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

LDL and HDL are well-established independent risk factors for cardiovascular disease. A report in the July issue of *Clinical Chemistry* hypothesized that patients with both elevated HDL cholesterol and ischemic heart disease may have a dysfunctional form of HDL or unrecognized, unconventional risk factors.

Dr. Amar Sethi is the lead author of the report and Vice President of Science and Technology at Pacific Biomarkers in Seattle, Washington. He is our guest in this podcast.

Tell us, Dr. Sethi, in your paper, you use the term, "dysfunctional HDL," what exactly do you mean by that?

Dr. Amar Sethi: Well, first of all, let me just thank *Clinical Chemistry* for organizing this podcast and having me here today. The word dysfunctional goes back to the original hypothesis that we tested in the study. We planned this study, because we wanted to understand why a minority of patients who have no other risk factors, and in fact have high HDL cholesterol that can develop ischemic heart disease.

So as you may understand, this is very unexpected. So we decided to test the hypothesis that the patient with ischemic heart disease and elevated HDL cholesterol levels and no apparent risk factors may have these HDL particles that are not functioning as they tend to physiologically and thus the term used, and they may have dysfunctional HDL.

Host: Now, could you explain the design of your study, especially the inclusion criteria? An inclusion criteria was no ongoing treatment with statins. In this day and age, how is that even possible?

Dr. Amar Sethi: Yeah, that's correct. That was difficult, but was very important for us. As we all know, statin therapy changes the lipid levels and perhaps also the actual functionality of the lipoproteins. So what we wanted to do was exclude the patients on statin therapy so that we could remove any potential confounding effects.

However, we also knew that this would decrease the study size, as majority of the patients would be on statins, but nevertheless, we found this to be too important to ignore, so we did it.

Let me explain the design. The study participants were patients referred to the Copenhagen University Hospital for coronary angiography due to an ischemic heart disease condition. So all of these patients were from the University

Hospital, and all the control subjects of the study were from the Copenhagen City Heart Study, which is a prospective cardiovascular general population study.

So initially, we had like 2,000 patients with ischemic heart disease to begin with, which then of course decreased dramatically to around 340 subjects when we applied it to the inclusion criteria, which were no ongoing statin treatment and normal levels of LDL cholesterol and triglycerides.

And then from those 340, what we did was we selected and separated them into two different groups; individuals with very high and individuals with very low HDL cholesterol levels, by choosing the 98th percentile and the 10th percentile as the delimiting factor.

So what we had was two groups with ischemic heart disease, one with very high HDL levels and one with very low HDL levels. Both of these groups were then age and gender-matched to subjects without ischemic heart disease. So basically we had four groups in a 2x2 design comparing ischemic heart disease patients to subjects without ischemic heart disease, with either very high or very low HDL cholesterol levels.

Host: With that in mind, you measured something called pre-β_1 HDL, which apparently was increased in patients with heart disease. Exactly what does this molecule do and why would you choose to measure it?

Dr. Amar Sethi: That's very interesting. Let me just take a step back here and mention that the very first thing we did was actually to measure or examine the composition of the HDL particle, as that also can relate to the function of the particle.

We examined the major apolipoproteins that are attached to the HDL particle. We also examined the subfractions of HDL by two different methods; one was the segment and gradient gel electrophoresis, another one was using a tube gel system.

But the surprising thing here was that, we didn't find any major differences, and that is why we turned our focus towards one of the HDL particles that are not normally identified with either a subfraction method, which is called the pre-β_1 HDL.

So what is pre-β_1? Well, pre-β_1 HDL is the nascent ApoA-I particle, whose main function I would say is to pick up cholesterol from the peripheral cells, like we know, macrophages and foam cells, when they are loaded with cholesterol, they are called foam cells.

So the whole process of picking up cholesterol and bring it back to the liver for removal is what we call the reverse cholesterol transport pathway.

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In this process, HDL maturation takes places and HDL size increases. Pre-β_1 is the initiator of this process and a very important particle. But there is another one called Lecithin–cholesterol acyltransferase, also called LCAT. This is an enzyme, which is further downstream of pre-β_1 HDL, and it's very important for the effective removal of cholesterol. As this enzyme, what it does is esterifies the cholesterol and then it packs it into the HDL particles.

Now, we found that the pre-β HDL was increased in subjects with ischemic heart disease, which initially did not make sense. Because why would a good particle, which is removing cholesterol, be increased in patients with ischemic heart disease, it should have been in patients with no heart disease.

Well, what we learned was that, although pre-β_1 in theory is a good particle, but its exact function cannot be determined before, after you have accurately assessed the other parameters down the stream of the HDL cholesterol removing process, like the action of the LCAT.

So in order to completely understand HDL's role in the reverse cholesterol transport, you need to closely examine every single step in this process, we believe. And if other process downstream are malfunctioning due to other reasons, it may affect the pre-β_1 HDL levels, which is exactly what we observed.

So our results indicated that high levels of pre-β_1 and low LCAT activity levels were associated with ischemic heart disease in subjects who otherwise did not have any other risk factor ischemic heart disease, and who also had high HDL levels.

So what this also means, and the bottom line here is that, the development of ischemic heart disease may be more closely related to a combination of factors involved in the reverse cholesterol transport pathway, rather than just one particular metric such as HDL cholesterol.

Host: So then, is your conclusion, do you suggest screening for the pre-β_1 HDL and LCAT biomarkers?

Dr. Amar Sethi: It depends on the patient's HDL cholesterol levels, on other risk factors, and family history. So certainly screening for

LCAT or pre-β_1 HDL makes lot of sense and would be suggesting specific cases.

Let me try and explain that. We examine subjects with isolated low HDL cholesterol levels and we know that this occurs in like about 30% of the population. And according to the guidelines of National Cholesterol Education Program, these subjects with no other risk factors would not be considered at increased risk for ischemic heart disease, and thus they would be not likely to be considered for treatment either.

So our study suggests that pre-β_1 HDL and LCAT, they are tests that can be useful in identifying the subset of patients that in fact are at increased risk for ischemic heart disease, but whom otherwise would be missed.

Secondly, individuals with increased levels of HDL, which is, in majority of cases, we have to stress that it protects against cardiovascular disease, of course, but you may have patients who nevertheless develop ischemic heart disease, as I said before. In those cases and in cases where there is a positive family history towards ischemic heart disease, I think our results suggest that measuring LCAT and pre-β_1 HDL could be beneficial to identify these individuals earlier, long before they would develop ischemic heart disease.

These subjects would never have been identified in the GP's office before, because all their lipid values would be within the reference range. And I think we need to start approaching HDL by not only assessing it by a quantitative measure, but also develop biomarkers that are useful in assessing its functionality, and high HDL may not always be protective.

Host: What do you see are the next steps following the study?

Dr. Amar Sethi: First of all, being a small study, our result needs to be confirmed in a much larger population study. I think that's very important. I do hope that future studies would include the LCAT and pre-β_1 HDL as tests in their clinical studies. If they include them, they will see how well they perform as diagnostic markers.

I think also, we need to explore subjects with normal HDL levels, also exhibit similar trends, as we have observed in patient with extreme levels. We only looked at the very high and very low, we don't know what happens in the middle. So I think that's important to examine that, and that would also determine the wide usability of these tests.

I think also an interesting spinoff from all these results could be to explore the HDL particles from the four different

groups in a study, where you look at it on a proteomics approach, where you would look at all the proteins attached to the HDL particle and explore whether they are abundant in the patient population with ischemic heart disease or not. This could help us later identifying novel biomarkers that would assess the HDL particles' functionality and development of these more traditional assays that can assess the HDL particles' functionality, rather than just relying on the HDL cholesterol levels, are also needed.

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Host: Well, finally doctor, would you care to elaborate on what tests you are considering?

Dr. Amar Sethi: Well, I think as the scientific community, we would need to understand that HDL is just much more complicated than first realized. It may not be surprising anymore, I think, when considering the numerous functions of HDL, that no single HDL feature, such as HDL cholesterol, can completely explain or capture all of the lipoproteins' antiangiogenic property. So I think we need to realize that we need to move on and beyond HDL cholesterol level.

I know a lot of people are focusing on developing tests that effectively can assess the function of HDL. Some of the tests that I am currently working on is to measure the antioxidative effect of HDL.

So we are trying to set up a method, which is called the Oxygen Radical Absorbance Capacity, which would be able to test the HDL's total antioxidative capacity. That's one of the major functions of HDL as well.

Secondly, we are also currently working on cell-based assay that can measure the anti-inflammatory capacity of the HDL, by examining its effect on some key inflammatory mediators.

Finally, the most commonly used right now is an assay called cholesterol efflux assay. It measures the capacity of HDL to efflux cholesterol. This is in its current form not really adaptable to a high throughput format, and therefore not properly utilized, but I hope that in the future it will be.

I think we would need to not only develop new and better test of HDL functionality, but also make them useful for clinical labs and pharmaceutical companies who currently are developing these HDL-raising drugs.

I think the pharmaceutical companies could use the same biomarkers to characterize the effectiveness of new and

existing drugs designed to modulate the HDL cholesterol concentration.

So biomarkers like pre-β and LCAT, they are just the beginning. Fully capturing all of the functions of HDL is still warranted and will hopefully be accomplished in the near future, by having more sophisticated high throughput biomarkers of HDL functions.

Host: Dr. Amar Sethi is Vice President of Science and Technology at Pacific Biomarkers, and he has been our guest in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.

Total Duration: 12 Minutes.