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Comparing Multiple Measures of Glycemia: How to Transition from Biomarker to Diagnostic Test?

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Guests:
Dr. Robert Cohen is from the University of Cincinnati, and Dr. David Sacks is at the National Institutes of Health in Bethesda, Maryland.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Although the underlying pathophysiology of diabetes varies, all patients have in common a metabolic derangement of carbohydrate metabolism, which results in hyperglycemia. Many patients with diabetes develop debilitating complications, ranging from retinopathy and nephropathy to myocardial infarction and stroke.

In the December 2012 issue of *Clinical Chemistry*, Drs. Robert Cohen and David Sacks wrote an editorial 'Comparing Multiple Measures of Glycemia: How to Transition from Biomarker to Diagnostic Test.'

Drs. Cohen and Sacks are our guests in this podcast. Dr. Cohen, tell our listeners a bit about this editorial.

Dr. Robert Cohen: So the editorial is entitled 'Comparing Multiple Measures of Glycemia: How to Transition from Biomarker to Diagnostic Test,' and it's really a commentary on the paper associations of alternative markers of glycemia with hemoglobin A1c and fasting glucose.

Let me talk a little bit about that paper first and then I will talk a little bit about our editorial.

So the paper is really addressing a comparison of different measures of glycemic control across a population and really looking for where the similarities are and the extent to which, there are basically commonalities between the populations demonstrable by regression.

And I think that the subtext to it is, there are situations in which hemoglobin A1c, which is a measure of glycemic control, is confounded by other variables and the question is whether

some of the other markers may be useful in those situations in which hemoglobin A1c is confounded.

I come from the background of comparing hemoglobin A1c to another marker and what I have used in the past has been largely fructosamine and so there are some differences in perspective on this thing.

My perspective is that these should be largely in agreement with each other, but that there are physiologic variables, which can cause them to deviate from each other, and the question is how much of the variation is due to physiology and how much is due to measurement variation.

Dr. David Sacks: Yes. I think that there certainly are limitations to hemoglobin A1c and to fasting glucose. In 2010, the American Diabetes Association recommended that hemoglobin A1c be used for diagnosis of diabetes and this has been accepted by other international clinical bodies, including World Health Organization, International Diabetes Federation, and it has been adopted in quite a few countries.

One of the reasons for doing that is that practically, fasting glucose is not that easy to get from patients, because often when the patients see their doctors they are not fasting. Fasting glucose and the oral glucose tolerance tests have been historically the gold standard for diagnosis of diabetes.

So the question that this paper addresses in some context is in those situations where patients aren't fasting and fasting glucoses cannot be used or hemoglobin A1c cannot be used, can one use other markers of glycemia? And the markers they chose were two glycated proteins, namely fructosamine and glycated albumin as well as 1,5-anhydroglucitol.

Now 1,5-anhydroglucitol is not a glycated protein, it’s a six carbon monosaccharide that’s not metabolized, and is normally reabsorbed by the kidneys, but when the blood glucose is high, glucose gets excreted in the urine. The glucose in the urine competes with 1,5-AG for reabsorption and the 1,5-AG – reabsorption in the kidney is reduced, so the circulating concentration of 1,5-AG goes down.

In terms of the fructosamine and glycated albumin, fructosamine is glycated protein in the blood. Now albumin is the protein in the highest concentration in the blood, but

fructosamine includes things other than albumin whereas the glycated albumin assay measures exclusively glycated albumin.

The lifespan of red blood cells averages with approximately 120 days in many people and the half-life of albumin and proteins in the blood is much shorter. So the fructosamine and glycated albumin reflect the glucose concentration of a much shorter time period than hemoglobin A1c.

Dr. Robert Cohen: So the argument is that in the vast proportion of people who have normal metabolism of red cells and normal metabolism of serum proteins, including albumin, there should be a pretty close relationship between those people, but part of the work that I have been involved in has been to show that there's more variation in the survival of red cells than has generally been understood. So that we've done that with some tracer techniques; I won't go into the details of that today.

But there are certainly situations with altered protein metabolism such as in protein-losing situations like the nephrotic syndrome which is very, in diabetes it's certainly one of the most common causes of nephrotic syndrome, where there is an over-excretion of albumen which shortens its survival in the blood and therefore alters its survival on the question of how well its metabolism allows it to reflect the level of glycemic control, because there aren't adequate corrections taken in the way the analyses are done.

Bob Barrett: You talked about the argument that has been made in the article. Can you talk about the particular strengths and weaknesses of that argument?

Dr. Robert Cohen: Certainly. There certainly is a correlation between these various measures besides hemoglobin A1c, with hemoglobin A1c on the one hand. On the other hand, they are not perfect because of physiologic variation and measurement variation. To narrow the scope of our discussion, the 1,5-anhydroglucitol is probably the least well correlated, and they show fairly convincingly that it would not make a very good substitute for hemoglobin A1c.

And then in terms of the analysis of the comparison of the other protein, I would say the weaknesses are that they try to interpret the relationship in terms of a certain form of mathematical modeling and I personally think that there is some tricky business to how you select the modeling and how you design the population from which you'd want to analyze those relationships. And so I think the combination of the

populations used in the modeling are somewhat of a limitation here.

What that means is that if I wanted to really find out the true biologic relationship between hemoglobin A1c and one of these other variables, I would want to have a dataset that really represents the whole spectrum of glycemic control.

And essentially, what they have done is they have used a population which is a real-world population. And so on the one hand the strength is that, that is a real-world population, that it's a population we actually deal with, but it may not be the ideal population for illustrating the relationship. So, I think that the population is not great for getting at the biology, but it is a fair representation of what's in the population.

Dr. David Sacks: I think you stated that quite clearly that the population is limited in some sense that it doesn't have a very wide representation of glucose concentrations because the number of patients with diagnosed diabetes was less than 20%.

There was also limitation in terms of the number of patients with renal failure which as we've discussed can influence some of these markers.

So I think that the concept is that perhaps the studies should be done in two phases; the first is the entire range of glycemic control, and then the second would be to apply this information generated from that to a general population cohort.

Dr. Robert Cohen: So, this concept of a two-stage process would be use the first stage to describe the biology, and then--in order to figure out the form of equation that best describes the biology, and then use equation that you validated that represents the biology and examine the real-world population, so that you can separate out: what are the problems with modeling, from what are the variations across the population.

When you do the whole thing with one population, you can't tell which of the issues are due to the population and which of the issues are due to the modeling.

Bob Barrett: It's a unique combination in this issue of the original investigation and then the editorial. What do you think readers are going to take away from that combination?

Dr. David Sacks: I think that one of the advantages of doing that, is an editorial puts the original paper in a broader context and hopefully will stimulate thinking in the mind of the reader about issues that are raised both in the paper, but also in the editorial which addresses obviously issues that can't be addressed in the paper.

Dr. Robert Cohen: And certainly we both have been medical school faculty and involved in teaching residents and fellows and we try to teach critical thinking in reading papers, but there's still an issue of, it's very tough to come up with all of the issues and all of the perspectives that would allow you to critically evaluate an individual paper when you're learning to do critical reading of papers.

And the hope is that by having an editorial here where you've got the perspectives of a couple people who work in the area, that we can bring out some ideas that wouldn't necessarily pop to mind for somebody who haven't spent as much time thinking about this as we have. And that it will broaden their perspective on how to read this paper and by inference will be a teaching device for more critical reading of other papers.

Bob Barrett: Dr. Cohen, about a year ago Dr. Sacks was the lead author of an editor entitled 'Gaps in the Glycation Gap Hypothesis' and you were the originator of the term glycation gap. So what is the glycation gap and how did the two of you happen to get together to co-write this editorial in *Clinical Chemistry* now?

Dr. Robert Cohen: So I started the term glycation gap about eight or nine years ago. I was looking at the comparison of one glycated protein to another and using it as a measure of the deviations of one from the other. And we use that first to determine whether there were reproducible variations in this relationship, and if there were variations in the relationship, what kind of significance they would have.

And so we first defined this measure we called the glycation gap, this deviation of the measured hemoglobin A1c from the prediction based on the related, the simultaneous, fructosamine measurement, and we used it to look at complications.

It turned out that the population that we looked at was a small population and we got somewhat anomalous results from our things, which I think confused people for many years.

It's a very tricky area to understand, because the basic concept is that we're talking about whether there are systematic

variations between hemoglobin A1c and other measures, and whether that relates to the underlying biology, whether the part of the reason that the correlation of diabetic complications with hemoglobin A1c is not just that hemoglobin A1c reflects glucose, but it also reflects the glycation and other metabolic processes that affect the relationship between glucose and protein.

So, it is a nonconventional idea and again, we had an odd population and I think part of the concept holds up, but there are some limitations to the concept which David and his co-authors hit upon, and it's really forced us to try to communicate a little bit better just what this means and what the limitations are to the technique.

Dr. David Sacks: I think one of the critical issues is how one actually identifies this gap, because clearly while using fructosamine is an easy way to do it, fructosamine has some limitations. In fact, if one thinks about fructosamine and glycated albumin, they have some advantages in terms of the assays, because the assays are rapid, technically easy. They can readily be performed on high throughput instruments that are available in most clinical laboratories and they're relatively inexpensive.

In contrast to those advantages, some limitations we've already discussed, that they're influenced by factors other than glycemia, but two of the other issues are that there are limited clinical studies and probably if one looks at PubMed, the literature, there are only certainly less than 5% of the number of papers have been published on this compared to hemoglobin A1c.

There is also an absence of outcome data that unequivocally link them to computations of diabetes, and in contrast to hemoglobin A1c, there is no agreed target values for glycemic control.

Now having said that, the hemoglobin A1c is obviously glycation of an intracellular protein in red cells. Fructosamine and glycated albumin are extracellular and so not subject to hemoglobinopathies, red cell lifespan, and other issues regarding transport of glucose into cells.

So, this is one way to evaluate this. The fructosamine assay has been around much longer than the glycated albumin, but is subject to more interferences and as indicated in the paper by

Juraschek et al., the glycated albumin actually correlates better with the hemoglobin A1c than does fructosamine.

Glycated albumin has been very widely used in Japan, but is not yet FDA approved, in the US, the assay that was used in this paper is not FDA approved. And it will be interesting to see how this evolves with time, and I anticipate that we will get more insight into this glycation gap as more studies are published. And I think the paper by Juraschek et al. does contribute to our knowledge by adding more information that is very valuable.

Dr. Robert Cohen: So, I think that David's point there brings us full circle back to the original thing which is that the way that criteria were set with hemoglobin A1c for the diagnosis of diabetes was that there were certain values of hemoglobin A1c that line up with the level of glycemic control at which there is the first appearance of complications of diabetes.

So, there is that kind of data for hemoglobin A1c. There doesn't currently exist the same kind of data for some of these other glycemic markers. I think that that will come out of some things that are in the works, but those would really be key to trying to use some of these other markers as substitute criteria.

I think if you derive a hemoglobin A1c for diagnosis based on retinopathy and then you derive the criteria for interpreting the next generation of markers against hemoglobin A1c, then you get further away from your original gold standard, from your original criteria, and you end up with creeping of the numbers away from the source of validation as to what they really mean. And you get into a lot of these differences that in the correlations that have been the sources of disagreement and confusion and sometimes emotional, and sometimes cool, calm, and collected.

So, I think that we're a ways away from really being able to use these other markers as direct substitutes, but we're moving in that direction. And my own view is that there is some very interesting biology and some more insights in the path of physiology that come from looking at these subtle variations and the relationships between these two.

We threw out the term glycomics in the paper as analogous to genomics and proteomics and metabolomics as a way of saying that there are really a lot of different ways of measuring these things in that there is a science to looking at the interactions

between these and where they agree and where they disagree and what information content comes from examining that agreement and disagreement.

Dr. David Sacks: Yes, I think that the term glycomics is in context with proteomics and genomics and would at least instill in readers' minds that there is a whole area about which we know very little. And the hope is that this would prompt further research to lead to an enhanced understanding of the molecular mechanisms of the glycation, and ultimately enhancing our comprehension of this is likely to result in better use of the laboratory tests of glycated proteins which should yield benefits for patients with diabetes.

Bob Barrett: Dr. Robert Cohen is from the University of Cincinnati, and Dr. David Sacks is at the National Institutes of Health in Bethesda, Maryland. They have been our guests in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.