Clinical Chemistry

Development and Validation of Apolipoprotein AI-Associated Lipoprotein Proteome Panel for the Prediction of Cholesterol Efflux Capacity and Coronary Artery Disease

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Guest: Dr. Cory Bystrom is Executive Director of Research and Development with Cleveland HeartLab in Cleveland, Ohio.

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.

High-density lipoproteins, or HDLs, are macromolecular assemblies that play a key role in lipid transport, but also exert effects in endothelial function, thrombosis, and inflammation. A recent focus by several groups on HDL function rather than HDL cholesterol revealed that efflux capacity was inversely associated with coronary artery disease. Traditionally, cholesterol efflux is measured using a cell-based assay where cultured microphages are treated with radioactively-labeled cholesterol and subsequently exposed to a cholesterol acceptor.

A recent study that appeared in the February 2019 issue of *Clinical Chemistry* took a completely different approach and assessed the lipoprotein proteome by using liquid chromatography and tandem mass spectrometry to measure 21 lipoprotein associated proteins. We are pleased to have the senior author of that paper as our guest in this podcast. Dr. Cory Bystrom is currently Executive Director of Research and Development with Cleveland HeartLab in Cleveland, Ohio. So, Dr. Bystrom, most people are familiar with HDL cholesterol as the "good" cholesterol. Why are you interested in looking at the proteins and function of HDL particles?

Dr. Cory Bystrom: Well, thanks for asking that question. Examining HDL function has been an area of interest for investigators that want to unravel the rather enigmatic behavior of HDL. Epidemiological studies have demonstrated an inverse relationship between HDL cholesterol and cardiovascular risk, giving rise to the HDL hypothesis. This HDL hypothesis is that if HDL cholesterol is as protective, then interventions to raise HDL cholesterol should have a health benefit.

Unfortunately, the body of evidence that now includes the number of randomized clinical trials had failed to demonstrate clinically significant reductions in adverse cardiovascular events even when HDL cholesterol has been raised substantially. This had led people to kind of raise the possibility of thinking about HDL in terms of -- it's not how much HDL cholesterol you have, it's actually how well it functions.

Bob Barrett: Well, let's dig a little deeper. What exactly is the HDL proteome and how is it related to HDL function?

Dr. Cory Bystrom: The HDL proteome is the term used to describe the collection of proteins that constitute the macromolecular structure we recognize as high-density lipoprotein. Studies to investigate the HDL proteome have explored the diversity and composition revealing a collection of 80 to 120 proteins which appeared to be consistently associated with HDL. For the listeners out there, I'd like to recommend HDL Proteome Watch website hosted by Sean Davidson at University of Cincinnati. It's a great compendium of mass spec-based HDL proteome research.

We know that HDL has biological activity. Some of this has been characterized by biochemical studies that predate mass spec-based proteome work. However, now that we have proteomic tools available, we can identify the proteins individually and by knowing their identity, we can associate them with function.

Clearly, there are proteins involved in lipid transport, but interestingly, there are proteins involved in acute-phase response, antioxidant function, and protease inhibition to mention a few. By looking at the changes in the protein composition, this does provide an insight into the basic functional properties of HDL. Most recently, these changes have been described in the relationship that's coronary artery disease. This work was done by Jay Heinecke.

Bob Barrett: Doctor, what was your approach to exploring the relationship between HDL structure and function?

Dr. Cory Bystrom: Our approach to examining these relationships was to examine an HDL function for which there is an associated disease and to recognize a well-developed analytical methodology. Then, our approach was to try to tie these two elements together to quantitative proteomic studies.

Bob Barrett: And why did you select cholesterol efflux as a key function of HDL for development?

Dr. Cory Bystrom: Our interest in cholesterol efflux was inspired by the insightful work published by Dan Rader and Phillip Shaw, in the *New England Journal of Medicine* in 2011 and 2014. They demonstrated that an in vitro measurement of the degree to which HDL cholesterol was independently and inversely associated with cardiovascular events. This provided a potential pathway to which the failure of the HDL hypothesis could be investigated.

The tool that was used to measure cholesterol efflux for these studies was biological assay, one not ultimately amenable to use in a clinical setting. So, we decided to investigate if the changes in the HDL proteome could be correlated with cholesterol efflux. When we found a number of strongly associated proteins, we then proceeded to build a predictor of cholesterol efflux based on a multiplex measurement of HDL-associated proteins.

And once we had a model in hand, we moved on to do validation, analytical validation studies, and ultimately clinical validity studies.

Bob Barrett: Well, finally, doctor, let's look ahead: what do you see in the future for your HDL proteomic assay?

Dr. Cory Bystrom: We think that future is really bright. I'm happy to say that we have a clinical validity study with an external calibrator that has just been accepted for publication, which offers additional insight into the clinical validity of the HDL functioned biomarker panel that we've developed.

In addition, we also now have a very nice proteomic pipeline to measure multiple HDL proteins with very good precision. The work in our *Clinical Chemistry* manuscript started with 20 proteins, but our second revision adds another 12. Given the large array of biological functions that are implied by the HDL proteome, we are interested in continuing to investigate opportunities that may yield more biomarkers associated with the HDL particles.

Bob Barrett: That was Dr. Cory Bystrom from the Cleveland HeartLab, and he's been our guest in this podcast about cholesterol efflux, the lipoprotein proteome, and coronary artery disease. That article appears in the February 2019 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.