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Clonotypic Light Chain Peptides Identified for Monitoring Minimal Residual Disease in Multiple Myeloma without Bone Marrow Aspiration

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Guest:
Dr. Robert Bergen is Associate Professor in the Department of Biochemistry and Molecular Biology and a Collaborative Scientist in the Proteomics Core at the Mayo Clinic in Rochester, Minnesota.

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.

Multiple myeloma is a disease that is characterized by amplification of a single plasma cell clone producing a monoclonal immunoglobulin. That clone proliferates in the bone marrow and can result in bone destruction.

The monoclonal LG produced by these cells can circulate as intact protein or as a free light chain, with no heavy chain. Multiple myeloma accounts for about 1% of all cases of cancer, and the diagnosis often requires obtaining and examining bone marrow specimens.

The January 2016 issue of *Clinical Chemistry,* a special issue devoted to mass spectrometry and the clinical laboratory, published a paper describing a liquid chromatography-tandem mass spec method with the ability to measure disease in patients that are negative by bone marrow-based methodologies.

The data indicate that this serum-based approach has more analytical sensitivity than traditional methods and requires no bone marrow aspirate.

The lead author of that paper is Dr. Bob Bergen, an Associate Professor in the Department of Biochemistry and Molecular Biology and a Collaborative Scientist in the Proteomics Core at the Mayo Clinic in Rochester, Minnesota.

Dr. Bergen is our guest in this podcast. Doctor, how is minimal residual disease in multiple myeloma currently determined?

Dr. Bob Bergen: There are three basic ways that clinically it's determined. The first one is they just take a bone marrow sample, make a slide and stain it, and then a pathologist looks at it and counts plasma cells.

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Another way, still counting plasma cells, is multicolor flow cytometry and they will label bone marrow plasma cells, put them through a flow cytometer and then do actual counting, and you get better statistics that way.

And finally, there is a new company that has started taking bone marrow samples, and doing next generation sequencing. They will find the sequence of the prominent clone, and then they monitor that in subsequent bone marrow samples for minimal residual disease measurements.

Bob Barrett: Do all current sensitive tests all require a bone marrow aspiration?

Dr. Bob Bergen: Yeah, all the most sensitive tests do. There are some serum-based tests, but they are not very sensitive, so the go-to tests all require a bone marrow aspiration.

And actually it's quite an expensive thing here at the clinic. It might cost about $5,000 just to collect the aspirate, which is then -- there are other charges on top of doing one of the tests that I just spoke about. So yes, it’s fairly expensive, and it’s an invasive thing, so it would be nice if we could get away from that.

Bob Barrett: Well, my understanding is that your new test does not require a bone marrow aspiration. Now talk us through that, how is that possible?

Dr. Bob Bergen: Yeah. So multiple myeloma is a plasma cell cancer and plasma cells are the cells that generate antibodies in your body. So when one of these plasma cells become cancerous, it’s producing a monoclonal antibody, and this is secreted into the circulation. And if you had a way to distinguish that from the polyclonal background, meaning all the other antibodies circulating in your blood, you can use that as a marker for the presence of these cancerous cells.

So what we have shown is that if you start with a high M-spike serum sample, and M-spike is actually the antibody coming from that clone, you start with that when the patient’s plasma cell clone is very abundant, so when the disease is first diagnosed, you have a good chance of identifying tryptic peptides that originate from that clone and that correspond to the complementary determining regions of this immunoglobulin, in our case the light chain only.

And this is then accomplished basically by two major advances. The first is that the mass spectrometers that are available today provide real high mass accurate fragment

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ion spectra, so the MS/MS spectra have high mass accuracy and are just spectacular. And more specifically the mass spec that I am talking about are these Q Exactive mass spectrometers that incorporate a quadrupole mass filter in front of an orbitrap.

And secondly, is that de novo sequencing software is available that allows for amino acid substitutions for sequences that are not in a database, so normally we search a database of known sequences, and try and match MS/MS spectra to that database.

So there is a lot of now de novo software packages, but we have used successfully this PEAKS software that comes from Bioinformatics Solutions.

Bob Barrett: What kind of sample preparation is required for this?

Dr. Bob Bergen: Well, all of our initial work thus far has been done utilizing 1D SDS Gel. So you take a high M-spike serum sample, and separate on a 1D gel about half a microliter of serum, and then you excise the light chain band and digest that with trypsin after reduction and alkylation. And that disease levels then are measured in subsequent samples the same way; we cut out the light chain band and separate it on a 1D Gel and process it as for the M-spike samples.

Bob Barrett: So we are all prepared, what happens next?

Dr. Bob Bergen: So as mentioned previously, the tryptic peptides from the M-spike protein need to be identified, and again, this is done at the beginning of the disease, when it's diagnosed, and to accomplish this you need this high M-spike light chain band. So you submit it for LC-MS/MS analysis on a Q Exactive mass spectrometer. You submit it to a database search and a de novo search. Then that data is filtered based on two criteria.

The first one is abundance. So we want to identify a tryptic peptide that belongs to the plasma cell clone, and these will be peptides that are high in abundance. So they rise above the polyclonal background in abundance. We don't want to misidentify tryptic peptides in a polyclonal background for those coming from the plasma cell clone.

And secondly, we want to identify tryptic peptides that ideally come from the complementary determining region of the immunoglobulin variable region. We want to do this because the M-spike protein is a mature antibody, typically, and the peptides mapping to the CDR regions will be the most unique or proprietary to that patient.

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In our initial work we have only looked at kappa and lambda immunoglobulin and light chain, so the abundant peptides we need to identify have two qualifiers. First they have to be high abundance; and secondly, they have to map to a CDR region of the variable gene.

And we found that abundance and mapping is most easily done in some new software, which was developed by Amol Prakash at a new proteomics software company called Optys Tech Corporation.

And what we are doing is taking the output of PEAKS, which does the database search and the de novo search and inputting this into Amol's Pinnacle software. And what Pinnacle does, it creates a base peak ion chromatogram, and by color highlights to what protein each peak in this base peak ion chromatogram maps to.

And if you take your cursor and hover over each of those peaks, a little pop-up will appear and it will detail what the sequence of that particular peptide is and the protein that it matches to. And then you can interrogate the peaks to determine if it is associated with a light chain variable region.

So inside Pinnacle there are several browser windows linked to different software tools; for example, UniProt and BLAST and PeptideAtlas, but for this work most importantly is this IMGT/DomainGapAlign algorithm. And when you have clicked on one of the peaks of interest in the base peak ion chromatogram, this DomainGapAlign algorithm aligns and maps the sequence of that particular peak to the light chain immunoglobulin variable sequences in the IMGT database, and you can immediately see if your target sequences maps to a CDR.

And ideally, we are looking for two or more tryptic peptides from each patient to monitor, and currently an MRD negative result would be when the target peptide could no longer be found in the serum.

Bob Barrett: Well, the obvious next question would be, how does this method compare with the current standard of care?

Dr. Bob Bergen: So we have initially looked at 62 myeloma patients, and of these, 20 were MRD negative by immunohistochemistry, and the remainder of the 42 were by 6-color flow cytometry. So they are all negative when we looked at them, and we were able to identify a target peptide in 57 out of 62 of these patients, so about 91% applicability.

And of these 57 we were able to detect disease by LC-MS in 52 of the 57, or again 91%. So it appears that we are still

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detecting disease when the current methods bring back a negative result in the majority of patients. You have to realize that multiple myeloma is currently an incurable disease and most of these patients will die of this disease.

So a couple of things could be going on here which we are trying to sort out. And the first is that the half-life of immunoglobulins range from about 5-21 days, with some of the IgGs having the longest half-lives of 21 days.

So if you start with this M-protein or M-spike concentration of a half a gram per deciliter, which is maybe an average value, it would require 262 days to achieve a level of 0.001 grams per deciliter, and at this level the LC-MS/MS sensitivities would never fail to detect a clone. So this is something that we are trying to address right now.

However, with the use of serum collection compared to doing a bone marrow biopsy, it will be easy to take a serial blood sample and just monitor levels with time and get a sense of the disease progression which is either going up or going down.

So the clinicians are really interested in where their patients are disease-wise. Is the chemotherapy that they are taking, is it addressing the level of the clone, knocking it back or not?

Secondly, is the apparent redundancy of the immunoglobulin sequences, and this is something that we are still trying to sort out.

So Dominique de Costa in a recent paper in *Journal of Proteome Research* showed that in seven normal individuals that they looked at, 83% of the tryptic peptides of IgG Fab fragments were found in all seven individuals.

So though we are detecting the plasma cell clones peptides as indicated by abundance, these same sequences could also exist in the polyclonal background. So we are trying to sort this out.

Although we are pretty sure that we are identifying the peptides that are coming from the clone, we are trying to ascertain that when we look at those, are they truly coming from the clone or are they also in the polyclonal background.

Bob Barrett: Well, finally doctor, let’s look ahead, where do you go from here?

Dr. Bob Bergen: Well, in our initial work we have only looked at the light chain, so immunoglobulins are comprised of heavy and light

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chains and they both have variable regions. So we want to look at, and add to our analysis, the heavy chain, so that's what we are currently working on.

And we think that if we add the specificity of adding CDR tryptic peptides from heavy and light chain variable regions, we will get additional specificity that we don't have with just looking at the light chain.

And finally, we want to define what is negative MRD by LC MS/MS. So is it when there is no detectable peptides so we just can't see them anymore in our analysis, or when they fall below some predefined signal-to-noise thresholds? So we are currently in the process of investigating some of these issues.

Bob Barrett: That was Dr. Robert Bergen, an Associate Professor in the Department of Biochemistry and Molecular Biology, and a Collaborative Scientist in the Proteomics Core at the Mayo Clinic in Rochester, Minnesota. He has been our guest in this podcast from *Clinical Chemistry* on using LC tandem mass spec in the diagnosis of multiple myeloma. His research appeared in the January 2016 issue of *Clinical Chemistry*, a special issue devoted to mass spectrometry and the clinical laboratory.

I am Bob Barrett. Thanks for listening!

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