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Interlaboratory Agreement of Insulin-like Growth Factor 1 Concentrations Measured by Mass Spectrometry

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Guests:
Dr. Larry Bowers is Chief Science Officer of the U.S. Anti-Doping Agency, and Dr. Andrew Hoofnagle is Director of Clinical Mass Spectrometry at the University of Washington in Seattle.

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.

Insulin-like growth factor 1 is a key mediator of growth hormone action and a biomarker of growth hormone abuse. Current immunoassays for IGF-1 suffer from poor agreement among different methods, which makes comparison of results among laboratories difficult.

In the March 2014 issue of *Clinical Chemistry*, a paper reported a new LC Tandem Mass Spec Method to quantify IGF-1 in serum and evaluated inter-laboratory performance for this analyte.

We are joined by two authors of this paper: Dr. Larry Bowers is the Chief Science Officer of the U.S. Anti-Doping Agency, and Dr. Andrew Hoofnagle, Assistant Professor and Director of Clinical Mass Spectrometry in the Department of Laboratory Medicine at the University of Washington. They are both our guests in this podcast.

Dr. Bowers, let's start with you. How did your group come together and how did all of this get started?

Dr. Larry Bowers: There are a number of attractive characteristics about LC-MS/MS measurement of protein analytes, including a specificity identification of the analyte and so on, that make them really attractive for anti-doping.

Harmonization of results is also an important consideration. So in 2008, USADA organized a working group by asking several researchers what it would take for them to develop a good LC-MS/MS serum assay for IGF-1, and the researchers said they wanted a homogeneous stable isotope-labeled internal standard, and a well-characterized reference

material, and so that was the beginning of the collaboration.

In 2009, USADA discontinued its independent research program and transferred the financial support of the working group to a group called the Partnership for Clean Competition, or PCC, which is a research-funding consortium that was founded by the U.S. Olympic Committee, the U.S. Anti-Doping Agency, Major League Baseball, and the National Football League.

So the working group, which consisted of five laboratories from three countries, participated in the study, and we actually held a teleconference, if you will, an international research group meeting, about once a month to review results and talk about what the next experiment would be. So that's how things came together.

Just one final comment, I really do have to compliment each of the five groups for putting aside personal interests and really working as a group. This research was a great example of research findings that can only be accomplished through group effort, and I really don't think that in general this kind of research gets enough credit.

Bob Barrett: Well, Dr. Hoofnagle, as a Medical Director of the Clinical Laboratory at the University of Washington in Seattle, why were you interested in a new assay for IGF-1?

Dr. Andrew Hoofnagle: Well, clinically we are using IGF-1 as a biomarker for growth hormone function, much like in the anti-doping world we are using it as a biomarker of growth hormone doping, and clinically, growth hormone deficiency in kids can result in short stature and delayed development, but in adults it really messes with metabolisms, specifically, bone metabolism. Because of its importance as a counter regulatory hormone and glucose metabolism it can have issues with diabetes, etcetera.

Growth hormone excess in kids causes gigantism and in adults can lead to acromegaly, both of which have their own health issues.

So together IGF-1 and growth hormone can be used to evaluate growth hormone biology, pituitary function, and most importantly, IGF-1 because of its higher concentration and it has a longer half-life, it's

a better marker overall, and if it's normal, we can rule out abnormalities of the excess.

So IGF-1 is a really great biomarker to understand and clinically evaluate growth hormone function.

What we know about immunoassays is that we are sometimes frustrated by immunoassays, and it really stems from how different manufacturers put their immunoassays together, and so for instance, different immunoassays, they have different antibodies that they use in their assays. They make their own calibrators, they have different calibrators and really just the addition steps of the reagents etcetera are all different. So really these are all just different methods.

The results are, we'll get different IGF-1 results from manufacturer to manufacturer to manufacturer. In addition, each sample, every sample that we take from a patient, has a different mix of potential interferences, so on different platforms we can get very different results, even as they are precisely very different from manufacturer-to-manufacturer.

So we might say that those results aren't entirely accurate even if they are extremely precise. The hope clinically is that we could get an assay, an IGF-1 assay, that could be rolled out in many different clinical laboratories that would give consistent results from patient to patient, really allow us to identify the reference range very specifically, and give us a better clinical tool for understanding growth hormone function in our patients.

Bob Barrett: From a basic science and analytical chemistry point of view, how did your study in *Clinical Chemistry* update the field of proteomics?

Dr. Andrew Hoofnagle: What we have learned, not only do different manufacturers get different results, we also learned and we actually published this in the *Clinical Chemistry* paper that we are talking about today, we published an example of using exactly the same immunoassay in two different laboratories, same everything. We bought the reagents from the same manufacturer, all the calibrators from the same manufacturer, and deployed that assay in two different laboratories. Now granted they were in two different countries but we don't think that that should really have anything to do with any differences that we might see. However, we saw up to 40% differences between results from exactly the same

immunoassay platform run in two different laboratories, and we decided to turn to bottom-up proteomics as a way to try to normalize and equalize the playing field.

Now bottom-up proteomics is interesting, first because it uses trypsin or other proteases to cut up proteins into pieces, and thereby removing a lot of the interferences that can cause those sample-specific slight inaccuracies and destroying them, turning them into peptides. The liquid chromatography can separate the peptides that we're interested in measuring from all the other peptides in the sample, and then finally mass spectrometry can specifically detect the peptide that we are interested in.

So one of the hopes and the dreams was that using this kind of technology, we would be able to improve concordance between laboratories because we could directly calibrate on the peptide that we were measuring, and as Dr. Bowers talked about, having an N-15 isotope labeled internal standard IGF-1 molecule, goes even one step further. We would be able to calibrate on the entire protein of interest because we would be measuring multiple peptides from that protein that we are interested in specifically detecting the peptides using mass spectrometry.

Now we know that one of the hardest things in bottom-up proteomics is that the trypsin digestion step is variable. It's variable from day-to-day but it's also variable from sample-to-sample, and that N-15 isotope labeled internal standard can control for a lot of that digestion variability.

What we were also hoping to do is to have good calibration between day-to-day and site-to-site and so we started making calibration curves using standard reference material available from NIBSC, and we did a pretty good job at pretty good precision, but we had a hypothesis that, well, maybe there is some variability associated with resuspending the standard reference material in making calibration curves each in our own individual laboratories. What if we tested the hypothesis that using a single point calibrator and each batch, could that reduce the variability, and this was the hypothesis that we had from a previous paper that had been published in *Clinical Chemistry* by my group in 2010, that showed that single point calibration could reduce variability from batch-to-

batch, thus removing some of the variability of the digestion.

And so what we did was used a single-point calibration approach in this paper and showed, indeed, that if you remove the steps involved in making a calibration curve, you could reduce the variability from laboratory-to-laboratory even further. And so what we demonstrated to the field was that bottom-up proteomics can be used to quantify proteins in clinical specimens, and it can be used on many different instruments in many different countries to get really precise results that can now be used clinically from my point of view, or from Dr. Bowers' point of view in anti-doping programs. But not only for IGF-1, this kind of approach can now be translated into any protein in human serum or other samples, which is really very exciting for us.

Bob Barrett: So Dr. Bowers, as Chief Scientific Officer of the U.S. Anti-Doping Agency, why were you and your agency interested in a new assay for IGF-1 that uses mass-spectrometry?

Dr. Larry Bowers: Anti-doping results are primarily used for forensic purposes, that is, they are used as evidence of a non-compliance with the rules of sport. We have been interested for more than a decade in developing a test for the abuse of growth hormone and actually two tests have been developed in parallel.

One test, which was described some years ago in *Clinical Chemistry* by Bidlingmaier, looks at recombinant versus pituitary-derived isoforms of growth hormone. The second test, which is the one we are interested in here, looks at biomarkers of growth hormone activity and one of those biomarkers is IGF-1.

Incidentally, there have been about 35 peer-reviewed publications on the biomarkers approach to growth hormone abuse detection. But for forensic purposes, it's preferable not only to quantify the IGF-1, but also to be able to show that you've identified the compound that you have measured as IGF-1, and the LC-MS/MS assay allows us to do both in one method.

Bob Barrett: Well finally, let's look ahead. Where is your group headed now?

Dr. Larry Bowers: Well, in addition to preparing a suitable stable isotope labeled internal standard, one of our goals

was to develop a certified reference material that would assist in harmonizing results both among the anti-doping laboratories as well as clinical laboratories.

We are currently with the National Institutes of Science and Technology to establish the IGF-1 concentration in a serum matrix for calibration purposes. We are hopeful that NIST will complete this project in the next 12 to 18 months, and then we'll have a good reference material to be used in all fields.

Our other goal, initially, was to develop both a top-down and a bottom-up assay for IGF-1; a top-down assay being measuring the intact protein essentially. We specifically made the N-15 stable isotope labeled IGF-1, so that we could actually use it as a good internal standard for both approaches. We've successfully published the bottom-up assay, that's what we are talking about today, and as Dr. Hoofnagle explained, we have been able to contribute knowledge about how to best calibrate peptide LC-MS/MS methods.

Our research also showed that a significant part of the differences between laboratories is related to the digestion steps. So we are excited about the possibility that a top-down assay might improve the accuracy and precision of the peptide LC-MS/MS quantification method even further, and those advances will benefit both anti-doping and the clinical world.

Bob Barrett: Dr. Larry Bowers is the Chief Science Officer of the U.S. Anti-Doping Agency, and Dr. Andrew Hoofnagle is Director of Clinical Mass Spectrometry at the Department of Laboratory Medicine at the University of Washington in Seattle. They have been our guests in this podcast from *Clinical Chemistry*.

I'm Bob Barrett. Thanks for listening!