

**Article:**

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Ceramide Risk Scores Can Bring Lipidomics to Clinical Medicine

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Guests: Dr. Paola Ramos from the Biorepository Laboratory at the Mayo Clinic and Dr. Jeffrey Meeusen from the Cardiovascular Laboratory Medicine program, the Clinical Specialty Laboratory, and Laboratory Services at the Mayo Clinic.

Bob Barrett:

This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. The prevalence and associated mortality of cardiovascular diseases is a major clinical challenge. The identification of patients at highest risk for cardiovascular events is essential for individualized treatment. The risk of those events in patients varies substantially among individuals.

Conventional lipid concentrations have been commonly used to assess this risk, as well as different lipoprotein subclasses. But what about other lipids? A ceramide, a phospholipid based cardiovascular risk score, has been found to predict the risk of cardiovascular disease events, particularly mortality. The December 2022 issue of *Clinical Chemistry* published a paper examining the association of ceramides and phosphatidylcholines with cardiovascular diseases in the elderly.

The same issue had an accompanying editorial highlighting that research. The authors of that editorial are Dr. Paola Ramos, a senior associate consultant in the Department of Laboratory Medicine and Pathology and co-director of the Biorepository Laboratory at the Mayo Clinic in Rochester, Minnesota; and Dr. Jeffrey Meeusen, co-director of the Cardiovascular Laboratory Medicine Program, the Clinical Specialty Laboratory, and the Laboratory Services, also with Mayo. We are pleased to have both of those authors with us as guests in this podcast. First of all, Dr. Ramos, you mentioned lipidomics in your editorial. Would you mind explaining just what that means?

Paola Ramos:

Sure. When we talk about omics as a field of study, we're actually referring to disciplines in biology that look into a collective characterization and quantification of molecules that are important for the function of organisms. Historically, the first omics term that was used was used for genomics when looking at information in the DNA. It has become a shorthand to describes any field.

We now hear terms of with omics, for example, proteomics, looking at all the proteins that are translated from RNA, and in our case, lipidomics, it refers to the study of the complete lipid profile that is present within a cell at a tissue level, organism, or even an ecosystem. It is regarded as a subset of the metabolome, which would also include other molecules such as sugars and amino acids.

Overall, lipidomics aims to capture temporal alterations in the concentrations and composition of lipid species when cells go through changes on their normal or pathological conditions. There are hundreds of kinds of lipids, and understanding more and more about the main players and their role in human disease is crucial for the discovery of biomarkers.

Bob Barrett: Well, how exactly do you measure a lipidome?

Paola Ramos: Lipidomics has been incredibly accelerated by spectrometric methods especially sub-ionization techniques. Mass spectrometry, in particular liquid chromatography mass spectrometry, is essential and it has made lipidomics more available for research laboratories as clinical laboratories as well around the globe.

We often think of lipidomics or metabolomics in two main ways, either a targeted or untargeted way. As it sounds, untargeted metabolomics is set to look for anything that is present within the sample under instrument specifications. We're looking for hydrophobic molecules within a size range, for example, while targeted metabolomics looks for more specific molecules in a predetermined way.

In general, lipidomics studies start with a control population and a disease population. After sample collection, in this case serum, serum is prepared to extract the lipids from it and then add the standards. During the analysis, lipids are then separated and quantified by chromatography and coupled with mass spectrometry or NMR. As you can imagine, this methodology generate enormous amounts of data. Informatics has also played an important role in the development of these fields.

Bob Barrett: Dr. Meeusen, how does lipidomics help diagnose disease?

Jeffrey Meeusen: As Dr. Ramos just mentioned, typically we are comparing and contrasting two different populations. It starts out with a well-characterized set of patients. Then you go through the technical bit of measuring in an untargeted fashion, these hundreds or even thousands of lipids. Then you have to go to the informatics level. You're looking at which lipids have increasing concentration versus those that have decreasing concentration in comparison to our outcome of interest.

In the case of ceramides, the outcome of interest has been cardiovascular events, like a heart attack or a stroke or coronary revascularization procedure. It turns out that even among patients that have similar risk factors that we know are associated with heart disease like older age, hypertension, diabetes, cholesterol, et cetera, the lipidome actually is very different among patients that go on to have an event compared to those that do not.

Bob Barrett: Do we have to measure the entire lipidome?

Paola Ramos: Well, it would be nice and this is how we add to our understanding, but many studies have focused in highlighting specific lipids that function as biomarkers, such that we can measure a few to get a snapshot of what's happening physiologically, for example, ceramides.

Bob Barrett: Well, why ceramides?

Jeffrey Meeusen: This most recent paper that our editorial was in response to looked at a lipidomic score based on a handful of ceramides and a few phospholipids. It began like the other descriptions we've discussed where lipidomics was untargeted but they found empirically that these handful of ceramides and some phospholipids were able to capture the majority of that signal for who was going to have the outcome.

What's also unique about this particular cohort was that the group was older patients that already had a history of heart disease, so they were theoretically at quite high risk and yet they were able to identify using these few ceramides and phospholipids who was at more risk of having a heart attack amongst that high-risk background.

Bob Barrett: That's one study. How much evidence is there for ceramides as important markers for cardiovascular disease?

Paola Ramos: Well, as mentioned by Dr. Meeusen, the availability of liquid chromatography mass spectrometry was necessary for the discovery of ceramides and other small molecules. Now, there has been many studies that have shown that ceramides and other sphingolipids accumulate in tissues in individuals with heart disease. There's been dozens of studies looking at more than 30,000 patients demonstrating an association of ceramides to cardio complications, including coronary artery disease, diabetes, heart failure, and even death.

It's worth mentioning that there's also been many studies looking at animal models that have shown that inhibiting the synthesis of ceramides can prevent the development of such diseases. Many different researchers have published associations between ceramides and heart disease. How does

this apply to the clinic where they're concerned with individual patients and outcomes?

Jeffrey Meeusen: One of the weaknesses of applying any omics technology where you measure so many different things is there's a chance that you could be confusing the clinician or the patient or just providing confounding results that confuse more than help the management decisions. When we measure biomarkers for the clinic, we want to be able to say, "Is this meaning that we're indicating high risk or are we indicating low risk?"

And so, what we've found is that when you have multiple markers, the best practice is to incorporate them into a score so that you can account for the fact that maybe one of them was suggesting high risk and the rest were suggesting low risk, and then overall incorporate that into a single answer that says you're either high, low, or indeterminate.

These scores are also good because as you measure so many different molecules or in lipidomics, you need to account for the variation that's involved with all the different measures as well as the variation involved with each different molecule just in a normal functioning day-to-day. Since that's so much different information to include, really you have to come up with some sophisticated algorithmic risk scores and then verify those scores at determining outcomes and helping to manage patient care.

We are somewhat used to this concept because right now, cardiovascular risk is typically gauged by incorporating a person's age, their sex, whether or not they have hypertension, whether or not they use tobacco products, and it gives out an estimate. What we're doing now is adding a layer on that where we would say, "Your lipidomics inputs are actually able to give you a more precise risk estimation."

Bob Barrett: Have there been many studies published on these sorts of risk scores?

Paola Ramos: Yes, there have been and the ceramide score has been well-studied, we can say. The original CERT1 score consisted of three single ceramides and three ceramides to ceramide ratios, whereas the newer CERT2 score had one ceramide to ceramide ratio, two ceramide to the phosphatidylcholine ratio in a single phosphatidylcholine. This score is the one that is mentioned throughout the management that we're talking about.

Now, the second CERT2 score was developed using the WECAC study that was the Western Norway Coronary Angiography Cohort, and was validated by very big studies like KAROLA and LIPID studies. There was a very large study

called STABILITY that included various places around the world and their results of the entire cohort showed that the CERT2 was able to improve the predictive value compared to the original.

Bob Barrett: Well, finally, when can we expect ceramides to be more routine in mainstream clinical diagnostics?

Jeffrey Meeusen: There's been a lot of effort spent on educating medical professionals and the general public about how we should manage our cardiovascular disease risk. Everybody now knows that they should watch their cholesterol and their diet and maybe even monitor their cholesterol with lab testing. This has taken extensive resources to get us to the point where everybody has this level of medical literacy. I don't think we're going to replace that anytime soon. However, there is a place and quite a few providers and now are starting to appreciate that amongst individuals where you have perhaps an indeterminate risk or if you're struggling to get a patient to see the benefit of starting a lipid lowering regimen like diet and exercise, or even a pharmacological lipid-lowering therapies, you could apply something like a risk score to help motivate the patient to see that their number of the risk score can actually be effective in the near-term by taking some of these steps.

That actually works well because ceramides can be lowered by the same things that we would do to lower cholesterol, the things we know that lower cardiovascular risk. That said, as that becomes more and more aware, I think it'll be more routine, but really, what's going to be the biggest driver that clinicians typically wait for before they start widespread use of a given test is we need to show that reducing ceramides without also reducing cholesterol is beneficial to patients, and until we have some pharmacological intervention, I think that might be slowing us down.

Bob Barrett: That was Dr. Jeffrey Meeusen from the Mayo Clinic in Rochester, Minnesota, and he was joined by his colleague, also from Mayo, Dr. Paola Ramos. They have been our guests in this podcast on ceramide risk scores and lipidomics in clinical medicine. Their editorial, as well as an original scientific paper on that topic, appears on the December 2022 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.