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Mass Spectrometry for Identification, Monitoring, and Minimal Residual Disease Detection of M-Proteins

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Guest: Dr. Hans Jacobs is a Laboratory Specialist in Medical Immunology at Radboud University Medical Center in the Netherlands and is the head of the Dutch reference center for M-protein diagnostics.

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

Monoclonal gammopathies are a group of clonal plasma cell disorders characterized by the excretion of a monoclonal immunoglobulin, the so-called M-protein. M-protein diagnostics play an important role in both the diagnosis and monitoring of multiple myeloma and other monoclonal gammopathies. Mass spectrometry is ideally suited for accurate mass measurements or targeted measurement of unique clonotypic peptide fragments making it an ideal candidate to aid in the detection of M-proteins.

A Review article appearing in the March 2020 issue of *Clinical Chemistry* provides a comprehensive overview of current mass spectrometry methods that can be applied to detect, characterize, and quantify M-proteins. The senior author for that article is Dr. Hans Jacobs. He is a Laboratory Specialist in Medical Immunology at Radboud University Medical Center in the Netherlands and is the head of the Dutch reference center for M-protein diagnostics. Dr. Jacobs is our guest in this podcast.

Doctor, first, tell us a bit about M-proteins and why their measurement is important.

Dr. Hans Jacobs: Yeah. Monoclonal gammopathies are a group of clonal plasma cell disorders, and they are characterized by the excretion of a monoclonal immunoglobulin. That is the so-called M-protein, and M-protein diagnostics plays an important role in the diagnosis and the monitoring of monoclonal gammopathies such as multiple myeloma.

Bob Barrett: Why should laboratories improve M-protein diagnostics with novel methods such as mass spectrometry?

Dr. Hans Jacobs: Well, M-protein diagnostics is routinely performed using electrophoretic methods. It is further supplemented with immunoassays to measure free light chains and total antibody concentrations, and M-protein detection and quantification is important for screening and monitoring of monoclonal gammopathies.

However, novel treatment strategies impose new diagnostic challenges. For example, therapeutic monoclonal antibodies may interfere with traditional electrophoretic methods to detect M-proteins, and novel improved treatments also lead deeper responses in increasing number of patients. The latest estimations of the International Myeloma Working Group show that more than 50% of all newly diagnosed patients with multiple myeloma reach a state of stringent complete remission. In these patients, residual disease can no longer be detected using routine diagnostics in blood or urine. Because most patients with stringent complete remission will eventually relapse, more sensitive assays capable of measuring minimal residual disease are urgently needed.

Bob Barrett: Well, currently, minimal residue disease is measured using specimens obtained by bone marrow aspiration. Is that not good enough?

Dr. Hans Jacobs: No, that's correct. Current methods to assess minimal residual disease in patients with multiple myeloma focus on techniques performed on a bone marrow aspirate. The three most used methods are multicolor flow cytometry, allele-specific oligonucleotide-qPCR and next-generation sequencing, and it is evident that among patients with multiple myeloma, these methods can detect disease activity with a sensitivity that reaches far beyond the detection limit of current methods in blood and urine.

Large studies show that MRD assessment can be applied to assess treatment effectiveness, and MRD status is also a major prognostic factor. The strongest limitation of these methods is that disease monitoring must be performed on bone marrow aspirates, and this introduces the risk of nonrepresentative sampling resulting from tumor heterogeneity The patchy nature of the disease has a direct negative impact on the reported results of these methods and extramedullary outgrowth of myeloma cells may give false negative results even after repetitive bone marrow sampling. Besides this, the need for repetitive bone marrow punctures for patient follow-up is a physical burden that reduces the quality of life of an individual patient.

Mass spectrometry performed on a blood sample would be an attractive alternative to the bone marrow-based methods that are currently applied for MR detection.

Bob Barrett: Okay. Measuring disease activity by mass spectrometry: how exactly does that work?

Dr. Hans Jacobs: Well, each M-protein is derived from recombination and somatic hypermutation events of both the heavy- and the light-chain loci of the clonal B cell. As a result, each M-protein has both a unique amino acid sequence and a unique molecular mass. And mass

spec is ideally suited for accurate mass measurements or targeted measurements of unique clonotypic peptide fragments. Based on these features, we can roughly differentiate two mass spectrometry-based methods to measure patient specific M-proteins.

The first one is called top-down MS, in which the unique mass and high abundance of the intact monoclonal light-chain distinguishes the M-protein from the polyclonal immunoglobulin background. In the Mayo Clinic, this method is streamlined into an automated high-throughput method that also allows M-protein characterization. In 2018, this method named MASS-FIX has been introduced in their routine clinical lab as an alternative for immunofixation electrophoresis.

The second is a so-called bottom-up MS method, in which a unique clonotypic peptides are measured for ultrasensitive M-protein quantification. For each individual patient, these clonotypic peptides need to be identified from the hypervariable regions, which make this method more complex. However, bottom-up MS does reach a new level of sensitivity and detects disease activity in blood samples that can potentially compete with MRD testing in bone marrow aspirates.

Bob Barrett: Can mass spec methods provide additional information about M-proteins that's not available through current M-protein diagnostics?

Dr. Hans Jacobs: Well, MS methods can measure the M-protein without interference from biologics. Even in case multiple therapeutic monoclonal antibodies would be administrated to one single patient. In addition, MS-based methods can reveal structural features of the antibodies such as sequence information, polyclonal mass distributions, antibody glycosylation, and other post translational modifications. Our current understanding on what makes some M-proteins pathogenic is very limited. It is interesting to find out why some M-proteins cause no pathology, while other M-proteins cause vasculitis or are highly toxic to organs such as the kidney.

Mass spectrometry may shed new light on this. For example, using top-down MS, it is observed that a relatively large proportion of patients with AL amyloidosis have atypical MS spectra that were caused by glycosylated clonal light chains. Because of this additional information, I anticipate that in the near future, mass spectrometry will play an increasingly important role in the field of M-protein diagnostics.

Bob Barrett: Well, finally Dr. Jacobs, do you believe mass spectrometry will replace myeloma minimal residue disease measurements on bone marrow aspirates in the near future?

Dr. Hans Jacobs: In my opinion, MS methods have rather complimentary value. MRD status evaluated in a bone marrow aspirate provides information that cannot be achieved by mass spec, such as clone evolution and bone marrow reconstitution. Also, MRD evaluated in bone marrow provides valuable information in the rare event that the myeloma clone does not secrete an M-protein, and it is important to realize that the M-protein is a surrogate marker of the cellular disease state. A confounding factor is the half-life of M-protein, which is on average 21 days for IgG and 10 days for IgA.

This causes a delay between the lysis of clonal plasma cells and the actual decrease of the M-protein levels in the blood. In contrast, an MS based method allows dynamic MRD monitoring by serial blood sampling without the trauma of repeated bone marrow aspirations, and also assures assessment of extra-medullary disease, which is not evaluated by bone marrow biopsy. As such, we anticipate that in the near future, mass spectrometry will not replace existing MRD tests in bone marrow, but they will have clinical value as a complimentary method especially for dynamic monitoring of MRD in blood.

Bob Barrett: That was Dr. Hans Jacobs from the Radboud University in the Netherlands, and he has been our guest in this podcast on using mass spectrometry for M-protein diagnostics. He is the senior author of a Review article describing that approach. His article appears in the March 2020 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.