

Bob Barrett:

This is the podcast from '*Clinical Chemistry*'. I am Bob Barrett. Low-density lipoprotein cholesterol is a major risk factor for cardiovascular disease. It is the primary target for lipid-lowering therapy and is used to classify patients into various risk categories.

The reference measurement procedures for LDL cholesterol and HDL cholesterol are based on ultracentrifugation to remove VLDL and chylomicrons followed by heparin-manganese precipitation to remove the LDL.

Although this method is impractical for routine use, it has been validated in large clinical studies and is the standard to which all routine methods are compared.

Until relatively recently, LDL cholesterol was estimated from the total cholesterol, HDL cholesterol and triglyceride using the Friedewald Equation.

This formula, one that first appeared in the classic paper in '*Clinical Chemistry*' nearly 40 years ago, becomes progressively less accurate with increasing triglyceride concentration and requires fasting prior to obtaining the sample.

In addition, bias and imprecision from the three separate measurements used in the calculation may also affect the accuracy of calculated LDL cholesterol.

To address these limitations, various homogenous reagents for the direct measurement of LDL cholesterol have been developed and are now widely used.

These methods do not depend on the measurement of triglycerides, making them less influenced by non-fasting samples. However, these direct measurement methods may not have complete specificity for LDL cholesterol and may not offer a significant advantage over simply calculating the LDL cholesterol.

In a study published in the March issue of '*Clinical Chemistry*', Dr. Alan Remaley, a senior staff member of the National Institutes of Health, and a team of researchers compared many of the direct LDL cholesterol methods on the market to the established reference procedure.

They found that the directly measured LDL cholesterol methods frequently fail to meet National Cholesterol Education Program total error goals on dyslipidemic samples when compared to the

ultracentrifugation reference measurement procedure.

Dr. Remaley and co-author, Dr. Manuel Deventer, are our guests in this podcast.

Dr. Remaley, a major finding in this study is that many of the available direct measurements of LDL cholesterol did not perform better than the calculated LDL cholesterol. Were you surprised by these results and would you recommend that the calculated LDL cholesterol replace direct LDL cholesterol measurement for all or most patients?

Dr. Alan Remaley:

Well, I don't think that the calculated LDL should replace the direct in all patients, but I think what we were surprised is that the calculated LDL is based on other laboratory parameters that the laboratory for most part is already measuring as part of lipid screen and that was used to calculate the LDL by the Friedewald Equation.

The direct LDL is an additional task. So everyone's expectation is that if you do the additional expense of doing this task instead of calculating it, that it should have value.

So we were surprised that in many cases that the calculated LDL did appear to match better the reference method, the gold standard, although the gold standard method, which is the beta-quantification, may be has some issues, which we will talk about in the subsequent questions.

So what we found was in patients that had triglycerides less than 200, which is probably the majority of the population, about five out of the eight direct tests that we measured that you were closer to matching the beta-quant, the estimation from the LDL cholesterol using the beta-quant by actually calculating rather than doing the direct. As I said, because the direct involves an extra test and extra cost, that was a surprise and a disappointment, because there is I think very little rationale there for doing the direct test.

But there was actually quite a range, however, and some direct tests did a fairly good job, but as I said, five out of eight, you are better off with the calculated.

But we did find two settings in which the direct LDL I think is important consideration or may add value.

One is those patients with triglycerides over 200; in that case, the direct LDL was better than the calculated in terms of matching the beta-quant for cardiovascular risk classification.

As I said, we are not assessing clinical endpoints here, and that's also an important limitation to the study we will talk about later. So if you have high triglycerides, you were probably better off getting a direct test, but that is the minority of the patients that most laboratories would see.

The other population which I think the direct LDL still may have an advantage is in the case where people aren't fasting. And it's always disappointing, but I would say a third of the patients that we have that come in through the Cardiology Clinic, oftentimes forget to fast and that really plays havoc with the calculated, because the calculated and Friedewald Equation uses triglyceride to estimate the VLDL cholesterol.

LDL cholesterol and HDL cholesterol doesn't change much after the postprandial state, but you need to have the triglyceride measured in a fasting state, because you estimate the LDL cholesterol by usually taking triglycerides when mixed or divided by five, and that relationship doesn't hold if you have a different kind of particle that you have during the postprandial state, which is chylomicrons.

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So that the direct LDL may be useful in people who aren't fasting, but there have been two studies; one was a large study from the Women's Health Study. For one particular assay, this showed that the direct assay in the postprandial state was not as good of a marker for cardiovascular disease compared to when it was used in the fasting state, and there was another smaller study that showed the same thing.

So although, operationally you don't need necessarily to not fast or direct; I am not quite sure whether there is a decrease in the predictive value as a biomarker for the direct LDL assays.

So in summary, I think in many cases one can use the calculated LDL and one doesn't have to do the additional expense, but for patients with high triglycerides and may be fasting, you may want to reserve the direct LDL test for those settings.

Bob Barrett:

Dr. van Deventer, in this study, LDL cholesterol was measured using the beta-quant ultracentrifugation reference measurement procedure at the CDC. It was further limited to a dyslipidemic population.

Do you think there are some patients where the reference measurement procedure may not accurately reflect true LDL cholesterol values?

Dr. Manuel van Deventer:

Well, thank you very much. I think it's important to realize that the beta-quantification procedure used to measure reference LDL cholesterol can be sensitive to cholesterol, both in intermediate-density lipoproteins and lipoprotein(a).

For direct LDL cholesterol methods that we evaluated are truly specific for LDL cholesterol and may very well show a negative bias compared to the beta-quantification reference measurement for LDL cholesterol, as we in fact observed in this study.

Although, reference LDL cholesterol measurement by beta-quantification seemed impractical for routine everyday use, it has been validated as a cardiovascular disease biomarker in many of the large clinical studies.

It's also important to realize that the apoB-containing lipoprotein fractions also contribute to calculate LDL cholesterol and non-HDL cholesterol, which may account at least in part from observed improved performance in cardiovascular risk score classification for these markers.

In my opinion, it's important for the future studies where clinical endpoints are performed to assess the clinical utility of the various direct measurements for LDL cholesterol and HDL cholesterol and to resolve the uncertainty by the clinical significance of the lipoprotein fractions that are being excluded or measured in these direct assays compared to ultracentrifugation reference measurement procedures.

Bob Barrett:

Now Dr., non-HDL cholesterol calculated using direct HDL cholesterol and total cholesterol results showed the best correspondence to the reference method procedure and better harmonization in cardiovascular risk score classification.

Non-HDL cholesterol was a better indicator than both direct and calculated LDL cholesterol methods for samples with both low and high triglyceride

concentrations. What do you think may be the reason for this?

Dr. Alan Remaley:

That wasn't actually a surprise, because as you said, non-HDL cholesterol is simply total cholesterol minus HDL, and the calculated LDL using the Friedewald Equation is LDL cholesterol equals total cholesterol minus HDL minus VLDL cholesterol, and the VLDL cholesterol is estimated by, as I said earlier, taking triglycerides divided by five.

So you can see from looking at the equations that the equation is very similar, but the calculated LDL has additional term where we are estimating VLDL cholesterol by using triglycerides.

So we described in this paper and it has been shown many times that, that is actually relatively poor relationship sometimes between VLDL cholesterol and triglycerides and it's highly variable depending on the patient.

In addition, you have additional error from having to make the additional measure of triglycerides. So I think the fact that non-HDL cholesterol better match the reference method for determining non-HDL cholesterol wasn't a big surprise given the uncertainties in estimating VLDL cholesterol.

Bob Barrett:

At this stage, would you then recommend that non-HDL cholesterol replace LDL cholesterol as a primary screening test for cardiovascular disease and are there any other advantages to non-HDL cholesterol?

Dr. Manuel van Deventer:

I think firstly, looking at this study, it's very important to realize that an important limitation of this study was that we didn't have any clinical outcomes. So looking at this study we can't answer that question.

I think before non-HDL cholesterol can be recommended as a primary screening test, it will be important to not only establish its superior correspondence to the reference measurement procedure, but also to show that it's at least equivalent to LDL cholesterol for cardiovascular disease risk prediction in diabetic patients.

If increased triglycerides in non-HDL cholesterol has been shown in several large studies to be superior to LDL cholesterol in predicting cardiovascular disease risk, and this may partly be true because of the apoB-containing lipoproteins such as remnant

lipoproteins also significantly contribute to the pathogenesis of atherosclerosis and diabetics.

Several epidemiological studies have also shown that non-HDL cholesterol in the general population is at least equivalent or better than LDL cholesterol and apoB, and similar to apoB in predicting cardiovascular disease risk.

Bob Barrett: What about the cost of the testing? Is there any difference there?

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Dr. Manuel van Deventer: So looking at non-HDL cholesterol from a cost perspective, it only includes total cholesterol and HDL cholesterol. So from a cost perspective, non-HDL cholesterol does have advantages when compared to direct LDL cholesterol or calculated LDL cholesterol as well.

Bob Barrett: Dr. Remaley, what are the current National Cholesterol Education Program's guidelines for the use of non-HDL cholesterol, and do you expect any changes with the upcoming fourth version of the Adult Treatment Panel guidelines in regard to non-HDL cholesterol?

Dr. Alan Remaley: Yeah, so the current ATP III guidelines, which have been in existence for almost ten years now, currently recommend the use of non-HDL cholesterol as a secondary treatment goal for those patients that have elevated triglycerides, I believe, over 200 milligrams per deciliter, otherwise there is no, I believe, other recommendations regarding non-HDL cholesterol.

But people have been talking for many years about non-HDL cholesterol and apoB and some other measures, particularly for people with intermediate risk.

The new guidelines have been -- actually we have been working on for a year. They are actually, I think, believed delayed. I hope and we are all hoping that we will have some new guidelines within the year.

I don't know what the outcome would be. I suspect that there will be increasing emphasis on the use of ancillary tasks such as CRP, non-HDL cholesterol, and apoB, particularly in patients with intermediate

risk in terms of helping us decide how to treat these patients.

One outcome from our result, which was a little bit alarming, is that we had a previous study where we looked at, in the direct LDL cholesterols, in this case, not looking at the reference method procedure, but just looking at the total error, looking at analytical goals for total error, and most of those assays did find in normal lipidemic individuals, but again, individuals with slightly elevated triglycerides or some other form of dyslipidemia, most of those assays do not do very well.

So I am concerned that the people who are at intermediate risk or maybe even high risk, that perhaps the direct LDL cholesterols really have to be followed up on with another test, such as maybe, perhaps non-HDL cholesterol or apoB to make sure we have a firmer foundation in terms of what in fact the risk factors are.

Bob Barrett: How do some other tests like apoB compared to non-HDL cholesterol for cardiovascular risk assessment?

Dr. Manuel van Deventer: So in this study, apoB were measured using a new one method, a nephelometric method on the Dimension. Vista System. As we all know non-HDL cholesterol is a measurement of cholesterol associated for all apoB-containing particles and therefore it wasn't surprising that in this study the coefficient of determination between apoB and non-HDL cholesterol was good. It ranged between 0.83 and 0.84.

And when referenced non-HDL cholesterol was compared to apoB, the coefficient of determination was 0.86.

On the other hand, apoB compared poorly with LDL cholesterol measurements and coefficients of determination ranged between 0.47 and 0.61.

I think if we look at the difference between apoB and non-HDL cholesterol, although apoB and non-HDL cholesterol are very strongly correlated, these measurements indicate important biological differences.

ApoB reflect atherogenic particle number of all atherogenic lipoproteins and therefore including VLDL cholesterol, and LDL cholesterol, and LDL particles.



We have some non-HDL cholesterol indicated with atherogenic cholesterol mesh in these particles.

There has been a number of studies that evaluated non-HDL cholesterol and apoB, and maybe just to mention two studies; there was a recent large meta-analysis in Emerging Risk Factors Collaboration study. And based on the prospective study of about 300,000 subjects, it showed that non-HDL cholesterol and apoB have similar predictive power for cardiovascular veins.

And on the other hand, just a very interesting article recently by Holewijn et al entitled Apolipoprotein B, non-HDL cholesterol and LDL cholesterol in identifying individuals at increased cardiovascular risk.

In this paper the authors measured noninvasive measurements of atherosclerosis such as intima-media thickness and found a strong relationship between apoB and subclinical atherosclerosis and for non-HDL cholesterol or even LDL cholesterol.

So I think to summarize, I think it's very important that further studies, specifically looking at clinical outcomes, evaluating apoB and non-HDL cholesterol are required.

There's also been a recent study by Schneiderman et al who proposed the diagnostic of globin, including apoB concentrations, triglyceride concentrations, and total cholesterol concentrations to diagnose this lipoprotein risk.

Dr. Alan Remaley:

In fact, if I could just said one more thing to what Manuel said, I agree with all that, is that there is a tremendous amount of residual risk for people treated with statins.

About a third of the people who retreat have reduction events, but two-thirds go on to develop an event. And part of that maybe related to the fact that statins do a very good job in lowering LDL cholesterol. They don't lower non-HDL cholesterol nearly as much, and they will lower apoB, but even less than what they lower non-HDL cholesterol. And some people believe that the residual risk that was remaining is related to the large number of the still elevated apoB levels, which reflects the total particle number.



So people are also proposing use of apoB for monitoring the patients and monitoring therapy.

So I think apoB has a lot to offer and I suspect that we will hear more about apoB in these non-HDL and other tests in the ATP IV guidelines,

Bob Barrett:

Dr. Alan Remaley and Dr. Manuel van Deventer are staff members at the National Institutes of Health. They have also been our guests in this podcast from '*Clinical Chemistry*'. I am Bob Barrett. Thanks for listening.

Total Duration: 16 Minutes