Clinical Chemistry

Comprehensive Assessment of M-Proteins Using Nanobody Enrichment Coupled to MALDI-TOF Mass Spectrometry.

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Screening Method for M-Proteins in Serum Using Nanobody Enrichment Coupled to MALDI-TOF Mass Spectrometry. Clin Chem 2016;62:1345-1352.
http://clinchem.aaccjnls.org/content/62/10/1345

Guest: Dr. David Murray, Protein Immunology Laboratory, Div of Clinical Biochemistry and Immunology, Dept of Laboratory Medicine and Pathology at the Mayo Clinic.

Bob Barrett: This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Monoclonal gammopathies are a group of plasma cell disorders defined by the presence of an over-expressed monoclonal immunoglobulin, also called M-protein. Assessing M-proteins is an essential component of detecting and managing disease. Traditionally, this has been done by using electrophoretic methods.

However, researchers at the Mayo Clinic have applied mass spectrometry to comprehensively and qualitatively measure M-proteins in patient serum and urine. The October 2016 issue of *Clinical Chemistry* published two articles detailing these methods and their benefits over traditional analyses.

Dr. David Murray, from the Protein Immunology Laboratory, Division of Clinical Biochemistry and Immunology, Department of Laboratory Medicine and Pathology at the Mayo Clinic, is the senior author on both of these articles and he joins us for this podcast. Dr. Murray, protein electrophoresis has been the mainstay for M-protein's testing. What prompted the development of a mass spec-based assay?

Dr. David Murray: Well, the mass spec-based assay was really based on the needs of some of the changes in the clinical testing for multiple myeloma. In the past, electrophoresis has really been the mainstay for myeloma testing or screening since probably their late 1960s, at least here at Mayo, but recent changes and deeper responses from patients, meaning they're going to lower and lower M-protein concentrations, the thought now is maybe we're actually getting close to cures for multiple myeloma, meaning that we need more and more sensitive tests to find the tumor markers for multiple myeloma, it sort of prompted our first look into mass spectrometry because it has better resolution and really, better analytical sensitivity. But not only that, a lot of the testing for clonality of plasma cells has been based on kappa and lambda ratio.

So, the reason we undertook a lot of the mass spec testing was to see if we could get away from the reliance on kappa/lambda ratio and actually measure the tumor marker like we do on other proteins in the body, like albumen or those kinds of things, where we actually measure the protein itself.

Bob Barrett: So, how do you envision these mass spec-based assays being implemented in the clinical lab?

Dr. David Murray: Well, there's going to be quite a shift. The mass spec-based assay is going to be a pretty big departure from a laboratory test that's been around since 1967. So, it's going to be a slow progress, I think. We're going to have to go carefully. We're going to look for -- I think, eventually, we're going to have to get some FDA clearance for these kinds of tests.

So, we're looking at the possibility of doing those kinds of things. For us, in the lab, we're going to start by looking at replacing immunoelectrophoresis to replace IFE in the lab.

Bob Barrett: Do these mass spec assays give new information that's not available by current laboratory methods?

Dr. David Murray: Well, one of the surprising things that we saw along the way as we are developing this assay, which in retrospect should have been intuitive, but it wasn't when we started. It was the ability to separate out the kappa and lambda chain for immunoglobulins.

So, a surprising result for us was the ability to get isotype-specific kappa and lambda ratios, which I think will be very useful in monitoring the immune response of patients receiving either immunotherapy or post stem cell transplant in myeloma to kind of look at the reconstitution of the bone marrow.

Bob Barrett: Why did you choose nanobodies instead of traditional sheep anti-human antibodies for this assay?

Dr. David Murray: Well, one of the things that happened to us earlier as we were developing this assay was that we were using traditional sheep antibodies to immunopurify serum before testing in the mass spec. What we learned was that sheep has the same mass as humans and especially in their lambda light chain with a sheep restricted to lambda light chain.

So, what we learned was, the reduction step, you could actually fool yourself into looking at the sheep lambda chains instead of humans. The nanobodies which are derived from camels or llamas in most cases that have a mass that's totally outside normal human immunoglobulin,

so therefore if they do accidentally come off the column or something like that, they don't interfere with the assay. So, I think that is one of the key things to make this test robust.

Bob Barrett: Finally, Dr. Murray, how are the mass spectrometers used in this article different from those used for micro-bacterial identification?

Dr. David Murray: That's a good question because one of the things that's going to have to happen for labs to adopt this technique would be to actually buy mass spectrometers, which aren't commonly available in a lot of labs.

So, one of the things that we sought to do when we develop this assay was to use platforms that are already making a headway into the clinical lab, and the Bruker Biotyper which is used for bacterial identification, has started to get acceptance into labs.

Actually, the mass spectrometer that we used in this paper and the series of papers that are in this publication is the exact same mass spectrometer; just that it has a different software.

So, we thought in the long run that then this would combine nicely in with the same mass spectrometer that's used for micro-bacteria. Therefore, the lab could maybe adopt this technique a little easier than have to buy a new piece of equipment.

Bob Barrett: Dr. David Murray is from the Protein Immunology Laboratory, Division of Clinical Biochemistry and Immunology, Department of Laboratory Medicine and Pathology at the Mayo Clinic in Rochester, Minnesota. He's been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett, thanks for listening!