Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Routine Lipid Testing

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Slide 2:
Routine lipid testing consists of a "Fasting Comprehensive Lipoprotein Profile" which includes analysis of: plasma total cholesterol, Triglycerides, Low Density Lipoprotein Associated Cholesterol (LDL-Cholesterol), and High Density Lipoprotein Associated Cholesterol (HDL-Cholesterol). It is recommended that all adults over the age of 20 undergo routine lipid testing at least once every 5 years. These recommendations are provided by the National Cholesterol Education Panel (NCEP). They were decided because of the causal risk associated with plasma lipids and coronary heart disease, or CHD.

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CHD is a considerable medical problem in the United States. In 2006, approximately 1 in every 6 deaths was due to CHD. In fact, CHD is the leading cause of death to cardiovascular disease. There is a direct relationship between the level of serum total and LDL-cholesterol and the rate of CHD. It has also been recognized that there is an inverse relationship between serum HDL concentrations and the risk of CHD.

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Based on clinical and laboratory evidence, the NCEP Adult Treatment Panel (ATP) III, in 2002, recommended the following cut points for triglycerides, total cholesterol, HDL and LDL. According to the NCEP ATPIII, for persons with no risk factors, LDL cholesterol below 100 mg/dL is optimal, between 100 and 129 mg/dL is near optimal, between 130-159 mg/dL is borderline high, between 160 and 189 mg/dL is high and > 190 mg/dL is considered very high. The NCEP considers total cholesterol <200 mg/dL to be desirable, total cholesterol between 200-239 mg/dL to be borderline high and cholesterol levels ≥ 240 mg/dL to be high. The recommendations for HDL are: <40 mg/dL is low and > 60 mg/dL is high. These cutpoints are the same for men and women.

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The NCEP recommends modified guidelines for patients with CHD or with risk factors for CHD. These patients are often on lipid-lowering therapy, and LDL-C and non-HDL-C are used as primary and secondary goals of therapy. Non-HDL-C is simply the Total Cholesterol value minus the HDL-C value. The set points for non-HDL-C are 30 mg/dL greater than those for LDL-C. So, the goals for LDL-C and non-HDL-C are: LDL <100 mg/dL and non-HDL-C < 130 mg/dL for patients with CHD and CHD risk equivalent; LDL <130 mg/dL and non-HDL-C <160 mg/dL for patients with more than one risk factor.

Slide 6:
Before we begin discussing how lipids are measured, we should briefly review lipid biochemistry. Lipids are organic molecules and insoluble in water. Lipids circulate as part of water-soluble macromolecules, which are known as lipoproteins. Lipoproteins consist of a hydrophobic core that contains hydrophobic cholesterol esters and triglycerides. Surrounding this core is a membrane composed of a monolayer of free cholesterol and phospholipids. Apoproteins are attached to the surface. There are five major lipoprotein classes, based on their size, density, composition and function: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein, and high density lipoprotein.

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This graph breaks down some of the physiochemical characteristics of the 4 major classes of lipoproteins. Density increases as protein content increases. HDL has the highest protein content. The amount of cholesterol in the lipoprotein particles varies, too, with LDL having the highest cholesterol content. In fact, 70% of total plasma cholesterol is carried by LDL. Chylomicrons consist almost entirely of triglycerides.

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Historically, measurement of lipids was a time-consuming and tedious process. The methods available, ultracentrifugation and electrophoresis, exploited the differences in physical properties of the different lipoprotein classes. Because these methods are so labor-intensive, they are not well suited for screening or routine clinical purposes. Now, there are fully automated homogenous assays available for the measurement of triglycerides, total cholesterol, HDL-C and LDL-C.

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Triglycerides are measured in the clinical laboratory by an automated enzyme-coupled reaction. In the first reaction, triglycerides are converted to glycerol by lipase. Then, the glycerol produced in the first reaction is converted to glycerol-3-phospate by glycerol kinase. Glycerol-3-phosphate is then converted to dihydroxyacetone and hydrogen peroxide in a reaction catalyzed by glycerol phosphate oxidase. The hydrogen peroxide reacts with 4-aminophenone to produce color.

Because plasma triglyceride concentration can increase transiently yet appreciably after a meal, a fasting specimen is required for the accurate measurement of triglycerides. It is recommended that the patient fast for at least 8-12 hours or overnight prior to the blood draw.

Slide 10:
Total cholesterol is also measured by an enzyme-coupled reaction. In this reaction, cholesterol esters are hydrolyzed by cholesterol ester hydrolase to free cholesterol. Free cholesterol is then converted to cholest-4-en-3-one by cholesterol oxidase. This produces hydrogen peroxide which can react with phenol and 4-aminoantipyrine to produce color.

A fasting specimen is not required for accurate measurement of total cholesterol, because plasma concentrations of cholesterol do not change appreciably after a meal. While this assay does not require a fasting specimen for accuracy, it is recommended that the specimen be drawn after fasting along with the rest of the "fasting comprehensive lipoprotein profile."

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There are direct (homogenous) HDL-cholesterol assays available for automated chemistry analyzers, and are widely used in clinical laboratories. There are different assays available but they have similar principles. For example, in one type of assay, the non-HDL-particles (chylomicrons, VLDL, and LDL) are blocked with a synthetic polymer. By the use of a selective detergent HDL is then solubilized and cholesterol is measured, with the use of cholesterol esterase and cholesterol oxidase. The end products are cholestenone and hydrogen peroxide. Like in the total cholesterol assay, the hydrogen peroxide formed in this reaction reacts with 4AAP to produce color.

Slide 12:
LDL can be calculated or estimated from measurements of total cholesterol, triglycerides and HDL-cholesterol. The equation used is called the Friedewald equation.

Using the Friedewald equation, LDL-C is estimated by subtracting the sum of HDL-C and VLDL-C from the total cholesterol value. VLDL-C is not measured, but determined from the triglyceride value by dividing the total trig concentration by 5. This is valid because the ratio of triglyceride to cholesterol in VLDL is 55%/12% which is equal to 5.

This does not hold up at high triglyceride concentrations; it is invalid when triglycerides are > 400 mg/dL. Advantages to using the Friedewald equation include:

1. Extensive experience with the equation,
2. It has been used in many clinical studies,
3. It has been well-established in determining clinical significance,
4. It is convenient, and
5. It is inexpensive

Disadvantages are that it does not hold up at high triglyceride concentrations; also, it is invalid when triglycerides are >400 mg/dL. Finally, the value is estimated from three independent measures, each with their own errors.

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There are now fully automated homogenous (direct) assays available for the measurement of LDL-C (these are third-generation assays). The premise is similar to the HDL-C assay. In all assays, the non-LDL

particles are removed by specific blocking or solubilization, in order to achieve specificity for LDL-C. Then, enzymes are added.

The assay shown here is the Kyowa Medex assay, distributed by Roche Diagnostics. In this assay, chylomicrons, VLDL and HDL are removed by complexing with synthetic polymer. Then, LDL is solubilized with a selective detergent and cholesterol esterase and cholesterol oxidase are added to produce cholestenone and hydrogen peroxide. As in the total cholesterol and HDL-C assays, the hydrogen peroxide reacts with 4AAP (catalyzed by peroxidase) to produce color. These assays have a few drawbacks. They have been shown to be unsuitable for patients with dyslipidemias and are less accurate within samples with high triglycerides. For patients with triglycerides >400 mg/dL, non-HDL-C is recommended over direct LDL-C assays.

Slide 14:
As mentioned before in this presentation, non-HDL-C is the difference of total cholesterol minus LDL-cholesterol. This calculation includes the VLDL and IDL fractions, and does not require fasting. Non-HDL-C is used as a secondary target of lipid-lowering therapy, especially in patients with co-morbidities such as diabetes mellitus or metabolic syndrome, who are likely to be dyslipidemic.

Slide 15:
It is important to understand that there is considerable intra-individual variability in lipid and lipoprotein measurements, due in large part to biological variation. Variation has been shown to be 6% for cholesterol, between3.6% and 12.4% for HDL-C and up to 20% for triglycerides. Thus, except in patients with marked abnormalities in lipid and lipoprotein concentrations, multiple measurements are recommended for accuracy. The NCEP guidelines recommend that the patient is sampled on several occasions at least a week apart within an 8 week period. These individual values can then be averaged.

Slide 16:
As stated by the NCEP, "The adoption of a single set of cutoffs imposes on laboratories the mandate to measure lipids and lipoproteins accurately and precisely." Therefore, the NCEP also proposed guidelines for total allowable error in assays for cholesterol, triglycerides, HDL- and LDL-cholesterol.
The bias is from the CDC reference methods.

Slide 17:
In conclusion, according to the NCEP ATP III Guidelines, routine lipid testing should be performed for every adult over age 20 at least once every 5 years. This should include measurement of plasma triglycerides, total cholesterol, HDL-C and LDL-C.

Lipids do not circulate freely but as part of water-soluble macromolecules called lipoproteins. Measurement of plasma lipids depends on the separation of the lipoprotein classes by their distinct physiochemical characteristics.

Labor-intensive lipid quantitation has been replaced by fully automated assays that are available in routine clinical laboratories.