EDUCATIONAL COMMENTARY – LIPID- AND LIPOPROTEIN-BASED RISK ASSESSMENT FOR Atherosclerotic Cardiovascular Disease

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LEARNING OUTCOMES

On completion of this exercise, the participant should be able to

• list five risk factors for atherosclerotic cardiovascular disease identified by the Framingham Heart Study.
• describe the structure of lipoproteins and classify the major subclasses of lipoproteins determined by ultracentrifugation as either atheroprotective or atherogenic.
• calculate non–high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol when given the total cholesterol, HDL cholesterol, and triglyceride concentrations.
• list target LDL cholesterol levels recommended by the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).
• interpret concentrations of the following lipids / lipoprotein components: triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein B, and lipoprotein (a).

Introduction

Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of death worldwide, even though increased emphasis on identification and subsequent treatment to reduce risk factors has led to decreased mortality rates. Although the process of atherosclerosis is not completely understood, it ultimately results in development of plaques, which can cause narrowing or complete occlusion of arteries. This plaque formation process includes endothelial dysfunction, inflammation, and buildup of cholesterol, calcium, and cellular debris.

Beginning with the Framingham Heart Study, which initially enrolled 4500 participants between 1948 and 1952 and followed them for decades, risk factors for developing ASCVD have been identified and treatments targeting the modifiable factors have been implemented. The Framingham Study identified age, sex, smoking status, diabetes, hypertension, and elevated serum cholesterol as important risk factors for ASCVD. Subsequent studies have established correlations between lipids and/or lipoprotein components and increased risk for ASCVD. The National Heart, Lung, and Blood Institute’s (NHLBI’s) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, known as the Adult Treatment Panel (ATP), has developed and published guidelines for physicians to use in the diagnosis, treatment, and monitoring of patients with ASCVD. These guidelines, first issued in 1988 as
ATP I and revised in 1993 (ATP II), 2001 (ATP III), and 2013 (ATP IV), include recommendations using lipid and lipoprotein-component concentrations to guide decision making in diagnosis, treatment, and monitoring.

**Lipids and Lipoproteins**

Cholesterol and triglycerides, the lipids identified as risk factors for ASCVD, are transported in blood as components of lipoproteins. Lipoproteins are spherical particles with a surface layer made of phospholipids, apoprotein molecules that help provide structure and stability to the particle, and free (unesterified) and esterified cholesterols. This surface layer surrounds an inner core of polar cholesteryl esters and triglyceride molecules. In the 1960s, ultracentrifugation was used to separate lipoproteins into the following subclasses: very low-density (VLDL), low density (LDL), intermediate density (IDL), and high density (HDL) lipoproteins. These major subclasses were arbitrarily assigned based on a density range; each consists of lipoproteins of differing size, composition, and density. The subclasses can be identified by several physical characteristics including cholesterol concentration, which became a surrogate for determination of the lipoprotein itself. High-density lipoprotein particles have been determined to be atheroprotective, whereas all VLDL, LDL, and IDL particles are considered atherogenic. Thus laboratory determination of VLDL-, LDL-, and IDL-associated constituents is considered measurement of positive risk factors for ASCVD, whereas measurement of HDL-associated constituents is considered determination of a negative risk factor.

**Triglycerides**

Measurement of triglycerides (TG) concentration is included in most lipid panels, and a positive association between incidence of cardiovascular disease and the total concentration of TG has been identified.\(^5\,6\) Measurement of triglycerides should be performed on samples collected after a 9- to 12-hour fast. Although TGs are present in all lipoproteins, the concentration divided by 5 is used in the Friedewald calculation (see discussion below) as an estimate of the cholesterol concentration in VLDL particles. The ATP III guidelines from the National Cholesterol Education Program (NCEP) include the classification of risk based on TG concentration as shown in the Table. Risk is defined as normal for TG concentrations less than 150 mg/dL and very high for concentrations of 500 mg/dL or more.

**Total Cholesterol**

As previously mentioned, the Framingham Study identified elevated serum cholesterol as a risk factor for ASCVD. Measurement of total cholesterol (TC) is included in typical lipid panels, although emphasis is now placed on the cholesterol content of atherogenic lipoproteins, particularly LDL. As shown in the Table, the ATP III classification defines the desirable level for TC as less than 200 mg/dL.
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Table. ATP III Classification of Triglycerides, Total Cholesterol, and LDL Cholesterol

<table>
<thead>
<tr>
<th>Triglycerides mg/dL</th>
<th>Category</th>
<th>Total Cholesterol mg/dL</th>
<th>Category</th>
<th>LDL Cholesterol mg/dL</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;150</td>
<td>Normal</td>
<td>&lt;200</td>
<td>Desirable</td>
<td>&lt;100</td>
<td>Optimal</td>
</tr>
<tr>
<td>150-199</td>
<td>Borderline high</td>
<td>200-239</td>
<td>Borderline high</td>
<td>100-129</td>
<td>Near/above optimal</td>
</tr>
<tr>
<td>200-499</td>
<td>High</td>
<td>200-239</td>
<td>Borderline high</td>
<td>130-159</td>
<td>Borderline high</td>
</tr>
<tr>
<td>&gt;500</td>
<td>Very high</td>
<td>≥240</td>
<td>High</td>
<td>160-189</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥190</td>
<td>Very high</td>
</tr>
</tbody>
</table>

Abbreviations: ATP, Adult Treatment Panel; and LDL, low-density lipoprotein.

SI conversions: To convert triglycerides to mmol/L, multiply by 0.013. To convert total cholesterol and LDL cholesterol to mmol/L, multiply by 0.0259.


Low-Density Lipoprotein Cholesterol (LDL-C)

Although early studies such as Framingham initially measured only serum TC, the fact that most of TC is contained in LDL implied that an elevated LDL concentration is a powerful risk factor. Subsequent studies confirmed that LDL is the most abundant atherogenic lipoprotein. The cholesterol content of LDL (LDL-C) may be obtained by direct measurement after separation of lipoproteins using physical, biochemical, or immunologic methods or may be estimated using the Friedewald calculation (see below). Epidemiologic studies of human populations have found a direct relationship between levels of LDL-C and the rate of new ASCVD: any concentration of LDL-C appears to be atherogenic. This association has led to the designation of LDL-C as the “bad cholesterol” in lay terms.

ATP III guidelines focus on measurement of LDL-C not only for risk assessment but also to guide and monitor treatment. Classification of risk based on LDL-C levels is shown in the Table, with levels below 100 mg/dL called optimal. Low-density lipoprotein cholesterol levels are used as treatment goals, and combinations of LDL-C levels and risk categories are used in the guidelines to recommend treatment to achieve desired LDL-C concentrations. For example, treatment (e.g., diet, exercise, LDL-lowering drugs) for persons with coronary heart disease (CHD) or CHD equivalents is based on the LDL-C concentration, with the goal of achieving an LDL-C level of less than 100 mg/dL. For patients with multiple risk factors
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and 10-year risk of less than 20%, the LDL-C goal is less than 130 mg/dL; and for patients with 0-1 risk
factor, the goal is <160 mg/dL. The guidelines provide detailed treatment protocols focused on achieving
target LDL-C levels.

In November 2013 when the ATP IV guidelines (now authored by the American College of Cardiology /
American Heart Association Task Force on Practice Guidelines) were released, one recommendation was
to replace the use of LDL-C and/or non-HDL-C treatment goals with statin therapy based on classifying
patients into statin benefit groups. In these recommendations, LDL-C levels are used as partial criteria
for identifying / defining the four major statin benefit groups and for defining the therapy intensity (e.g.,
high-intensity statin therapy lowers LDL-C by 50% or more). The impact of these changes on clinical
laboratory medicine is yet to be determined.

Friedewald Formula for Calculation of LDL-C

The Friedewald formula, published in 1972 as a method for estimating the LDL-C concentration, assumes
that the ratio of triglycerides to cholesterol in VLDL particles is 5:1.8

\[
\text{Friedewald formula:} \\
\text{LDL-C} = \text{TC} - \text{HDL-C} - \left(\frac{\text{TG}}{5}\right)
\]

A fasting sample is required for accurate measurement of TG. As total TG concentration increases, the
ratio of TG to cholesterol in VLDL increases to greater than 5:1, which causes underestimation of LDL-C
at higher TG concentrations. For this reason, the formula should not be used when TG concentrations
are greater than 400 mg/dL; some recommendations state that the formula is not accurate when TG
concentrations are greater than 250 mg/dL. The formula has also been shown to be unreliable at
accurately classifying patients near the LDL-C treatment threshold of 70 mg/dL.9

High-Density Lipoprotein Cholesterol (HDL-C)

The lipoproteins in the HDL subclass have been shown to have a number of atheroprotective properties
that help slow the atherosclerotic process. They have a role in removing excess cholesterol from
macrophage foam cells in the arterial wall and transporting it to the liver, where it is excreted in the bile.
Other atheroprotective properties of HDL include anti-inflammatory, antioxidative, and antithrombotic
activities. High-density lipoprotein cholesterol is known as the "good cholesterol," and the ATP III
classification assigns a negative risk factor for HDL-C levels of 60 mg/dL or greater. HDL-C
concentrations of less than 40 mg/dL are categorized as high risk. The apolipoproteins in HDL particles
are designated as A apolipoproteins (A-I and A-II), with the predominant apolipoprotein (apo) being apo...
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A-1. Measurement of apo A-1 has been proposed as an alternative to HDL-C measurement, but in most studies measurement of apo A-1 and HDL-C give equivalent predictive values.\(^2\)

Non-HDL-C

Because non-HDL particles (VLDL, LDL, IDL, and chylomicron remnants) are considered atherogenic, calculation of the cholesterol content of these molecules would seem to correlate with ASCVD risk. This calculation involves subtraction of HDL-C concentration from the TC concentration.

**Non-HDL-C formula:**

\[
\text{non-HDL-C} = \text{TC} - \text{HDL-C}
\]

ATP III guidelines recommend calculating non-HDL-C (atherogenic cholesterol) whenever TG levels are 200 mg/dL or greater.\(^6\) If non-HDL-C levels are used as treatment goals, the target concentrations are approximately 30 mg/dL higher than the corresponding LDL-C levels in the guidelines.

Apolipoprotein B (apo B)

All atherogenic lipoproteins [VLDL, LDL, IDL, and lipoprotein (a)] contain one molecule of apolipoprotein B (apo B). This glycoprotein exists in two isoforms: apo B\(_{100}\), the full-length protein; and apo B\(_{48}\), the N-terminal truncated protein, which is approximately 48% of the size of apo B\(_{100}\). Methods for determining apo B levels typically measure either apoB\(_{100}\) or total apo B. The structure of apo B is flexible, which allows adoption of different conformations according to the size of the lipid component to which it is attached. Thus, within a subclass, the cholesterol and TG content may vary, but each particle contains only one molecule of apo B. Very low-density lipoprotein particles may convert to smaller and denser IDL and LDL particles without protein exchange, as the apo B adapts to changes in particle size.

Because the intact atherogenic particles, not solely the cholesterol in the particle, are involved in the atherosclerotic process, the concentration of apo B is a more accurate measure of the total concentration of atherogenic particles than the LDL-C concentration. Apo B testing offers the following advantages: nonfasting sample, standardization of assays, noninterference from TG, and availability on automated instrument platforms. Measurement of apo B should be considered as an alternate measure of LDL-related risk (as measured by LDL-C and non-HDL-C), not as a risk factor that is distinct from these other measures. Guidelines from several professional organizations, consensus conferences, and working groups include recommendations to include apo B testing in lipid panels, either as a replacement for or in addition to LDL-C and/or non-HDL-C testing.\(^{10-12}\) When apo B levels are used for monitoring treatment, goals equivalent to those for LDL-C are used. An apo B level of 80 mg/dL is equivalent to the LDL-C
treatment goal of 100 mg/dL, and apo B levels of 100 and 120 mg/dL are equivalent to the LDL-C goals of 130 and 160 mg/dL, respectively.

**Lipoprotein (a) [Lp(a)]**

Lipoprotein (a), pronounced “lipoprotein little a” and abbreviated as Lp(a), are LDL subclass molecules with a glycoprotein, apo(a), attached covalently to the apo B₁₀₀ molecule of the particle. There are multiple apo(a) isoforms, and some trials have shown higher risk associated with smaller apo(a) isoforms. Lp(a) levels are genetically determined and remain relatively stable over an individual’s lifetime. Elevated levels of Lp(a) (mass values >30 mg/dL) have been shown to be independent risk factors for ASCVD. Because Lp(a) levels are not associated directly with elevated LDL-C, identification of persons with elevated Lp(a) concentrations allows physicians to treat these patients using more aggressive LDL-C goals.¹⁰

**Risk Factor Calculations**

Large population adult cohort studies such as the Framingham Heart Study have led to the development of equations with online or downloadable tools that allow calculation of risk for a future cardiovascular event (often within 10 years). The Framingham CHD Risk Calculator (http://cvdrisk.nhlbi.nih.gov/) requires input of age, gender, TC and HDL-C, systolic blood pressure, current smoking status, and current blood pressure medication status.

Laboratory results used in one or more of the published risk equations include total, LDL, and HDL-C; c-reactive protein (CRP); and hemoglobin A₁c. Other factors used in one or more risk equations include age, sex, systolic blood pressure, blood pressure medication, diabetes, smoking, family history, body mass index, social deprivation, and geographic region. The ACC / AHA Work Group that published the ATP IV document concluded that the variables statistically meriting inclusion for its Pooled Cohort Equations were: age, TC and HDL-C, systolic blood pressure, use of blood pressure-lowering medication, diabetes, and current smoking status.⁷ A downloadable spreadsheet and a web-based calculator enabling estimation of 10-year and lifetime risk for ASCVD using these Pooled Cohort Equations are available at:

http://my.americanheart.org/professional/StatementsGuidelines/PreventionGuidelines/Prevention-Guidelines_UCM_457698_SubHomePage.jsp

and

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Summary

High-density lipoprotein particles have an atheroprotective role concerning atherosclerosis, whereas VLDL, LDL, and IDL particles are atherogenic. Data collected during multiple longitudinal large population adult cohort studies have demonstrated correlation between concentrations of lipids (TC and TG) and/or lipoprotein components [HDL-C, LDL-C, apo B, Lp(a)] and relative risk for ASCVD. Although the primary focus in recent years has been on the use of LDL-C for risk assessment and treatment goals, the measurement of other components such as HDL-C, non HDL-C, apo B, and Lp(a) has contributed to risk assessment and treatment monitoring. While determination of individual lipid and lipoprotein component concentrations is used to determine risk factors for ASCVD, the balance between atherogenic and atheroprotective lipoproteins may be a better predictor of the likelihood that a cardiovascular event will occur. Several ratios of lipid to lipoprotein component concentrations have been utilized as a means of expressing this balance. These ratios include the following: TG/HDL-C; TC/HDL-C; apo B/apo A-I; non-HDL-C/HDL-C; and HDL-C/TC. Comparison of risk based on any one of these ratios to risk based on other lipid or lipoprotein components previously mentioned has been mixed, with some studies showing an advantage to using a ratio and others finding no benefit.

References


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