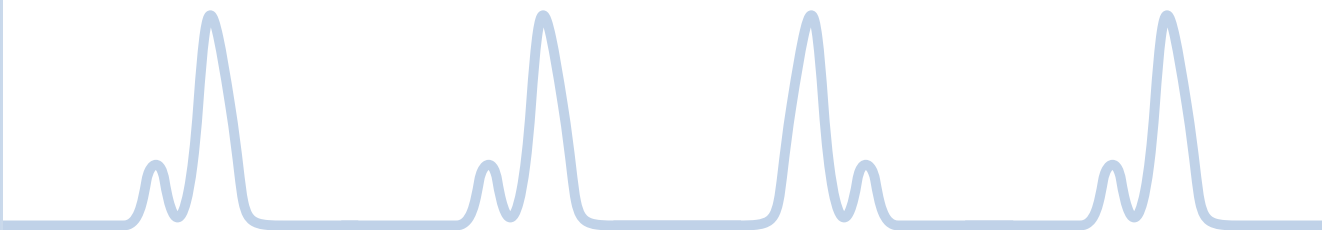# Clinical Value of Routine Determination of Lp(a): Clinical and Methodological Considerations

Symposium Proceedings

AACC • ASCLS • NACB Meeting

July 27, 2004

Introduction

Helena Laboratories is pleased offer this educational monograph edited from the expert panel discussion, "Clinical Value of Routine Determination of Lp(a): Clinical and Methodological Considerations". This workshop was held July 27, 2004 at the AACC • ASCLS • NACB Annual Meeting. Faculty included Dr. Robert Galen, Dr. Sanda Clejan, Dr. Herbert Naito, and Dr. Joseph McConnell. Comments from Dr. Jane Emerson are also included in this monograph.

The purpose of this workshop was to discuss emerging cardiac risk factors including the role of Lp(a) in the routine evaluation of patients for CAD risk, the problems associated with different analytical methods for the determination of Lp(a), and the relative significance of Lp(a) and CRP in assessing atherosclerotic burden and vulnerable plaque. Of note was the presentation of data from a 4-year study of angiography patients comparing Lp(a) methods and CRP. Recommendations for extended risk marker panels were also provided.

Quantitation of Lp(a)-C is an integral part of the Helena Cholesterol Profile assay. One key advantage of this method is its simultaneous, direct determination of HDL, LDL, VLDL and Lp(a)-C in one run. The assay provides a "broadcut" LDL like the CDC reference method with no fasting required. It allows up to 100 samples to be analyzed in 80 minutes with little hands-on time, but also offers smaller-sized gels for lower volume laboratories, and a highly favorable reimbursement margin.

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Bob Galen

Robert S. Galen, MD, MPH
Professor,
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Let me introduce myself. I'm Bob Galen, pathologist and professor at the University of Georgia and consulting Medical Director of Helena Laboratories. I'm going to be moderating the program this evening. It's an exciting opportunity for those of us that have worked in this area to get together and to share with you some of our progress. A number of years ago, I became interested in atherosclerotic heart disease and couldn't help but notice that there were significant problems with what we were doing. Many years ago I was very interested in issues of sensitivity, specificity and normal ranges for laboratory tests. While a pathology resident, I was also getting my masters in public health at Columbia, and was very struck by the fact that in the United States, the mean value for cholesterol, for men, was about 225 mg/dL. Much of the world had levels that were significantly lower and in those populations, there was no or very little coronary disease. And so, together with Ray Gambino, I wrote "that with regard to morbidity and mortality, although statistically normal, a number of 225 might be actually quite abnormal."[1] And this was radical. We did autopsies on patients dying of acute M.I. and if you looked at the lab reports, the upper limit of normal for cholesterol in the United States in hospital laboratories in 1970 was 300 mg/dL. We were defining normal based on what we found, and normal went up to 300, and so clinicians were not too worried about heart attacks driven by cholesterol. It was kind of interesting, as you might imagine, trying to suggest that we lower the cut-off. And so that's what we were doing in the 1970s. More recently, Tony Gotto, who is now the Dean of the medical school at Cornell, wrote that "few investigators or clinicians were willing to extrapolate and predict that lesser degrees of hypercholesterolemia might predispose to atherosclerosis."[2] As you know, we have guidelines now that try to manage the clinical situation and those guidelines are a work in progress. Recently, these cut-off points have been lowered which, of course, amuses me to no end. Along with these guidelines, comes the idea of emerging risk factors, and it's one of the emerging risk factors that we're going to be talking about this evening – lipoprotein(a) or Lp(a). We are also going to explore methodological issues with lipid testing. Dr. Herb Naito will be our first speaker.

> *"And so, together with Ray Gambino, I wrote "that with regard to morbidity and mortality, although statistically normal, a number of 225 might be actually quite abnormal."*

> *"Recently, these cut-off points have been lowered which, of course, amuses me no end"*

> …*"few investigators or clinicians were willing to extrapolate and predict that lesser degrees of hypercholesterolemia might predispose to atherosclerosis."*[2]

Herbert K. Naito

Herbert K. Naito
Chief of Clinical Chemistry
Louis Stokes VA Medical Center
Cleveland, Ohio

My objectives for this presentation are to discuss: (1) the accuracy of the current lipid panel; (2) the need to increase the utilization of emerging risk factors; and (3) problems with Lp(a) measurement.

The ATP III report came out during 2001, which is now three years old. The report suggested more aggressive LDL and HDL cholesterol goals and also lowered the triglyceride classification cutpoints. The report raised diabetic patients to a higher risk status. For some reason, healthcare providers still seem to not embrace this point more seriously. The report also identified metabolic syndrome as an important new risk factor, which many clinicians still do not address. We need to make an assessment of whether or not a patient has metabolic syndrome because of the growing evidence that it increases the risk for CHD events. The ATP III report emphasized the need to recognize other CHD risk factors, called emerging risk factors, which include homocysteine, high-sensitivity CRP, remnant lipoproteins, lipoprotein particle size or density, lipoprotein subfractions and Lp(a).

In addition, the Framingham database for the 10-year absolute CHD risk projection is not being used for risk assessment as was recommended. As a reminder, the ATP III report still places emphasis on LDL cholesterol reduction as the primary target of therapy. For treatment intervention strategies, therapeutic life-style changes (increased physical activity, weight reduction, diet) should be primary. Also, greater uses of the lipid-lowering agents, such as the statins are being recommended.[3]

Russ Warnick put some things into perspective about the impact of the ATP III guidelines on the clinical laboratory. I'd like to go over those very briefly because I think he made some important key points. The lower and more closely spaced lipid cutpoints will emphasize the need for accuracy and require increased efforts to standardize lipid and lipoprotein measurements for reliability of testing. There should be increased testing for emerging risk factors and secondary dyslipidemia, especially in patients with diabetes and/or thyroid dysfunction. Also, more attention should be given to identifying patients with metabolic syndrome, patients with high triglycerides, and the concept of non-HDL cholesterol as a risk factor.[4]

Now, if we look at this slide containing the constellation of CHD risk factors, the list keeps growing. My point on this slide is that we really need to look at the total package. There are really three major categories. One is the major risk factors that exist beyond a reasonable doubt, i.e., the data strongly shows that total cholesterol LDL, HDL and what I don't have on here, age, diabetes and tobacco smoking are the major risk factors. These others listed here are emerging risk factors along with another group we call underlying risk factors, some of which are modifiable through lifestyle changes. These include being overweight, physical inactivity, atherogenic diets, and even socioeconomic and psychosocial stresses. There's another category in this third group that is non-modifiable, which would include family history for premature CHD, various genetic and gender-related, as well as racial factors that cannot be modified.

A New Look at CHD Risk Factors

- Total Cholesterol
- LDL-Cholesterol
- HDL-Cholesterol
- Lipoprotein(a)
- Homocysteine
- Particle size/density
- Lp subclasses
- Triglycerides
- Inflammatory factors
- Oxidized-LDL (Ox-LDL)
- Metabolic syndrome
- Prothrombotic factors
- Apolipoproteins
- Glucose impairment
- Subclinical atherosclerotic diseases
- Diabetes mellitus
- Life-habit risk factors

slide 1

That's also part of this total CHD risk package. We take a very myopic look, at the present time, in trying to control heart disease by focusing on the traditional risk factors. The presentation today is to get you to look further, beyond that point. We need to take a more comprehensive approach in assessing all the patient's CHD risks. (slide 1)

Now I'd like to go back to something we've ignored for a long time and it's about time

that we faced the problem of inaccurate LDL-C measurements. How many of you are still using the Friedewald equation to estimate LDL-C? That's too many! As you well know, this method of estimating LDL-C has been around for a long time. The NIH NCEP Laboratory Standardization Panel recommended the Friedewald calculation method. Admittedly, at the time, we thought it was the best solution because homogeneous, direct methods were not available when the Laboratory Standardization Panel that I chaired at NIH made the recommendations. However, over time, technology has progressed in that we have new methods of obtaining more accurate LDL-C values. The Laboratory Standardization Panel recommended that with the LDL-C measurements, the laboratory accuracy goal should have a total error of 12% or less. Ideally, that means that the precision of the method should be 4% or less and the analytical bias should not exceed 4% to achieve that 12%. One should also remember, you're measuring a package of Lp(a), IDL and remnant lipoproteins as well as LDL with current direct LDL-C methods. This nonspecific LDL-C measurement creates other problems, e.g., if treating an abnormal LDL-C value with a statin, the result can be misleading. If you have a LDL-C of 160 mg/dL, how do you know if it is all LDL-C? What happens if high Lp(a), i.g., 25 mg/dL, contributes to part of that LDL-C value by the direct measurement method? (slide 2)[5]

Measure LDL-C More Accurately

Friedewald Calculation:

- LDL = TC − (HDL + VLDL)
  (TG/5 *approximates* VLDL)
  Recommendation: Not valid with TG > 400 mg/dL
- LDL = Lp(a) + IDL + Remnant Lp + LDL
- Total Error = Bias + (1.96 x $CV_{imprecision}$)
  < 12% (ideal: + 4% & CV < 4%)

From: *Clin Chem* 41:1414-1420(1995)

slide 2

Another point should be made about inaccuracy of LDL-C estimation by the Friedewald method. This data has been around since 1990, and if you look at Russ Warnick's work on the calculation method with different concentrations of triglyceride, you can see that the higher the triglyceride value, the greater the inaccuracy. If the LDL-C calculated values were within plus or minus 10% of the preparative ultracentrifugal values, you can see that very quickly we begin to have values that are not agreeing. In fact, even below 200 mg/dL, you already have 7% that are not agreeing. At 201 to 300 mg/dL, 25% error is seen and 301-400, it's at 39% error rate. With triglyceride 401-500 mg/dL, the error is close to 60% and above 500 mg/dL, the error exceeds 80%. As you can see, the error can really get very large. (slide 3)[6]

Accuracy of LDL-c Calculation Method

Triglycerides Mg/dL	Percent Correct (Within + 10%)	Percent Incorrect
< 200	93%	7%
201-300	75%	25%
301-400	61%	39%
401-500	41%	59%
> 500	< 20%	> 80%

From: Warnick GR et. al. *Clin Chem* 36:15-19 (1990)

slide 3

And this work was repeated by Judy McNamara's group at Tufts and the data are almost identical. They had the errors begin to accumulate very rapidly as the triglyceride levels increased. They demonstrated that with triglycerides between 201-300 mg/dL the error was 23% and 301-400 mg/dL, they had 41% non-agreement with the preparative ultracentrifugal data. (slide 4)[7]

Accuracy of LDL-c Calculation Method

Triglycerides mg/dL	Correct LDL-C (Within + 10%)	Incorrect LDL-C
< 200	85%	15%
201-300	77%	23%
301-400	59%	41%
401-600	41%	59%

slide 4

A few years ago, in 2001, similar data was published[8] and these authors indicated that the Friedewald calculation underestimates the LDL, as did previous authors.[6,7] As the triglycerides increase above 200, (in fact, they said 177 mg/dL and above), they begin to see significant deviation from the true value. The LDL-C calculation method underestimates if you do not fast, and they gave this as an example: The patient has two beers the night before, the next morning the triglyceride was 50 milligrams higher. How does that impact the Friedewald estimate? You can see it's 10 mg/dL falsely lower. The importance of fasting and also being careful about those that are sensitive to triglyceride increase due to alcohol intake are stressed.

Their study also showed that calculated LDL-C is underestimated if LDL-C values are below 100 mg/dL. This was one of the unique things about this paper that I wanted to go over. Now, this slide illustrates the error observed in their data when the triglycerides exceed 177 mg/dL. Very quickly you can see that the percent of patients that were misclassified due to the calculated LDL-C values was 52% in this NCEP category of 130-160 mg/dL and about 46% in the 160-190 mg/dL LDL-C risk category. These are significant errors, which can lead to the misclassification of the patient. (slide 5)[8]

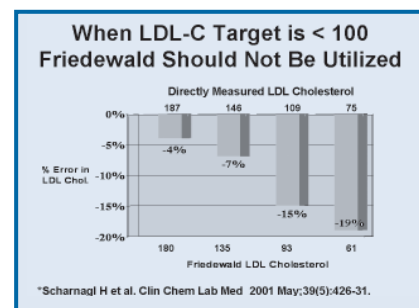*"So the shortcomings of the LDL calculation method are enormous"*
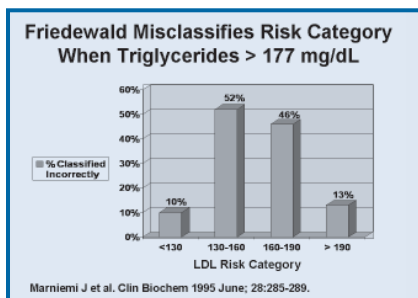
Now what happens if the LDL-C values are less <100 mg/dL? You can see that at a level of 75 mg/dL by direct measurement, the calculated method is on the average at 61 mg/dL or about a 19% lower value than one should get compared to the direct measurement. Likewise, with the direct measurement of 109 mg/dL, the calculated method value of 93 mg/dL represents about a 15% lower value.) (slide 6)[9]

Friedewald Misclassifies Risk Category When Triglycerides > 177 mg/dL

Marniemi J et al. Clin Biochem 1995 June; 28:285-289.

slide 5

So the shortcomings of the LDL calculation method are enormous. There are significant inaccuracies going on with the calculated method when the triglycerides are above 200 mg/dL or when the LDL-C values are below 100 mg/dL. The magnitude of the errors is c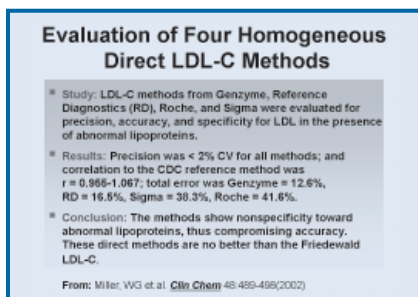ompounded because of the three different analytical measurements that you must make (total cholesterol, triglycerides, HDL-C) that require accuracy. If any one of the three measurements is inaccurate, the error's reciprocal. In other words, if the triglyceride values go up, whether it's analytical or biological error, the calculated LDL will go down. A 12-hour fasting specimen is required to use the equation.

The bottom line is, these LDL-C inaccuracies can lead to misclassification of CHD risk and can lead to very difficult evaluation of the efficacy of treatment intervention. So I think it's time that we need to acknowledge these studies and abandon the Friedewald method of LDL-C estimation. The ATP III Panel recommended the use of direct LDL-C measurement methods.

We now have alternatives. We now have many direct LDL-C measurement methods. However, I found this study by Greg Miller and his group somewhat disturbing. They looked at four different third-generation methods, i.e., Genzyme, Reference Diagnostics, Roche and Sigma. They examined the precision, accuracy and specificity for LDL. However, they used specimens with abnormal lipoproteins, i.e., those that had remnant lipoproteins, Lp(a), IDL, etc. The precision for all four methods was very good, as one would expect, because they're primarily done by automated methods. They all were less than 2% CV. The

When LDL-C Target is < 100 Friedewald Should Not Be Utilized

*Scharnagl H et al. Clin Chem Lab Med 2001 May;39(5):426-31.

slide 6

overall correlation with all four methods, from an accuracy standpoint, to the CDC Reference Method for LDL-C, appears good with r-values of 0.9 or better. However, when you look at the total error of the Genzyme method, it does not meet the NCEP Laboratory Standardization Panel guidelines (for total error <12%). The Genzyme method total error was 12.6%, Reference Diagnostics was 16.5%, Sigma was 38%, and Roche was 41.6%. Since that time, several of these companies have focused on better antibodies and better precipitation techniques to be more specific for the isolation of the LDL. The authors concluded that, "the four

Evaluation of Four Homogeneous Direct LDL-C Methods

- Study: LDL-C methods from Genzyme, Reference Diagnostics (RD), Roche, and Sigma were evaluated for precision, accuracy, and specificity for LDL in the presence of abnormal lipoproteins.
- Results: Precision was < 2% CV for all methods; and correlation to the CDC reference method was r = 0.966-1.067; total error was Genzyme = 12.6%, RD = 16.5%, Sigma = 38.3%, Roche = 41.6%.
- Conclusion: The methods show nonspecificity toward abnormal lipoproteins, thus compromising accuracy. These direct methods are no better than the Friedewald LDL-C.

From: Miller, WG et al. *Clin Chem* 48:489-498(2002)

slide 7

methods showed non-specificity toward abnormal lipoproteins, thus compromising the accuracy. These direct methods are no better than the Friedewald LDL cholesterol calculation." (slide 7)[10]

Now, if you review all of the LDL-C methods that are available, you have the ultracentrifuge, which is considered the "reference method". However, it's very time consuming, very tedious, very difficult to do and you really need highly-trained individuals. It is also very costly. The electrophoresis method is another technique that can be used. It simultaneously separates major lipoprotein fractions, which can then be quantitated. It can be technically difficult to use unless it's completely automated. It can be tedious and time consuming and it can be costly unless, again, it is highly automated. With an automated system like the Helena High-Resolution Lipoprotein System, you circumvent a lot of these issues. (slide 8)

Review of LDL-C Methods

1. Ultracentrifugation:
   + Reference method, accuracy based
   - Time-consuming, tedious, technically difficult, costly
2. Electrophoresis:
   + Simultaneous separation of major lipoprotein fractions, can quantitate, visualize some unusual bands
   - Technically difficult to do; unless automated, it can be tedious and time consuming, can be costly

slide 8

The third-generation direct methods are very attractive. There's no pretreatment, they're fully automated, have improved analytical precision, fasting specimens are not required, and you can save on labor costs. The labor cost estimation savings due to the automation is around 15 to 20%. You still can have the lack of LDL specificity and inaccuracy because of the analytic interference from triglycerides, bilirubin, intermediate-density-lipoprotein, Lp-X, VLDL cholesterol, Lp(a), apo E-rich HDL and/or hemoglobin. (slide 9)[11]

Review of LDL-C Methods (Cont.)

3. Third Generation Direct Methods:
   + No pretreatment, full automation, improved analytical precision, fasting specimen not required, save on labor cost
   - Can have lack of LDL specificity; inaccuracy because of analytical interferences from TG, bilirubin, IDL-C, Lp-X, VLDL-C, Lp(a), apo E-rich HDL, and/or hemoglobin; can be costly

From: Nauck M et. al. *Clin Chem* 48:236-254 (2002)

slide 9

Now let's switch our attention to HDL cholesterol testing. The laboratory goal set by the NCEP for total error is 12% or less. To achieve this goal, the precision of the method should be 4% CV or less, and the analytical bias should not exceed 4%. The CDC HDL-C reference method is a combination of ultracentrifugation (to remove the chylomicron and VLDL) and heparin-manganese chloride precipitation followed by the measurement of cholesterol by the Abell-Kendall chemical method. (slide 10)

HDL-c Testing

- NCEP Laboratory Goals
  - Total Error = ≤ 13% (analytical bias +1.9% CV)
  - TE = Ideal: ≤ 5% and ≤ 4% CV
- CDC Reference Method: 3 Stage Procedure
  1. Ultracentrifuge = remove chylomicron + VLDL
  2. Heparin MnCl$_2$ ppt. = remove apoB lipoprotein
  3. Abell-Kendall cholesterol assay on HDL
- CRMLN Designated Comparison Method
  - Dextran Sulfate (50,000 DA) ppt.
  - Abell Kendall cholesterol assay on HDL

slide 10

A review of four major categories of HDL methods (immunologic, polyethylene glycol, synthetic polymer and enzymatic approaches) suggests that some of these methods do have, in fact, problems. Most of these methods are rather robust. They've really come a long way to handle higher triglycerides, i.e., around 900-1,000 mg/dL. However, they do suffer from lack of specificity with some apoproteins and I'll go over that very shortly. My recommendation is to be sure to select systems that have the cholesterol reference method laboratory network (CRMLN) certification suggesting that accuracy is possible with proper instruments, reagents, and calibrators. However, I must remind you that the certification by the CDC CRMLN Program is a one-time shot and applies only to the instrument, reagent and calibrator lots used for the certification process. Whenever the lot of the calibrator or the reagent changes, it's your responsibility to validate the continued accuracy of the measurement process. (slide 11)[12]

Homogenous HDL-c Methods

- There are 4 types of homogeneous HDL-C methods: immunologic, PEG, synthetic polymer, enzymatic.
- All can be automated & directly measure HDL.
- Most have excellent precision (< 3% CV).
- Many have CRMLN certification, suggesting that accuracy is possible with proper instrument, reagents, and calibrator.
- Specificity and interferences: most are robust and can tolerate TG < 900 mg/dL, but biases can occur with atypical lipoprotein patterns.

From: Warnick et. al. *Clin Chem* 47:1579-1596 (2001)

slide 11

In review of the HDL-cholesterol methods, again, ultracentrifugation has a high degree of

accuracy. Like the LDL-C, it has many negative issues centering on cost and technical difficulty. As we indicated with the electrophoresis system for LDL-C, it does separate the

major fractions and quantitates the lipoprotein fractions. Technically, it's rather difficult to do unless it's automated. However, it can be less tedious, less time consuming, and less costly if it's done by a completely automated system. With today's new generation, high definition agarose gel and the completely automated system, you can circumvent many of these issues and I will elaborate on this system later. (slide 12)

Review of HDL-c Methods

1. Ultracentrifugation:
   + Comparison method for accuracy
   - Time-consuming, tedious, technically difficult, costly, can have interference from Lp(a) and others
2. Electrophoresis:
   + Simultaneous separation of major lipoprotein fractions, can quantitate, visualize some atypical bands
   - Technically difficult to do; unless automated, it can be tedious and time consuming, can be costly

slide 12

The third generation direct method is attractive because (1) you have no pretreatment, (2) it can be fully automated, which improves analytical precision, (3) a fasting specimen is not required, and (4) there's a savings in cost because of the automation. On the other hand, there are a couple of papers that I found that because of the lack of specificity for the HDL, inaccuracies can occur due to the interference from the HDL variants such as the apo A-I$_{Milano}$, apo E-rich HDL and, of course, the standard interfering substances such as bilirubin, hemoglobin and high triglycerides (particularly the chylomicron and VLDL cholesterol). (slide 13)

"So I think it's time that we need to acknowledge these studies and abandon the Friedewald method of LDL-C estimation."

Now, when you think about electrophoresis, and we used to do hundreds of them in the laboratory at the Cleveland Clinic during the 1980s, it brings up a lot of negative issues, even though it was a nice qualitative system. Back then, the separation of the major lipoprotein bands on agarose gel was not always ideal. The bands did not always agree with the preparative ultracentrifuge method. Back then, the different lipid dyes used resulted in different affinities for the different lipids in the lipoprotein fractions. Also, the different lipoproteins could result in different mobilities, unlike where the lipoproteins customarily migrate. This phenomenon occurred particularly if the patient was stressed due to the epinephrine effect. The stress hormones caused the breakdown of fat, which resulted in the release of fatty acids, which hitched up to the lipoproteins and caused an increase in electronegativity. This caused a change in the mobility of each lipoprotein fraction in an electrical field. The precision was okay back then. However, precision sometimes was very difficult to control, calibration to the gold standard was not easy and verification of accurate test results was difficult because of lack of adequate reference materials. Today, lipoprotein

Review of HDL-C Methods (Cont.)

3. Third Generation Direct Methods:
   + No pretreatment, full automation, improved analytical precision (<3% CV), fasting specimen not required, save on labor cost (20% savings)
   - Can have lack of HDL specificity; inaccuracy because of analytical interferences from bilirubin, hemoglobin, high TG (chylomicrons and VLDL-c), Lp(a), apo E-rich HDL, and/or HDL variants (Apo A-I$_{Milano}$)

slide 13

electrophoresis is better-controlled and more automated to give better precision and accuracy. The negative issues that plagued the old technology have changed with the improvements in electrophoresis technology, the gel composition and the equipment that is used today.

New Approaches to Quantitative Lipoprotein Electrophoresis

* Completely automated; better precision
* Better gel/buffer system (high-resolution)
* Precision control of temperature during electrophoresis
* Densitometer designed for higher sensitivity
* Simultaneously measures VLDL-c, LDL-c, HDL-c & Lp(a)-c
* LDL-c and HDL-c compares well with CDC reference methods
* Lp(a) compares well with Lp(a) candidate reference method

slide 14

Today's systems are designed to be completely automated. You can run hundreds of specimens in just a few hours. You have much, much better precision on totally automated instruments — from sample application to the actual reading of the gels by the scanner. Today's gel/buffer system has been optimized to give better separation and resolution of the lipoprotein bands. You have precise control of the

temperature during the electrophoresis process. The better design of the densitometer enhances the precision and accuracy of the scanning process. In addition, the densitometer

Within-Run with Helena Electrophoretic Method

Pool	VLDL-C mg/dL	LDL-C mg/dL	HDL-C mg/dL
B	8.1 ± 0.4 (5.3% CV)	149.1 ± 2.0 (1.3% CV)	41.4 ± 1.5 (3.6% CV)

From: Naito HK, et al. Handbook of Lipoprotein Methods. AACC Press, Washington D.C., Pgs. 477-495(1997)

slide 15

has increased sensitivity. The Helena electrophoresis system simultaneously measures four major fractions [VLDL-C, LDL-C, Lp(a)-C, and HDL-C]. The HDL and the LDL compare well with the CDC reference methods and the Lp(a) compares with the reference method. (slide 14) We used the REP 3, which is no longer being made. We did our study about five years ago. Today, this system is replaced by the SPIFE 3000. We still had excellent results with this older system. It's rapid; I believe we did 64 in matter of an hour and fifteen minutes. This is the total time required.

The system was linear up to 400 mg/dL for cholesterol. They used the new high-definition agarose and we got very good, precise and accurate results.

This data was published in the *Handbook of Lipoprotein Methods*, AACC Press (1997). You can see that the LDL-C and HDL-C precision is quite respectable and meets NCEP guidelines.) (slide 15)[13]

Comparison of Methods: Helena Electrophoresis vs. CDC Reference Method

Fractions	Regression	r
VLDL-C	y=0734 + 0.389x	0.97682
LDL-C	y=1.783 + 1.07x	0.99809
HDL-C	y=2.449 + 1.1x	0.99241

From: Naito HK, et al. Handbook of Lipoprotein Methods. AACC Press, Washington D.C., Pgs. 477-495(1997)

slide 16

Concerning accuracy, look at the regression formula and the correlation coefficient and one can see excellent results. (slide 16) Again, these values resemble works of others, including that of Nauck et. al. In fact, he has results on Lp(a) which show excellent correlation. (slide 17)[14]

Comparison of Methods: Helena vs. Ultracentrifugation

Fractions	Regression	r
VLDL-C	y= 0.929x - 6.39	0.947
LDL-C	y= 1.006x + 5.27	0.962
HDL-C	y= 1.158x - 8.00	0.918
Lp(a)	y= 0.23x + 0.421	0.906

From: Nauck M, et al. Clin Chem 1995; 41:1761-1767.

slide 17

So for the LDL-C, HDL-C summary: the LDL-C calculation method is not dependable when compared to some of the current direct LDL-C methods or alternative methods that are available, such as the ultracentrifuge and third generation electrophoretic methods. I suggest to you that it's time that we abandon the Friedewald calculation technique. When I say to abandon the calculation method and move on to something that's more dependable, I say it because it does have an impact on patient care. The third generation direct, or homogeneous, methods have some issues about specificity and the various manufacturers are working on this. I hope they resolve these issues quite soon, because a lot of laboratories are using the direct or homogeneous LDL-C cholesterol methodology, as well as the direct HDL-C cholesterol methodology. (slide 18)

LDL-c and HDL-c Summary

♦ LDL-C calculation method is not dependable when compared to some of the current direct LDL-C methods.

♦ Ultracentrifugation methods for LDL and HDL still remains the gold standard for accuracy.

♦ The 3rd-generation electrophoresis methods for lipoprotein-C quantitation have several attractive features.

♦ The 3rd- generation direct or homogenous LDL-C and HDL-C methods have better precision but may suffer from lack of LDL or HDL specificity; thus, accuracy may be compromised.

slide 18

LDL-c and HDL-c Summary (cont)

♦ The selection of your instrument-reagent-calibrator system for LDL-C or HDL-C determinations should be based on the approved list of the CDC CRMLN certificate of traceability.

♦ Periodic verification of accuracy of LDL-C and HDL-C should be done, particularly if the reagent and/or calibrator lot number changes.

♦ More robust LDL-C and HDL-C methods should be employed that are not affected by interfering substances (i.e., abnormal lipoproteins, hypertrigly-ceridemia)

slide 19

The bottom line is, if you are going to use the homogeneous methods, make sure that they are CDC CRMLN certified methods for accuracy. And then do your part — whenever you change lots of reagents or calibrators validate that the accuracy is maintained. And finally, one should be concerned about interfering substances such as hypertriglyceridemia. There's the possibility that you will run into the situation when patients, particularly diabetic patients who are poorly controlled, have triglycerides in the 2,000 to 3,000 mg/dL range. You need to dilute those specimens. You need to double check your measurements just to be sure that you have

accurate LDL and HDL cholesterol measurements. The more robust your method, the greater assurance you have for accurate measurements. (slide 19)

Lipoprotein(a) Chemistry

- Structurally resembles LDL
- Has a second large polypeptide, Apo(a)
- Is polymorphic in size; Molecular weight 420-840 kDa
- Has 10 types of kringle 4, which is the basis of the different isoform size variability

slide 20

Now let me focus on another topic that Dr. Galen wanted me to cover, i.e., Lp(a). It structurally resembles LDL, has a second large polypeptide called Apo(a), is polymorphic in size and has ten types of kringle 4's which is the basis of the different isoform size variability. (slide 20)

From a physiologic standpoint, it's an acute phase protein, which is made by the liver. It's assembled with apo B-100 on the hepatocyte surface. The catabolism is still not really clearly elucidated. It competes with plasma plasminogen for the binding sites, resulting in decreased synthesis of plasmin and causing inhibition of fibrinolysis. Lp(a) increases cholesterol deposition in the arterial wall. It enhances foam cell formation. It makes oxygen-free radicals in monocytes. It promotes muscle cell proliferation. It induces monocyte-chemotactic activity in the subendothelial space. All of this enhances the development of atherosclerosis. (slide 21)

Physiology of Lp(a)

- Is an acute phase protein. Apo(a) is made by the liver and is assembled with apo B-100 on the hepatocyte surface. Lp(a) catabolism is unclear.
- Compete with plasma plasminogen for binding sites, resulting in decreased synthesis of plasmin and inhibition of fibrinolysis
- Increases cholesterol deposition in the arterial wall
- Enhances foam cell formation
- Makes O_2-free radicals in monocytes
- Promotes SMC proliferation
- Induces monocyte-chemotactic activity in subendothelial space

slide 21

Well, what does this all mean from a coronary atherosclerosis standpoint? Well, high Lp(a) causes CAD by two mechanisms: (1) by way of atherogenesis and (2) by thrombogenesis — two pathways. Most of the studies (prospective, case-control, and cross-sectional studies) demonstrate that Lp(a) is an independent risk factor for CHD. And if you add it to other global risk factors, it has an even greater impact as a risk factor. Different studies have shown that in stent implant patients, premature CAD patients, or revascularized patients, the Lp(a) levels tend to be high. In response to intervention therapy, diets generally do not work to lower Lp(a). Exercise has no effect. The statins do not have real impact on the Lp(a), while niacin and aspirin have been documented to be effective. As you well know, niacin is not a very easy pharmacological agent to use because of the many side effects. The pharmaceutical industry is working on specific drugs to help control the Lp(a). However, that's a ways down the path. (slide 22)

Physiology of Lp(a) (Cont.)

- Mechanism of CAD: Atherogenesis and Thrombogenesis
- Emerging Risk Factor for Vascular Disease
  - Most prospective and retrospective studies suggest an independent association between Lp(a) and presence and extent of CAD, premature CAD, MI, restenosis after balloon angioplasty, and CVD.
  - There is evidence for a benefit of lowering Lp(a)
- Response to Intervention Therapy
  - Diet and exercise have no effect: (maybe with monounsaturated fats or caloric restriction with weight loss)
  - Effect of statins are controversial
  - Niacin and aspirin will lower

slide 22

Now this is one of the better studies that pulled all of the major research papers together. This is a meta-analysis of 27 prospective studies with a large population (about 5,500 individuals). They had a mean follow-up of ten years and it showed that if an individual in the general population is in the upper third at baseline, one was a 70% increased risk for coronary heart disease compared to persons that were the lower one-third percentile. They concluded that the Lp(a) association with CHD risk is significant and is independent of the standard vascular risk factors. (slide 23)[15]

Lp(a) and CHD: Meta-analysis of 27 Prospective Studies

- The study (n = 5436) with a mean follow-up of 10 years showed that if an individual in the general population is in the upper third at baseline, you are at 70% increased risk for CHD compared to persons at the lower one-third.
- The Lp(a) association to CHD risk is significant and is independent of the standard vascular risk factors.

From: *Danesh J . Circulation* 2000; 102: 1082-1085.

slide 23

This paper was published in 2001. The PROCAM Prospective Study involved 788 male subjects who were followed for 10 years. The overall risk of CHD was 2.7 times greater if Lp(a) was >20 mg/dL. The risk increased further if there were other risk factors such as high LDL-C, low HDL-C or elevated blood pressure. In summary, the bottom line is that if you add the Lp(a) to other major CHD risk factors, it even adds a greater risk for coronary heart

disease. So if adding other risk factors does compound the problem, it seems prudent, that high-risk individuals with any one of the major CHD risk factors should be evaluated for the possibility of elevated Lp(a). (slide 24)[16]

As an overview, most prospective and retrospective studies suggest an independent association between Lp(a) and the presence and extent of CAD, premature MI, restenosis after balloon angioplasty and stent implants, peripheral vascular disease, stroke and deep-vein thrombosis. Lp(a) and cholesterol work synergistically as a risk factor. For some reason, African-Americans seem to have a 4- to 5-fold higher Lp(a) concentration in the blood as compared to the white population.

> **Lp(a) Increases CHD Risk In Men With Other Risk Factors**
> - PROCAM Prospective Study: 788 males were followed for 10 yrs; [Lp(a) measured on fresh blood]
> - The overall risk of a coronary event was 2.7 times higher if Lp(a) was > 20 mg/dL. The risk increased further if there were other risk factors, I.e., LDL-C > 160 mg/dL, HDL-C < 35 mg/dL, BP > 140/90 mmHg
> - CONCLUSION: Lp(a) is an important independent CHD risk factor that aggravates the coronary risk exerted by elevated LDL-C, low HDL-C, hypertension or the combined effects of multiple risk factors (TG, smoking, diabetes, angina pectoris, and family history of MI)
> From: *Am Coll Cardiol* 2001;37:434-439

slide 24

Then the question is, who should have Lp(a) tests done? My belief is the following patients warrant having their Lp(a) evaluated:

1. Patients who have a normal lipid profile, but have documentation of definite CHD.
2. Patients with a past MI or angina.
3. Patients who have had CABG, angioplasty or stent implants.
4. Patients with parents or first-degree relatives who died of premature CHD.
5. Patients with known elevation of Lp(a), or their parents have high Lp(a).
6. High-risk African-American males.
7. Post-menopausal women and women before age 55 with high Lp(a).
8. Men with traditional and/or global CHD risk factors. This includes diabetic patients and patients with renal disease. (slide 25)

Concerning the measurement of Lp(a), I just wanted to highlight the fact that size heterogeneity presents a problem because it leads to biases with most immunological methods. The epitopes and isoform size recognition is very difficult under those conditions. The current immunoprecipitation techniques are causing different reactivities, and therefore, different Lp(a) values. The selection of monoclonal antibodies needs greater attention and the manufacturers are focusing on that right now. They are making more specific antibodies that are directed to the apo(a) antigenic determinants expressed in kringle 4 type 2. One can use preparative ultracentrifuge techniques or one can use a high resolution electrophoresis method to circumvent this size heterogeneity problem. This problem makes it difficult because the different immunochemical methods cause different biases. Because of this issue, (1) you cannot compare the different population studies using different Lp(a) methods, (2) you cannot use recommended cutpoints suggested by these different studies, (3) this leads to misclassification of risk, and (4) longitudinal monitoring of the patient by different methods is very difficult.

> *"One can use preparative ultracentrifuge techniques or one can use a high resolution electrophoresis method to circumvent this size heterogeneity problem"*

> **Who Should Have an Lp(a) Test Done?**
> - Patients with a normal lipid profile, but have documentation of definite CHD (MI, angina, CABAG, angioplasty, stent implants)
> - Patients with parents or 1st-degree relatives who died of premature CHD
> - Patients with known elevation of Lp(a) or parents with elevation of Lp(a)
> - High-risk African American males
> - Postmenopausal women
> - Men with traditional and/or global CHD risk factors; diabetics and patients with renal disease

slide 25

The next question I want to address is, do we use Lp(a) mass or Lp(a) cholesterol? The West of Scotland Coronary Prevention Study helped me answer that question. They looked at both methods of testing and reporting and came out with the conclusion that neither assay method had the advantage for the prediction of risk. I have

further thoughts on that. If the predictability was the same, then the Lp(a) cholesterol by electrophoresis or preparative ultracentrifuge would avoid the present analytical issues associated with size polymorphism. This should suggest that we favor the use of techniques that are not influenced by size differences.[17]

NHLBI conducted a workshop on Lp(a) and published a report in 2003. I just want to highlight a couple of things. They looked at 22 different methods and they found that none of them compared well with the reference, or the candidate reference method. It's not surprising. There are many factors that lead to this. For example, they indicated that besides the different Lp(a) sizes that the methods are sensitive to, biases were due to the differences in antibody properties, the assay precision and robustness, the sensitivity of the assay to sample handling, storage conditions, length of storage of the specimens, etc. These factors all played a role in the outcome of the study. They recommended that the manufacturers should direct their major efforts towards minimizing the impact of apo(a) size variability as well as assay imprecision and batch-to-batch antibody variability. If the methods are sensitive to apo(a) isoform size, the panel recommended that samples with values >50 mg/dL should be remeasured by a referral laboratory using validated methods. The paper also indicated that screening of the general population was not recommended at this time; however, individuals with increased risk of cardiovascular disease, particularly those with borderline LDL cholesterol or high apo B, should be evaluated. Lp(a) values exceeding the 75th percentile are at risk for CVD.[18]

> *"They looked at 22 different methods and they found that none of them compared well with the reference or the candidate reference method. It's not surprising."*

In conclusion, the standard lipid profiling technique used today:

1. Is compromised because the reliability of testing (i.e., the LDL-C calculation method) is inaccurate.
2. Is not adequate to evaluate the presence of emerging risk factors. It does not provide a comprehensive lipoprotein analysis to accurately assess the entire spectrum of CHD risks.
3. Is insensitive to detect additional risk factors in high-risk patients with normal lipid levels.
4. Is insufficient to develop effective treatment of many potential lipoprotein abnormalities.
5. Not adequate for identifying risk factors that many high-risk patients have.

Recently, I believe it was last week, the new position paper from the NCEP group came out. Since 2001, there have been five major clinical trials using statin therapies with clinical endpoints which are altering our strategy for cholesterol management for the high-risk patient they have given us new therapeutic options. The latest publication in *Circulation*, the panel of experts established a new group called the very high-risk patient. For that group, the LDL cholesterol goal should now be <70 mg/dL. And how did they define the very high-risk patient? This group includes persons with acute coronary syndrome or diabetic patients with cardiovascular disease. We need to keep that in mind, and we should not get overzealous with that goal for the entire population that we're treating.
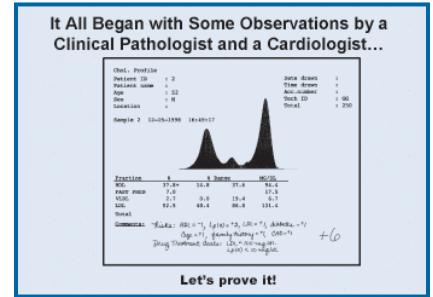
And finally, I thought this comment by a cardiologist was appropriate on where things are going. This was published by Dr. Fonarow in the Cleveland Clinic Journal of Medicine. His conclusion was, "Any patient who presents with atherosclerosis is never 'no longer at risk', even after surgical intervention. Atherosclerosis is not only sneaky, it is ruthless, i.e., 80-90% of patients who manifest atherosclerosis eventually die from it. In patients with known cardiovascular disease and those at high-risk for it, (including patients with diabetes), physicians must begin to treat aggressively."[19]

Sanda Clejan

Sanda Clejan, PhD.
Prof. Pathology & Biochemistry
Director, Core Laboratory
Tulane Univ. School of Medicine
New Orleans, Louisiana

I will limit my talk to the clinical value of Lp(a) and I will try to answer two important questions: "Are we there?" Have we really established the clinical value of Lp(a) as an independent risk factor and for what diseases? And the second question that is much more interesting for clinical chemists: "What methodology will we use in the future for Lp(a)?" Well, some history…in the 90s, we were beginning to use what was called then, the fast pre-beta fraction. Then, I wanted the cardiologists to look at Lp(a) and see what the significance was. So I always showed them the electrophoresis and the fact that when you have a very nice fraction of Lp(a) that is very high, this is important. (slide 26) And they looked at this, and one of the cardiologists, the chief of cardiology, after one year said, "You are fantastic. This is so important. Give me this every time there are the other risk factors, but let's score Lp(a) cholesterol "plus two" on the Framingham risk assessment". We also devised from these calculations treatment goals (e.g. to reduce LDL to <100 mg/dL if the Framingham score was +4 or above), and this was in 1995-2000! And this is what, for many years, we really did. At the end of the last century, my cardiologist disappeared from Tulane and a new chief of cardiology (who was very current with the literature on Lp(a) and the controversies) arrived and I was still doing the same routine. He said, "show me, really prove it, you have accumulated enough data in the last 10 years."

slide 26

Clinical Studies

*   Retrospective study of 1124 subjects with lipoprotein electrophoresis (including Lp (a)) performed in a 26 month period. Lipoprotein electrophoresis performed only when abnormal lipids where found apriori and/or in cardiac patients with high hsCRP/histamine. These patients are followed to date.
*   Prospective study of CHD compared with healthy subjects matched for age and sex: Distribution according to race and Lp(a).100 patients, (72 w, 28 aa), and 50 healthy subjects.
*   Prospective study of 51 patients with poorly controlled T2DM before and after treatment with infusion with a external insulin pump.

slide 27

So in this slide (slide 27), you see the clinical studies. The first one was a retrospective study of around a 26 month period which included the lipoprotein electrophoresis. The lipoprotein electrophoresis was performed only in patients with abnormal lipids found apriori (high LDL, low HDL) or in all our cardiac patients that had high hsCRP or, high histamine or patients with CHD without established risk factors. These patients are followed to date. In addition, we included a prospective study of coronary heart disease where we compared healthy subjects, matched for age and sex distribution according to race and we looked at Lp(a). Furthermore, we included another prospective study of a few patients with very poorly controlled type 2 diabetes before and after treatment with infusion with an external insulin pump. Patients from the two prospective studies were assayed, both by the Helena electrophoresis method for Lp(a) cholesterol and by an Lp(a) mass method (diaSorin immunoturbidimetric method).

Major Diagnosis

2 major groups: Caucasians + (Hispanics) and African Americans were considered for statistics.

	T2DM	CHD	CVD	Other
Caucasians	82	90	20	10
African Amer.	88	84	24	14

CHD: coronary heart disease (MI, angina, coronary insufficiency, angioplasty, bypass surgery, restenosis)
CVD: cerebrovascular disease (ischemic stroke, TIA, brain infarction)
T2DM: Type 2 diabetes mellitus (± nephrotic syndrome)
Other: PAD, chronic renal failure

slide 28

Distribution of Lp(a) Cholesterol According to Race

	Lp(a) < 4.0	Lp(a) 4.1-10.0	Lp(a) > 10.1
Caucasians	344	85	184
African Amer.	40	89	210
Hispanics	49	12	20
Asians	42	6	4
Pakistani+ Indians	34	2	3
Total (%)	509 (45.28)	194 (17.26)	421 (37.45)

slide 29

Distribution of Lipids in the Cohort with Lp(a) > 4.1

	Chol > 200	TG > 150	HDL < 40
	# (%)	# (%)	# (%)
Caucasians	130 (37.8)	110 (32.0)	86 (25)
African Amer.	29 (72.5)	14 (35)	16 (40)
Hispanics	15 (30.6)	10 (20.4)	14 (8.6)
Asians	12 (28.6)	8 (19.1)	6 (14.3)
Pakistani+ Indians	30 (88.2)	14 (41.2)	2 (5.9)

slide 30

We decided to concentrate on two major groups: Caucasians (+Hispanics) and African-Americans. In these groups, you see the major diagnosis. (slide 28) New Orleans is a dream for any researcher looking into the distribution and profile of lipids by race and ethnic backgrounds because of the population diversity. (slides 29, 30, 31)

This slide (slide 32) shows the distribution of Lp(a) according to race. From what I showed you in the previous slides, what we began to think maybe normal values for Lp(a) are very different for African-Americans. And thus we proposed a study with electrophoresis looking at Lp(a) in 150 African-Americans, male and females. We collected the samples from every place that we could, churches in New Orleans, around New Orleans, at a black college so young African-Americans are included. And to our surprise we found very high Lp(a), but these people really had serious maladies. Many were never seen by a physician, so we had people at age 20, who didn't know that they had a cholesterol of 400! So to make a long story short, we had to eliminate a lot of people. So we still don't have enough African-Americans to decide what are the normal values of Lp(a) for African-Americans. Notwithstanding the small numbers of African-Americans, you see that the mean Lp(a) is slightly higher. The number of African-Americans with Lp(a) above 10 is higher and when you see the maximum, you may think that there is some difference. But, I am still not convinced that what we found is due to the fact that the African-Americans may have initially higher normal values of Lp(a). In fact we found another way to look at descriptive statistics, and for this I have to thank Bob Galen because he had a fantastic idea. I didn't know what to consider the best cutoff for clinical specificity and sensitivity. It's very hard because you have so many risk factors. We looked at the Framingham risk assessment, not the ten years, but rather the regular one that considers the age, sex, family history, diabetes, cholesterol or LDL and so on. We had to recalculate the Framingham scores. Remember we included Lp(a) in our initial score, so we subtracted it. Now everything made sense! (see the slide on Lp(a) stratified according to the risk score). For Framingham 4 or below, the 95% is a Lp(a) of 9.8 ± 3.4 (SD), whereas at a score of above 8, the 95% is a Lp(a) of 26.3. Very significant! Now we have real established reference ranges for the patient population we evaluated. (slides 33, 34) These results showed that there is a relationship between the severity of the clinical signs and symptoms of CHD, CVD, or T2DM and the median cholesterol Lp(a) concentration, demonstrating that the cholesterol Lp(a) can be used as an aid in the diagnosis of all degrees of severity of CHD including asymptomatic patients.

The ROC (Receiver Operating Characteristic Curve) of Lp(a) cut-offs versus clinical sensitivity and specificity from the clinical study data is shown next. (slide 35) The area under the curve is 0.94. So, at a sensitivity of 82.5 and approximately 95% specificity the Lp(a) value really is 9.9. It probably doesn't matter if he's African-American or if he's a Caucasian, if he's an Asian or from Timbuktu, this will be the cutoff value that I will consider right now. Now we didn't think that we had enough years of follow up but when we looked at the Kaplan-Meier estimates of the incidence of the primary end point of a major additional cardiovascular event or death, we saw very good difference

> "These results showed that there is a relationship between the severity of the clinical signs and symptoms of CHD, CVD, or T2DM and the median cholesterol Lp(a) concentration."

Distribution of Increased Lipoproteins in the Cohort with Lp (a) > 4.1

	VLDL > 35	LDL > 120	HDL < 40	BROAD B
Caucasians #(%)	48 (23.5)	62 (30.4)	40 (19.6)	34 (16.6)
African Amer. #(%)	50 (23.8)	71 (33.8)	38 (18.1)	10 (4.8)

slide 31

Distribution According to Race & Lp(a) (36w, 21aa)

Lp(a)	Caucasians	African American
	# (%)	# (%)
0 – 4	25(69.4)	12(57.1)
4.1 – 10	4(11.1)	4(19.1)
> 10.1	7(19.5)	5(23.8)

slide 32

Normal Lp(a) Values

Lp(a) Non-Diseased Population (Caucasian & African American)

	Caucasian			African American		
	All	Males	Females	All	Males	Females
Mean	2.6	2.4	2.9	3.4	3.2	3.8
SD	2.8	2.8	2.7	3.4	3.4	3.6
Median	1.9	1.6	2.3	2.2	2.0	2.4
95th Percentile	6.2	5.2	6.3	8.6	8.3	8.3
% ≥10 mg/dL	98.3	98.8	97.7	97.9	98.2	97.0
Min	0	0	0.2	0.4	9.2	0.4
Max	11.4	10.9	11.8	11.3	10.9	11.5
N	254	146	108	102	56	46

slide 33

Lp(a) Stratified by Risk Score

Diseased Population

	Framingham Risk Assessment				
	All	≤4	5 and 6	7	≥8
Mean	11.1	3.2	6.6	10.9	14.9
SD	8.7	3.4	5.9	8.8	8.0
Median	7.2	1.9	4.4	9.0	10.6
5th Percentile	2.4	1.5	2.3	4.2	5.4
95th Percentile	22.4	9.8	14.8	18.0	26.3
% ≥10 mg/dL	51.6	7.2	22.5	59.4	65.2
Min	0.2	0	0.6	1.6	3.3
Max	24.4	14.6	22.4	23.2	28.8
N	530	103	105	195	127

slide 34

by 30 months. We also stratified 0-4 and 4-10, but 0-4 and 4-10, looked very similar, so we combined it in one group and compared with Lp(a) above 10 as the second group. Look how significant the difference is. So we are very sure that the estimates of survival were similar in the first two tertiles, but remarkably lower among persons in the higher tertile of Lp(a).

ROC of Lp(a) Cut-offs vs Clinical Sensitivity & Specificity at Framingham Risk Assessment Cut-off of < 4
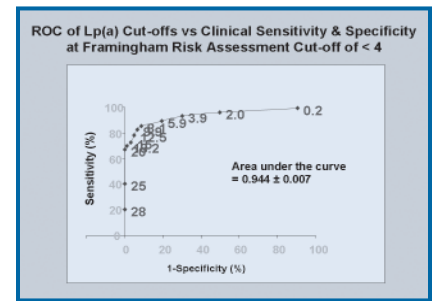
slide 35

What We Learned from these Studies?

✦ Elevated cholesterol Lp(a) levels are commonly observed in patients and families with premature CHD, but also in CVD and T2DM.

✦ Both in retrospective and prospective studies, we identified Lp(a) as an independent risk factor and a more relevant predictor of patients at risk for major CHD, CVD, T2DM.

✦ Special attention should be given to African Americans with elevated Lp(a) who have an increased risk of developing major CHD, CVD due to an Lp(a) which is very heterogeneous.

✦ Conflicting results may be attributed to analytical issues.

✦ Non-concordance between electrophoretic cholesterol Lp(a) and immuno mass Lp(a) has to be analyzed further.

✦ In our hands, cholesterol Lp(a) is specific and sensitive (see ROC Lp(a) cut-offs vs clinical sensitivity and specificity).

✦ Our findings (not discussed) indicate that patients with high cholesterol Lp(a) benefit from early and continued lowering of Lp(a) by a combination of intensive lipid lowering statins, together with Niacin (slow release) and apheresis.

Questions Still to be Answered :

What is Lp(a)? Every one has a different definition !

Who needs the Lp(a) test? Only patients with other lipid abnormalities? What about patients with only abnormal Lp(a)?

What do I do to treat a patient with high Lp(a) levels?

What are the levels of Lp(a) that I should be concerned about?

Are Lp(a) and indices of inflammation or oxidation (hsCRP, histamine, and/or isoprostane) related?

Joseph P. McConnell

Joseph McConnell, PhD
Co-Director, Biochemical
Genetics Lab
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Let me begin with a mandatory slide: cardiovascular disease is the number one killer in the United States. (slide 36) About 1.5 million heart attacks occur in the U.S. every year with 500,000 deaths. About 500,000 strokes with 150,000 deaths occur, and what's of interest is that one third of the individuals who experience an ischemic event will die from that event. Many of these individuals have no prior symptoms. (slide 37) Prevention is key. We want to prevent those events from happening. How do we do it? We use risk factors as Dr. Naito has already discussed in a significant amount of detail. Risk factor guidelines have been put forth by the National Cholesterol Education Program: ATP-III guidelines. (slide 38) Briefly, everybody who's over 20 years old should be screened for risk factors (fasting total cholesterol, HDL, triglycerides and calculated LDL) and it should be done every five years. Treatment guidelines, as Dr. Naito pointed out, are based on the LDL cholesterol. (slide 39)

This is a picture of the Easter Bunny and there's a doctor in the background and he's looking at the eggs and he says, "Cholesterol," and really, from a laboratory perspective, right on! LDL cholesterol is what we base our guidelines and treatments on. I'm going to talk a little more about novel risk factors and why we might want to look beyond LDL cholesterol. (slide 40)

If you take a look at the distribution of patients who have no coronary heart disease and patients who have coronary heart disease and you look at their total cholesterol values, you see there's a significant amount of overlap. In actuality, 35% of coronary heart disease events occur in people with total cholesterol of less than 200. (slide 41)[20] A very similar diagram could be made for LDL, with one third to one half of ischemic events occurring in individuals with LDL cholesterol less than 130 mg/dL, and the current guidelines target an LDL of <130 mg/dL for primary prevention. (slide 42) Dan Rader pointed it out very eloquently in a New England Journal editorial, writing that there's a need for additional risk factors, and additional risk factors would improve the accuracy of decisions regarding preventative therapies. (slide 43)[21]
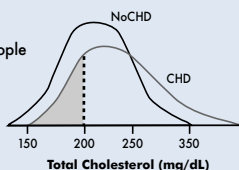
That's all well and good, but….where do we start? Over 200 potential cardiovascular risk markers have been suggested in the literature. This figure depicts a blood vessel and some of the interactions that

slide 36

slide 37

slide 38

slide 39

slide 40

slide 41

slide 42

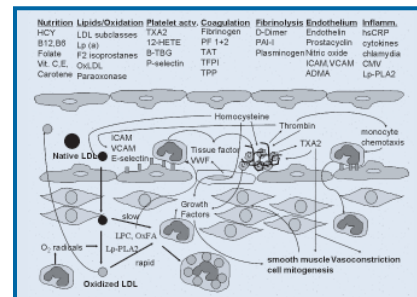# Joseph P. McConnell, continued...

Cardiovascular Risk Factors

- There is a need for additional risk factors.
- Additional risk factors would improve accuracy of decisions regarding preventative therapies

Rader DJ. NEJM. 2000;343:1179-82

slide 43

Emerging Risk Factors Proposed Panel

- Homocysteine
- Lipoprotein(a) [Lp(a)]
- High-sensitivity CRP
- Fibrinogen
- Small dense LDL

Currently no guidelines for measurement; may guide intensity of risk reduction therapy in selected patients.

slide 45

Use of Novel Risk Markers

Mayo Test Volumes: 2003

- Homocysteine   58,000
- hsCRP   26,000
- Lp(a)   22,000
- LDL subfractions   3,600
- Fibrinogen   ?????

slide 47

Mayo Recommendations for Use of Extended Risk Marker Panel

- When to Measure
  - Use to enhance clinical decision making in persons at intermediate risk for developing an ischemic event as assessed by the Framingham 10 year risk score: 10-20% risk.
- Provide with Request
  - Age, gender, smoking status, blood pressure (treated or untreated)

slide 49

occur, contributing to the formation of atherosclerotic plaques. This is a very incomplete list showing only some of the markers of oxidation, platelet activity, coagulation, fibrinolysis, endothelial function, and inflammation that are of interest. Where do we really want to start? (slide 44

The National Cholesterol Education Program did give us a little help. They identified some emerging risk markers of risk and indicated that clinicians can utilize them in selected persons to guide the intensity of risk reduction therapy and to modulate clinical judgment when making therapeutic decisions. That's good, but they didn't tell us which group of patients should have these measurements, or what what to do when an abnormal value is obtained. So that is where we are left. (slide 45)

slide 44

Close inspection of the literature reveals that some markers have emerged as those that are most likely to be useful in the clinical setting. They include homocysteine, Lp(a), high sensitivity to C-reactive protein, fibrinogen (this is an interesting one that I'd like to spend some time talking about but don't have time here), and small dense LDL particles. But again, there are currently no guidelines for measurement or treatment based on abnormal values. (slide 46) There are, however, lots of physicians using them. These are the 2003 test volumes at the Mayo Clinic. 58,000 homocysteine, almost 26,000 high sensitivity CRP, 22,000 Lp(a), and 3,600 LDL size determinations. We could not determine the number of fibrinogen assays ordered to assess cardiovascular disease, since fibrinogen is used in other clinical circumstances. The take home message: novel risk markers are being used by clinicians without official guidelines. (slide 47)

Emerging Risk Factors

The NCEP ATP-III panel identified these novel markers and indicated that clinicians may utilize them in selected persons to guide intensity of risk reduction therapy and modulate clinical judgment when making therapeutic decisions.

They do not however, identify in which group of patients these markers are best used or how to respond to elevated values.

JAMA 2001;285;2486-2497.

slide 46

At Mayo, we have developed a novel cardiovascular risk marker panel. This was developed with input from individuals in Laboratory Cardiology (Drs. Alan Jaffie, George Klee, Mary Burritt, Paula Santrach, and John O'Brien), the Mayo Clinic Cardiovascular Health Clinic, and the Department of Laboratory Medicine Clinical Practice Committee. Also acknowledged are the very important laboratory technologists who perform the actual testing in my lab. We developed recommendations for use of the extended risk marker panel.

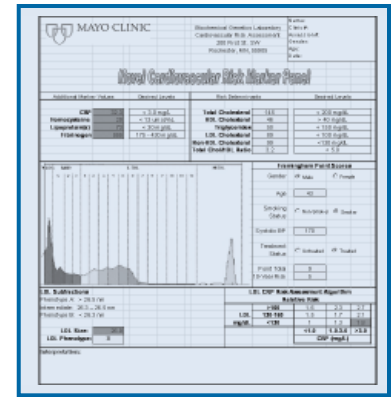**Mayo Recommendations for use of Extended Risk Marker Panel**
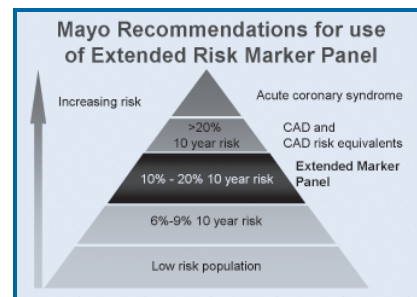
slide 48

This pyramid depicts increasing risk as you go up the pyramid. (slide 48) The individuals most likely to benefit most from measurement of novel cardiovascular risk markers are those who

are at intermediate risk for developing cardiovascular disease or events as defined by the Framingham 10 year risk score. We recommend measurement in the intermediate risk group, 10-20% (some say 6-20%) 10 year risk. If a patient has acute coronary syndrome or a coronary disease risk equivalent (>20% risk or diabetes), you're going to treat aggressively (pharmacotherapy, etc). If the patient is in the low risk group, you're going to try more traditional therapy (diet, exercise, etc). (slide 49)

slide 50

This is an example of our novel cardiovascular risk marker panel at the Mayo Clinic, and it's what we've devised to really begin to investigate these particular markers in the setting of risk assessment. (slide 50) This is a particularly interesting example of a 43 year old male. Total cholesterol 145, not bad at all; HDL cholesterol 46, I'd take that; triglycerides 50; LDL cholesterol 89. He does have a high blood pressure which is treated. Even treated, his systolic blood pressure is 170. This is a 43-year-old man who came in with a myocardial infarction and if you look at the risk markers, CRP is 32.3. CRP is an acute phase reactant and the high CRP can be attributed to the myocardial infarction. As you know, fibrinogen also an acute phase reactant, also elevated. But the homocysteine is 20 and Lp(a) is 73, both elevated, and despite the fact that this guy has pretty good HDL and low triglycerides, he does have small dense LDL. An interpretation is provided with each report. The interpretations are made by doctoral staff in laboratory medicine or in the Mayo Cardiovascular Health Clinic. Interpretations include a description of abnormal values as well as suggestions for appropriate treatment given the noted abnormalities. (slide 51)
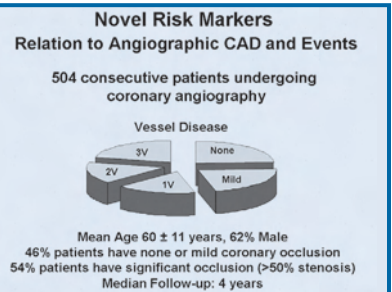

Mayo Recommendations
Clinical Response to Elevated Markers

- Interpretations are provided with every report of the novel risk marker panel.
- Interpretations are made by doctoral level staff in Laboratory Medicine or in the Mayo Cardiovascular Health Clinic.
- Interpretations include a description of abnormal values, as well as suggestions for appropriate treatment, given the noted abnormalities.
- A separate document describing up to date information on the background, interpretation and recommended therapy for abnormalities of each of the risk markers is included with the report.

slide 51

Novel Risk Markers
Relation to Angiographic CAD and Events

504 consecutive patients undergoing coronary angiography

Vessel Disease

Mean Age 60 ± 11 years, 62% Male
46% patients have none or mild coronary occlusion
54% patients have significant occlusion (>50% stenosis)
Median Follow-up: 4 years

slide 52

Patient Demographics

Age (yr)	60±11
Male (%)	62
Hypertension (%)	46
BMI (kg/m^2)	29±6
Prior infarction (%)	15
Heart failure (%)	12
Significant CAD (%)	54
ACS (%)	34

slide 53

Now I'll change gears and discuss a Mayo study in which we are evaluating these new markers. This study involves 504 patients who underwent angiography at the Mayo Clinic. Patients were categorized as having no disease, mild disease which was defined as >10% but less than 50% stenosis, and 1, 2, or 3 vessels disease with >50% stenosis in 1, 2, or 3 vessels respectively. We also divided them into 2 groups, none or mild disease vs. 1, 2, or 3 vessel disease. (slide 52) The mean age of the patients was 60, and 62% were male. (slide 53)

Study Population

- Indication for coronary angiography
  - Acute coronary syndrome (34%)
  - Positive stress test (25%)
  - Exertional dyspnea (27%)
  - Other (14%)

slide 54

These patients were seen for a number of different reasons as described here (slide 54).

We designed the study to look at the novel risk markers including Lp(a), CRP, lipoprotein-associated phospholipase A2, including how they related to other cardiovascular risk markers, acute coronary syndrome, angiographic coronary disease, and clinical outcomes. (slide 55

Aims

To analyze for associations between novel risk factors including Lp-PLA2 and:

- Other CAD risk Factors
  - (both traditional and emerging)
- Acute coronary syndromes
- Angiographic CAD
- Clinical outcomes

slide 55

Lp-PLA2 is also known as platelet-activating factor

acetylhydrolase. It's a 50 kDa, calcium-insensitive lipase produced by macrophages. 80% is bound to LDL particles. It's not responsive to cytokines like C-reactive protein is, so although it is often grouped among the inflammatory markers; it's not as sensitive an inflammatory protein as some others like CRP. Lp-PLA2 hydrolyzes oxidized phospholipids. (slide 56)

So what does Lp-PLA2 do? What is its function? Lp-PLA2 circulates bound to LDL, and it cleaves oxidized phosphatidylcholine present in LDL (oxidized LDL) to produce oxidized free fatty acid and lysophosphatidylcholine.

In our population of 504 patients with angiography, Lp-PLA2 was found to be significantly associated with cholesterol, triglycerides, and LDL cholesterol. It was negatively associated with HDL in the population. It was also correlated inversely with particle size. There was no association with C-reactive protein. It was weakly associated with Lp(a), fibrinogen, gender and smoking status, but was not correlated with homocysteine or hypertension.

In a univariate model, Lp-PLA2 was significantly associated with degree of vessel disease or the extent in vessel disease. However, when we put it in a multivariate model, adjusting for age, gender, smoking history, hypertension, cholesterol, HDL cholesterol and triglycerides, Lp-PLA2 was no longer independently associated with angiographic coronary disease. (slide 57) Of note, C-reactive protein was not associated with angiographic coronary disease in either univariate or multivariate models. Now that may come as a surprise to you, because many of you may know that there are multiple studies linking C-reactive protein to events. However, several of the studies that have looked at CRP have not shown a strong association between C-reactive protein and angiographic coronary disease or atherosclerotic burden, rather CRP and Lp-PLA2 may be better predictors of vascular events. (slide 58)

So then we went ahead and queried the 504 patients to get follow-up data. Mean time to follow-up was 4 years. We found 58 cardiovascular events in 49 of the patients. Some had multiple events. Events included cardiac death, myocardial infarction, coronary revascularization and stroke.

Here are the results. Of all the laboratory measures tested, only CRP, fibrinogen and Lp-PLA2 were significantly associated with events on multivariate analysis adjusting for age, gender, smoking, hypertension, total and HDL cholesterol, triglycerides and Lp(a).

However, if we added the inflammatory markers (fibrinogen, CRP and Lp-PLA2) to the model, Lp-PLA2 maintained its statistical significance, as was observed in the WOSCOPS data, while C-reactive protein completely lost its association with endpoints and fibrinogen's association was also attenuated.
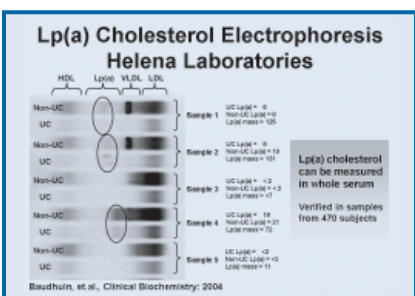
Let's move on to Lp(a). This figure represents a Helena agarose electrophoresis gel stained for cholesterol. (slide 59) Lipoprotein regions are labeled as LDL, VLDL, Lp(a) and VLDL. This represents five patient samples, three of which contains detectable Lp(a): 1, 2,

Lipoprotein Associated Phospholipase A2 (Lp-PLA2)

- AKA: Platelet-activating factor acetylhydrolase
- 50kDa, Ca-insensitive lipase
- Produced predominantly by macrophages and contributes to foam cell formation
- > 80% bound to LDL
- Not responsive to IL-1, IL-6, TNF-alpha.
- Hydrolyzes oxidized phospholipids

slide 56

Multivariate Logistic Regression Analysis

- After adjusting for age, gender, smoking history, hypertension, cholesterol, HDL cholesterol, and triglyceride, Lp-PLA2 was no longer was no longer independently predictive of angiographic CAD.
- CRP was not predictive of CAD in either the univariate or multivariate model.
- Data Presented at American College of Cardiology Annual Meeting, March 2003.

slide 57

HR For Angiographic CAD: Multivariate

Variable	HR	95% CI	P-Value
Age	1.05	1.03 - 1.07	<0.0001
Male Gender	4.03	2.46 – 6.60	<0.0001
Hypertension	1.60	1.06 - 2.42	0.026
Smoking	1.45	0.95 - 2.22	0.087
Total cholesterol	1.01	1.004 - 1.016	0.001
HDL cholesterol	0.96	0.94 - 0.98	0.0002
Log triglyceride	0.84	0.52 - 1.37	0.481
Log CRP	1.06	0.87 - 1.30	0.532
Fibrinogen	1.16	0.95 - 1.42	0.154
Lp-PLA2	0.91	0.71 - 1.17	0.466
Lp(a) protein (per 20 mg/dL)	1.14	0.91 - 1.43	0.25
Lp(a) cholesterol (per 5 mg/dL)	1.56	1.15 - 2.11	0.004

slide 58

Lp(a) Cholesterol Electrophoresis Helena Laboratories

slide 59

and 4. Each patient sample is analyzed in two lanes, the first represents whole serum, the second represents the bottom fraction or the ultracentrifuged serum, which contains LDL, HDL, and Lp(a), but not VLDL. At Mayo we routinely ultracentrifuge any sample on which we perform Lp(a) cholesterol analysis, because of the potential interference from VLDL. About five years ago, Dr. Naito was at AACC presenting data generated using the Helena electrophoresis system. It was said that you can measure Lp(a) cholesterol in whole (uncentrifuged) serum. I stood up and said "you better be careful because I don't think that's true, VLDL will interfere…you need to ultracentrifuge before we measure Lp(a) cholesterol". Dr Galen visited me after that at Mayo and suggested I test it for myself. Although I was reluctant, he convinced me to analyze Lp(a) cholesterol in a couple of studies. We compared Lp(a) cholesterol measurements using both ultracentrifuged and whole serum in 470 patient samples, and we determined that we can reliably measure Lp(a) in whole serum. We published the data this year in Clinical Biochemistry.

> *"We compared Lp(a) cholesterol measurements using both ultracentrifuged and whole serum in 470 patient samples, and we determined that we can reliably measure Lp(a) in whole serum"*

This (slide 60) represents the correlation between the ultracentrifuged Lp(a) cholesterol and Lp(a) mass determined by immunoassay. It looks exactly the same if we use whole serum Lp(a) cholesterol versus the Lp(a) mass. We see a fairly strong correlation, but if we look at the clinical decision making cut point for Lp(a) mass, which is typically 30 mg/dL, we see an interesting finding. If we drew a line right here at the 30 mg/dL Lp(a) mass, you can see that anytime we had an Lp(a) mass greater than 30, there was measurable Lp(a) cholesterol in the sample. However, there were 54 patients that had an
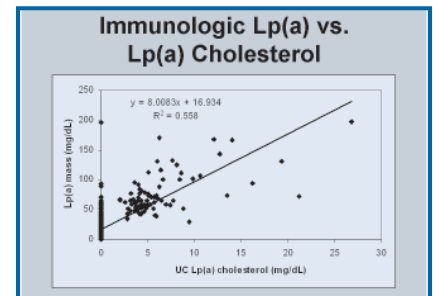
slide 60

Lp(a) mass greater than 30 but no detectable Lp(a) cholesterol. Well, what does that mean? What are the possibilities? 1) Maybe Lp(a) cholesterol is a less sensitive method. 2) Maybe these 54 patients have a type of Lp(a) particle that produces a large signal by immunologic method, without actually being present in high concentration. It is well known that apolipoprotein (a) is size heterogeneous, based on the number of kringle4 type 2 repeats it contains. It is also known that immunoassays are influenced by the isoform size of Lp(a).

slide 61

To test this, we measured Lp(a) cholesterol and Lp(a) mass in the angiography patient samples. This slide represents data from 425 patients in the study. We excluded those patients who had acute myocardial infarction at presentation (time of sample collection) because we didn't want to have the acute phase response influence results of inflammatory parameters like C-reactive protein. Results for Lp(a) mass and cholesterol were the same if all patients were included. This (slide 61) represents a univariate analysis. Association with events was observed for age, C-reactive protein, homocysteine, fibrinogen, Lp-PLA2, and Lp(a) cholesterol, but not LPA mass.

slide 62

Now what happens if we put this in a multivariate model and we look at all of these things? I would start by saying that this is over modeled. (slide 62) We only had 58 events

HR For Angiographic CAD: Multivariate

Variable	HR	95% CI	P-Value
Age	1.05	1.03 - 1.07	<0.0001
Male Gender	4.03	2.46 - 6.60	<0.0001
Hypertension	1.60	1.06 - 2.42	0.026
Smoking	1.45	0.95 - 2.22	0.087
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Lp(a) protein (per 20 mg/dL)	1.14	0.91 - 1.43	0.25
Lp(a) cholesterol (per 5 mg/dL)	1.56	1.15 - 2.11	0.004

slide 58

and we're looking at multiple parameters. When you over model, typically things tend to drop out, as is the case here. But as you see, significance was maintained for a few analytes, with Lp-PLA2, fibrinogen, and Lp(a) cholesterol being significantly associated with cardiovascular events.

Now, let's go back and look at angiographic coronary disease or atherosclerotic burden. Now what's associated with angiographic coronary disease? This, again, is a multivariate model.
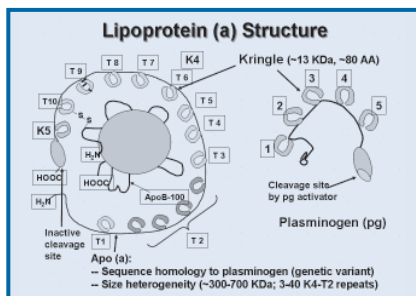
"It turns out that Lp(a) cholesterol is the laboratory measure that appears to be associated with both angiographic cornonary disease and events."

(slide 58) You will note some very different associations. Namely, significant associations with angiographic coronary disease were observed for age, male gender, hypertension, total cholesterol, HDL cholesterol, LDL cholesterol (not shown), and Lp(a) cholesterol but not Lp(a) mass. It turns out that Lp(a) cholesterol is the laboratory measure that appears to be associated with both angiographic coronary disease and events.[22]

And that begins to make a little sense if we begin to think about what Lp(a) is. It's an LDL particle which has an apo(a) molecule attached to it. So it has the properties of LDL cholesterol but it also has this apo (a) molecule that's attached to it. Apo(a) is similar in structure to plasminogen except that it doesn't have the active site responsible for lysing fibrin clots. It may therefore, bind to forming clots in place of plasminogen, thus inhibiting fibrinolysis. And so, in theory, it has the negative properties associated with it's similarities to LDL, but also contributes to atherosclerosis via inhibition of fibrinolysis.

slide 63

The immunologic Lp(a) mass assays are not standardized, but I won't belabor the point here. However, at a recent midwest cardiovascular diseases convention Dr. Angelo Scanu, an Lp(a) guru, indicated that unless you really understand the assay you are using he could not recommend measuring Lp(a) because of the lack of standardization of methods. I believe that Dr Scanu is very much sure that Lp(a) is a cardiovascular risk marker, but the methods are so unstandardized, he suggests that unless you really know what your assay is measuring, don't play with Lp(a) analysis. That's sobering and I think we heard the same thing from Dr. Clejan just a minute ago. But why is there a problem? (slide 63)
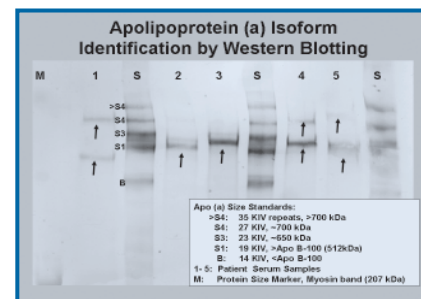
slide 64
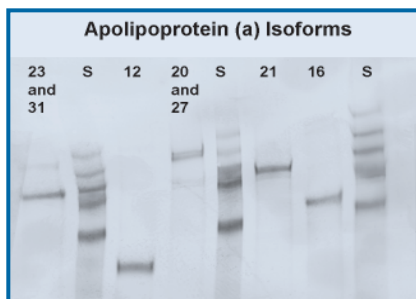
slide 65

This represents a western blot analysis of apo (a), demonstrating that apo (a) size isoforms exist. A kringle 4 type 2 repeating structure is present with only one to as many as 40 repeats. It may be that the immunoassays are influenced by the number of kringles. To prove this, we are now in the process of measuring the isoforms in these study samples (slide 64).

This slide demonstrates the variation of apo(a) size in patients. (slide 65) In lane 3 for instance, one predominant isoform that is expressed is very small in size with 12 total kringles, while in lane 4

the predominant isoform is larger in size with a total of 27 kringles. We hope the data we generate will further demonstrate the need for isoform independent assays. In theory, measurement of the cholesterol content of the Lp(a) molecule, should not be influenced by apo (a) isoform size, but that remains to be proven, which we hope to do in our study.

"In theory, measurement of the cholesterol content of the Lp(a) molecule, should not be influenced by apo (a) isoform size."

Overview and Conclusions

- Prevention of Cardiovascular events is Key!!!
- Know Your Cholesterol, Know Your Risk
  - Lipid profile in everyone > 20 years of age
  - Use ATP III guidelines
  - Determine Framingham Risk Score
  - Educate Others
- Novel/Emerging Risk Markers
  - Primarily in patients at intermediate risk
  - Guide intensity and type of therapy
  - Much more work needs to be done

slide 66

In conclusion. Prevention of cardiovascular events is key. Know your cholesterol, know your risk. Lipid profile everybody over 20 years of age. Use the ATP III guidelines. Novel and emerging risk markers should be performed primarily in patients at intermediate risk or in those with a strong family history of early atherosclerosis without conventional risk factors. There are some other situations where that can occur as Dr. Naito pointed out. Much more work needs to be done to determine the most appropriately measured novel markers and/or panel of markers. (slide 66)

Lp(a) cholesterol is a strong marker of angiographic coronary diseases, as well as cardiovascular events. Differences observed between Lp(a) cholesterol and immunologic Lp(a) mass need to be further investigated, but are likely due to isoform size differences. Efforts to standardize Lp(a) need to continue. And it's very important, if you're measuring Lp(a) in your laboratory, that you understand the limitations of the method that you're using. (slide 67)

Conclusions

- Lp-PLA2 is emerging as an independent risk marker for cardiovascular events: orally active specific inhibitors make it a potential therapeutic target.
- Lp(a) cholesterol is a strong marker for angiographic coronary disease as well as cardiovascular events.
- Differences observed between Lp(a) cholesterol and Immunologic Lp(a) mass assays need to be further investigated, but may be due to dependance of the mass assays on apo(a) isoform size
- Efforts to standardize Lp(a) methods need to continue.

slide 67

There is a difference between atherosclerotic burden and the vulnerability of an atherosclerotic plaque. Laboratory measures that predict atherosclerotic burden may be different than those that predict an impending ischemic cardiovascular event. **Remember, that Lp(a) cholesterol is the laboratory measure that appears to be associated with both angiographic coronary disease and events.**

Dr. Jane Emerson

Jane Emerson,
MD, PhD, FASCP
Chief of Clinical Pathology
University of California-Irvine
Medical Center, Orange, CA

We looked at 266 consecutive specimens coming into the laboratory on which our comprehensive lipid profile was ordered. Our comprehensive lipid profile, at the time, consisted of the Medicare panel plus lipoprotein electrophoresis for phenotyping, Frederickson classification and detecting intermediate bands, Lp(a) and immunoassays for APO-A and B. And that's what we had. And we said, all right, what does this comprehensive panel give us that the Medicare panel doesn't? (slide 68) Because, you know, it's an ongoing debate about which analytes are best in terms of assessing risk. At a minimum, which of these are giving you new information and alerting clinicians to something they wouldn't otherwise know? At what point can you dismiss a patient as having a good lipid profile without need to worry about it further? We decided to compare the added information of each of these analytes to the Medicare panel.

So, first of all, what if you measure only a total cholesterol or only a total cholesterol and triglyceride? That is something that many health fairs and drug stores and even doctor's offices do. If you only measure those and the patient had desirable levels by NCEP, ATP-III, what would you miss? Well, we found out that, of those, 44% had Lp(a) above the 55th percentile and 28% had Lp(a) above the 75th percentile. And, generally, when you look at the studies on Lp(a), you see level of risk associated with different quartiles or quintiles, but almost every

Lipid Profiles

- Medicare-approved panel
  – Total Cholesterol, Triglycerides, LDL-c, HDL-c
- Candidate additional analytes
  – ApoA, ApoB, IDL, Lp(a), LDL Sub, HDL Sub, RLP

slide 68

> "If you only measure those and the patient had desirable levels by NCEP, ATP-III, what would you miss? Well, we found out that, of those, 44% had Lp(a) above the 55th percentile and 28% had Lp(a) above the 75th percentile."

study, certainly above the 75th, says there is significantly increased risk. We used percentiles as cutoffs with percentiles determined in our laboratory from looking at 500 apparently healthy subjects. Now suppose their entire Medicare lipid screen is desirable; how many of those have an abnormal Lp(a)? We eventually found that half of them had Lp(a) above the 55th percentile. (slide 69)[23]

Next, we wanted to know if anything in the profile would predict the Lp(a). To make a long story short, nothing did. (slide 70)

For measuring Lp(a), we had the options of doing mass or Lp(a) cholesterol measurements. We decided we wanted to use the Lp(a) cholesterol after doing a comparison of three methods for several reasons. One reason is the cholesterol assay is more easily standardized because of the inter-individual variation; there I was just adhering to the general dictum that uniform standards are generally better standards. Well-standardized measurements are more likely to be correctly used, interpreted and correlated appropriately with risk eventually, if not now. The other is that, for us, it was important to be able to do a one-shot measurement of what we now thought was the ideal lipid screen. The method we went to was automated enough that, operationally, even though we do a high volume of screens, we would be able to work it into our workflow and work it into our other electrophoresis workflow, too.

Panel Comparison

- 266 consecutive subjects
- 45% had desirable TC, TG by NCEP
  – Of those, 44% had Lp(a) above 55th percentile
  – 28% had Lp(a) above 75th percentile
- 28% had desirable TC, TG, LDL-c, HDL-c
  – Of those, 49% had Lp(a) above 55th percentile

slide 69

Lp(a) Correlates

- None of the following are correlates or predictors of Lp(a) values:
  – Total cholesterol
  – LDL-c
  – HDL-c
  – TG
  – Apo A1
  – Apo B100

slide 70

Now, the sticking point was raised by our coagulation expert who said, well, if the basis of the increased risk, especially for stroke or even CHD, is the prothrombotic features of the particle, then maybe we would be missing something if we're looking at Lp(a) cholesterol rather than the Lp(a) mass assay. We don't really have the answer there in the literature or anywhere else, but we decided we could, at least, determine whether endogenous levels of Lp(a) are correlated with platelet function, or essentially a better measure of bleeding times. If a patient's endogenous Lp(a) or Lp(a) cholesterol measurement is not

Lp(a)/Lp(a)-c and Platelet Function

- Study of 100 subjects with existing Lp(a) and Lp(a)-c measurements
  - Lp(a) ranged from <5-231 mg/dL
  - Lp(a)-c ranged from 0.1-33 mg/dL
- Measured platelet aggregation to collagen/ADP and collagen/EPI by PFA
- Medication history: 34% on ASA or NSAIDS

slide 71

reflected in any thrombotic state that we can measure, or there's no difference between the two, then we wouldn't go to a mass assay over cholesterol. We decided to use the PFA-100 which uses whole blood under flow conditions going through a collagen-coated membrane and then subject to either ADP or epinephrine agonists. This is essentially a simulated bleeding time absent the vascular constriction factors and skin factors that figure into a template bleeding time. We looked at a subset of those initial 500 patients, and it was a representative subset in that the Lp(a) values by both the mass and the cholesterol assays, ranged from very low, undetectable, desirable to markedly elevated. (slide 71) Since we had the data for both the mass assay and the cholesterol assay, we tested their platelet function to collagen/ ADP and

"…for us, it was important to be able to do a one-shot measurement of what we now thought was the ideal lipid screen"

Results

- Closure times with either EPI/collagen or ADP/collagen were not decreased as Lp(a) concentrations increased
- No difference between Lp(a) and Lp(a)-c

slide 72

collagen/epinephrine. What we found was that we didn't really see any decrease in closure time with even the highest Lp(a) values. If there was any effect, and I'm not sure whether it's statistically significant yet or not because we haven't completed that part of the analysis, it's not impressive. If there was any effect, there was a slight prolongation of the closure time to ADP with the subjects with the higher Lp(a). But, in any case, there was no difference between Lp(a) mass and Lp(a) cholesterol. (slide 72)

Dr. Robert Galen: All right. So now I have some questions. I'm interested in the process whereby a pathologist can actually play a role, as you have, in changing clinical practice, because what we do in the lab ultimately affects how clinicians take care of patients. A lot of our colleagues think there's nothing we can do about that. And very few are proactive. So this is a perfect case study of how you've changed the clinical practice here. How did you get from understanding what you wanted to do, to effectuating a change, and having a routine lipid profile that included Lp(a)?

Dr. Jane Emerson: Okay, this all came, basically, from what you were just saying, profiles and compliance, how people order, and what can you do to satisfy them. The goal is to decrease medically unnecessary testing yet satisfy patient and clinician convenience and medical necessity. So we redesigned requisitions. We created cascades and implemented policies for processing and holding specimens, in case we needed them further down the cascade, such as we would for an anemia cascade. I had gotten the feedback from a formal study that, yes, it

"I've always been interested in lipids because it's the model condition for widespread screening of the number one health problem - it's silent, has proven morbidity, mortality, proven effective intervention. If we're going to test anything on people, it ought to be lipids."

significantly matters how you present things to physicians. With lipids, what is the ideal profile that should be offered? I've always been interested in lipids because it's the model condition for widespread screening of the number one health problem- it's silent, has proven morbidity, mortality, proven effective intervention. If we're going to test anything on people, it ought to be lipids. I wanted to optimize what it is clinicians did for their patients.

Dr. Robert Galen: What next?

Dr. Jane Emerson: I actually approached the cardiologists and other internists about establishing a cholesterol center or a risk reduction center. I said I was interested in laboratory support for that kind of thing and that I wanted to support it on several levels. One was that we wanted to offer walk-in lipid testing as a mechanism of capture for these patients to then refer to specialists in lipid clinics. And then the other was to offer the right kinds of testing in the right manner and format. We proposed to establish and maintain a database with the idea that clinical and laboratory services should build the center together. It's very slowly gotten off the ground. So we do have a walk-in lipid testing program. We've had over 600 people come through and it's not advertised. Testing is offered in very limited hours; patients just kind of show up and pay out of pocket, even though they're insured. I send them a letter with their results and call them if they want to be called.

Dr. Robert Galen: And so what do they get? How much do they pay out of pocket?

Dr. Jane Emerson: They pay $20 and they get a lipid screen consisting of the Helena Cholesterol Profile along with a triglyceride and a letter reporting results along with general recommendations.

Dr. Robert Galen: What are your secrets here in promoting the enhanced lipid profile?

Dr. Jane Emerson: It's a slow process. People just kind of have to get to know you and trust your motives. I did have some resistance because patients get these letters with an explanation of Lp(a) and what the associated risks are. That's one thing a lot of patients aren't familiar with. Clinicians are then faced with patients coming to them about their Lp(a) values, so they may have to be updated on how to interpret and act on these values.

Dr. Robert Galen: Okay. So that's one piece of the puzzle. The other one is the compliance and billing issues. What was the process—do you have somebody or a committee in the hospital that deals with that?

Dr. Jane Emerson: For us, the biggest challenge is making sure that the billing matches the physician order. So as long as we can, on an audit, show that the physician has indicated this is what they want, then that's the way we bill it. But, yes, we have a laboratory compliance committee and our lab compliance officer runs that committee, and serves on the medical center compliance committee. That's where we discuss all the logistics of what it is we're trying to do medically, what we have to satisfy operationally, and then how we make sure that we're fine about the billing. We've had no problems at all.

Dr Robert Galen: I want to thank everyone on the panel. Let me close with a comment from Dr Claude Lenfant, former Director of the NIH. "The real challenge of the new millennium may indeed be to strike an appropriate balance between the pursuit of exciting new knowledge and full application of strategies that already are known to be extremely effective, but considerably underused." [24]. I would submit to you that including

"The real challenge of the new millennium may indeed be to strike an appropriate balance between the pursuit of exciting new knowledge and full application of strategies that already are known to be extremely effective, but considerably underused."[24]

Lp(a) in the routine lipid profile is an example of one of those strategies. Laboratory tests continue to be the most cost effective screening tool in the battle against heart disease. We in the laboratory have a unique opportunity to advance clinical practice.
Thank you.

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