

Bob Barrett:

This is the podcast from '*Clinical Chemistry*'. I am Bob Barrett. Many deaths related to Type 2 diabetes are attributed to coronary artery disease, and much research is centered on the connection between poor glucose control and negative cardiovascular outcomes. Individuals with Type 2 diabetes are frequently monitored by assessing circulating concentrations of hemoglobin A1c. Although epidemiological connections remain under debate, recent large randomized trials have failed to translate tight control of hemoglobin A1c into any cardiovascular benefit.

The risk of myocardial infarction has even been reported to increase in association with certain classes of anti-diabetic therapies. In response, the U.S. Food and Drug Administration issued a Guidance for Industry suggesting that developers of new anti-diabetes drugs demonstrate that therapies will not result in an unacceptable increase in cardiovascular risk. Consequently, there is a need for new markers used in the monitoring of Type 2 Diabetes and related cardiovascular complications.

In a paper published in the May issue of '*Clinical Chemistry*', Dr. Chad Borges and Dr. Randall Nelson of the Molecular Biosignatures Analysis Unit at The Biodesign Institute at Arizona State University reported the biomarker development studies undertaken to characterize protein microheterogeneity and evaluate its use in creating multidimensional biomarker views related to the pathobiologies of Type 2 diabetes and cardiovascular disease comorbidities.

Dr. Borges and Dr. Nelson are both our guests in this podcast. So Dr. Nelson, what are you referring to in the title when you state '*Multidimensional Biomarker Views of Type 2 Diabetes*'?

Dr. Randall Nelson: Yeah. So multidimensional, in our case, has at least two different uses. First off, multidimensional in the first order we are using multiple markers to track across from healthy to diabetes to cardiovascular, so instead of just one marker, many.

But more importantly, the way that we kind of work this out is that we have aligned the biomarkers with different pathobiologies in your body. For instance, oxidative stress, glycation, and each one of these different modalities represents in our mind a different dimension.

And so when we go into this term multidimensional analysis, we are really trying to relate that to the multiple pathobiologies in your body, and it seems to work at least in our hands and it has shown a lot of promise to start to pry these diseases apart from each other.

Bob Barrett: Can you comment briefly on the added utility of using markers that indicate biological processes that extend beyond the control of blood glucose alone?

Dr. Randall Nelson: Oh, yeah, yeah, yeah. In fact, that's a general working paradigm in diabetes right now. Glucose control, obviously, if you are diabetic, got to do it, right? But then it hits its limit, and I wish it didn't, but it hits its limit in trying to anticipate other outcomes, i.e., in our case, cardiovascular disease, renal failure, et al. And so you are going to have to switch to a different -- looking at a different dimension in your body, some other pathobiology, and so that's what we are tapping into right now.

In the utopian world, you look at all the pathobiologies in your body, in every disease. What we are doing is we are starting with one and moving to two and three. How many are there? Probably many. So we are just getting started on that.

Bob Barrett: Well, now, what exactly is protein microheterogeneity?

Dr. Randall Nelson: First off, it's a hard word to say.

Bob Barrett: I understand.

Dr. Randall Nelson: Technically it refers to, if you will, post-translational modifications, genetic variance, really anything that happens to the protein. It's sometimes environmentally induced, i.e., a protein is not, often not, an exact and only one species in your body. It can have post-translational modification variant that might be at a lower level.

And so that's what it refers to. It's kind of if you name a protein, hemoglobin for instance. It can have a number of different variants, and all those variants collectively are referred to microheterogeneity.

Dr. Chad Borges: And of course the microheterogeneity can alter the function or the pre-described nominal function of whatever that protein may be?

Dr. Randall Nelson: Absolutely! That was Chad Borges, by the way.

Bob Barrett: And we will get to him later. How common is this microheterogeneity? Does it happen with all proteins?

Dr. Randall Nelson: The way we have been seeing it, it seems to be on all proteins. I am not going to say it's on every single one, but it's very, very common, and often the microheterogeneity -- now remember, it's just not the qualitative definition of their being in other species, you know another variant form, but

also the quantitative variation in these, i.e., the stoichiometries, and so some of these variants might be at a part per thousand relative to the predominant species in your body.

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So when you have the ability, for instance, in the mass spec approach, like we use, to look at dynamic ranges of a thousand. Let's say you start to discover more and more of these low level variants are occurring. I am not going to say it's in everything, because that's just setting myself up for failure, but it's pretty much in everything we have seen so far.

Bob Barrett: Well, why did you decide to employ mass spectrometry as your analytical approach?

Dr. Randall Nelson: That goes back almost 20 years. Mass spectrometry or proteins showed up on the scene about 20 years ago, in early 90s. And so I have been studying proteins, and all of my group has been studying proteins with mass spectrometry for years. So in our case it's an exceptional analytical tool.

Why did we decide to employ it? Well, we had it. What this give us? It turns out to be an excellent way to look at this microheterogeneity. So in a strange way it's a match made in heaven.

Bob Barrett: Well, Dr. Borges, what's different about your approach using mass spectrometry compared to other studies that use mass spectrometry to look for biomarkers of disease?

Dr. Chad Borges: So when we talk about using mass spectrometry to look at biomarkers of disease, generally where it focused on analyzing proteins, that's generally what people are talking about.

And so at the protein level there are at least three different modalities at which you might identify a biomarker. The first modality, and the one that's most commonly focused on in a mass spec based analysis of biomarkers, is changes in protein concentration; the protein concentration go up or down in disease state.

The difference, the key difference I think that we are focusing in on here are qualitative variations in proteins, not that other approaches can't look at these, but the way that we look at them, by analyzing the intact protein, we can at sort of a 20,000 foot level, see in essence every modification that's going on in the protein and then look at each modification in and of itself as a possible marker.

As Randy was saying, one of the key things that we find are changes in protein post-translational modification, i.e., different bells and whistles that are attached on to the protein backbone after it's made, often caused by environmental changes.

Another thing we can get at is changes in the genotype of a person. So we can look at both the protein phenotype and the genotype at the same time.

Bob Barrett: Are you saying you can get genetic information from your analysis?

Dr. Chad Borges: That's correct, actually we can. So numerous human proteins, many of them, have several common different genetic variants and in many of these cases the DNA changes that encode for the genetic variants cause a change in the amino acid sequence of the protein. That change in the amino acid sequence of the protein in turn causes a mass shift of the intact protein. It's often very subtle, but using advanced mass spectrometric techniques we are able to detect that mass shift and determine, for example, whether a person is homozygous for a particular allele, heterozygous for particular allele, or even possess a rare and very uncommon genetic variant by looking at the protein level.

Bob Barrett: Can unanticipated protein microheterogeneity interfere with mass spectrometry immunoassay-based assays?

Dr. Chad Borges: It's actually sort of the opposite of interfering. In other words, as we see it, when something like this occurs, when some sort of unanticipated protein modification occurs, what we do is we see it, we can identify it using mass spectrometry, and then by virtue of how mass spectrometry operates we are able to put it in its own analytical register or bin, if you will.

So in general, the answer to that is no, because we can immediately see it, tell what it is, and in the vast majority of cases it's in a distinct position within the mass spectrum and so arises as a distinct signal and does not end up interfering.

Bob Barrett: Well, once you identify a protein variant of interest, do you recommend continuing to use mass spectrometry or switch to another platform?

Dr. Chad Borges: So yeah, we actually do think that the best approach is to indeed continuing to use mass spectrometry, and that's for a couple of reasons. I guess if one were to consider switching platforms probably the first candidate in line as an

analytical platform would be a conventional ELISA assay. So in other words, convert this assay, where we are looking at a particular protein species to an ELISA assay.

Well, the difficulty is that with some of these post-translational modifications and genetic variance that we are seeing, there would be some extreme barriers in terms of technical difficulty and cost to build an ELISA assay that could actually be specific for these unique protein variants that we are looking at.

So that's almost a nonstarter for many of these protein variants that we are looking at, just in terms of the technical difficulty and the cost of building the ELISA.

In addition, once let's say you were successful in building the ELISA, there is still a disadvantage in that if some new protein variant comes along, it could still interfere with that ELISA and you would have no idea about it.

The nice thing about continuing to use mass spectrometry is that if some new variant within the population shows up, in most cases as I was saying, we can see it, identify it, and put it in its own register and have it actually not interfere with the assay.

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Bob Barrett: Are there any similarities between the types of markers you are studying and conventional biomarkers already used in clinical practice today?

Dr. Chad Borges: Well, the short answer to that is yes. In our paper, we appear to be exclusively focusing on this microheterogeneity, and for the most part, we are, but it's not something that's entirely novel to clinical medicine. In fact, it's very commonplace.

HbA1c in fact is the best example. So for those who aren't familiar with what HbA1c is, HbA1c is a modified version of hemoglobin. It's a version of hemoglobin in which a glucose molecule, because of its build up in the bloodstream, has become covalently or permanently attached to the protein backbone of hemoglobin. And so this is the type of modification that we are looking at, and yes, indeed, it is employed widely in clinical practice today. In fact, the American Diabetes Association recently changed their guidelines to be able to diagnose diabetes based on measurements of HbA1c.

So it's a very common, well-accepted, clinically mode of biomarker, it's just up until recently there aren't that many examples.

Bob Barrett: Well Dr. Nelson, this work appears promising for diabetes. Do you think your approach to biomarker development would be applicable towards other diseases, such as cancer?

Dr. Randall Nelson: Sure, because what we are really doing here, and I will answer this in the general and then the more specific, what we are really doing here is studying protein behavior. More of an esoteric way of thinking about it is, how does a protein behave? What are its idiosyncrasies when its circumstances are helping diabetic, cardiovascular disease, cancer, just keep that list rolling.

So in a broader sense what we are really doing is studying proteins in a mode we call Population Proteomics. Now, you see enough of this idiosyncratic behavior, that's where your biomarkers come from. And then putting them together in the multidimensional views, like we described in the '*Clinical Chemistry*' paper, is kind of the next step to it.

Inside of that whole thing though is, once we get a good biosignature for let's say diabetes, by necessity we have to look at it in cancer, just to see whether it has any mimics. If there is any confounders in between the two diseases, and just flip that over.

If we started studying in cancer, as our populations, we would sooner or later have to look at diabetic, so on and so forth. So what we have done is we have set up programs, of course around the diseases; diabetes, cardiovascular disease, and cancer, and we are doing the same thing right now in cancer cohorts, not necessarily with the exact same proteins that we described in this paper, but essentially looking for this idiosyncratic behavior of these proteins in the microheterogeneity realm relative to cancer, healthy, and then challenged cohorts on the side.

The short answer to what your question is, is yes.

Bob Barrett: Dr. Chad Borges and Dr. Randall Nelson are from the Molecular Biosignatures Analysis Unit at the Biodesign Institute at Arizona State University. They have been our guests in this podcast from '*Clinical Chemistry*'.

I am Bob Barrett. Thanks for listening!

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