priscilla Yeung: Well, the most widely used method for determining free light chains are immunoassays, which quantify the total kappa and total lambda free light chains, and then use a ratio between the kappa to lambda free light chains to infer clonality. Like other immunoassays, free light chain immunoassays can suffer from the hook effect, law-to-law variation in the reagent antisera, mutations in patient epitopes that affect reagent binding, and artifacts from free light chain aggregation. But the core limitation of free light chain immunoassays is that they don't directly measure clonality. That means that the interpretation of clonality is heavily reliant on the reference interval, which is not easily established, nor generalizable, and actually has been an

active topic of discussion within the clinical chemistry community.

Specifically, in patients with elevated polyclonal backgrounds, like those with chronic kidney disease, infection, or autoimmune disease, both the total kappa and total lambda light chains increase, so the ratio can be hard to interpret. For example, one common scenario is that of a patient with renal failure. Even if their kappa light chain is slightly high, or if both the kappa and lambda light chains are high, those abnormal values can be caused by the kidney disease and might not be related to an underlying plasma cell disorder.

Bob Barrett: Previous studies have used mass spectrometry to measure free light chains. Can you tell us what they found?

Priscilla Yeung: Yeah. In the past few years, there's been a wave of studies using mass spectrometry to address this clonality issue. This field was first pioneered by a group at the Mayo Clinic, who developed a MALDI-TOF-based method called MASS-FIX for measuring serum monoclonal proteins, including free light chains. They showed that MASS-FIX can directly determine the clonality of free light chains within elevated polyclonal backgrounds with much better analytical sensitivity compared to immunofixation electrophoresis.

On the other hand, around 16% of samples with negative electrophoresis and negative MASS-FIX results had abnormal results if we use the immunoassay method interpreted using the manufacturer's reference intervals. More recently, along with my research mentor, Dr. Ruben Luo, we developed a method that combines an on-probe immunocapture step with high-resolution mass spectrometry called OPEX-MS to determine the clonality of unmodified serum-free light chains. Like the Mayo Clinic study, we also found some minor discrepancies between the MASS-FIX and immunoassay results. Specifically, we could identify monoclonal free light chains in around 14% of immunoassay negative samples. At the same time, we found that 33% of kappa-elevated samples by immunoassay, as well as 83% of dual-elevated kappa and lambda samples by immunoassay didn't have monoclonal free light chains by mass spec.

Bob Barrett: So, considering the recent studies, what advantages does mass spectrometry bring to serum free light chain testing?

Priscilla Yeung: That's a great question. I think mass spec based methods can add a few things, the main one being the direct detection of all the light chains, which allows for clonality determination without relying on a reference interval for the kappa to lambda ratio. Also, both Mayo Clinic and our group found that there is often no monoclonal proteins in samples with mildly elevated or dual-elevated free light chain immunoassay

results, which makes mass spec a useful tool in determining clonality to avoid over diagnosis of these patients.

On the other hand, the improved sensitivity compared to immunoassay makes these methods valuable in monitoring patients who have been treated to a low level of residual disease, potentially serving a similar role as gene-based MRD testing. There are a few clinical trials evaluating this idea now. And lastly, there was around 10% prevalence of glycosylated light chains in the tested cohorts. Because light chain glycosylation status has been shown to have prognostic value, it can be used along with other factors for patient risk stratification.

Information about post-translational modifications is something that's currently not accounted for in the available immunoassays.

Bob Barrett: Well, finally, Dr. Yeung, looking ahead, how do you envision these mass spectrometry-based methods being used in the clinical lab?

Priscilla Yeung: At least in the near future, I think the majority of free light chain testing in clinical labs will still be done using immunoassays because they work well for most cases. Immunoassays do a pretty good job of measuring the total amounts of kappa and lambda light chains, and there's not really an issue in samples where the interpretation of the reference range is straightforward. Mass spec has many strengths, but one challenge is accurate quantitation. Because each light chain clone has a different ionization efficiency, it's hard to have a reliable internal standard.

MASS-FIX now has a quantitative assay that incorporates a nephelometry component to measure the total immunoglobulins in order to normalize the relative amounts of clonal proteins. Another consideration is that in the clinical lab, mass spec tests are often more technically complex to implement, and in many cases, can have longer turnaround times compared to immunoassays, depending on the batching workflow. But given the advantages that I highlighted earlier, mass spec can definitely serve as a complementary testing method to free light chain immunoassays in evaluating patients with monoclonal gammopathies. Whereas immunoassays can quantify kappa and lambda free light chains, mass spec is helpful in directly determining clonality and in identifying post-translational modifications for additional prognostic information.

Bob Barrett: That was Dr. Priscilla S.-W. Yeung from Stanford University in Palo Alto, California. She wrote a new research article on monoclonal free light chain measurement by mass spectrometry in the December 2024 issue of *Clinical*

Chemistry, and she's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.