Laboratory Investigation of Dyslipidemia

ABSTRACT

Cardiovascular disease is the leading cause of illness and death in the United States. Disorders of lipid and lipoprotein metabolism (dyslipidemia) predispose patients to premature atherosclerosis. Dyslipidemias can be classified as hypercholesterolemia, hypertriglyceridemia, combined hyperlipidemia, and low levels of high-density lipoprotein cholesterol. Elevated cholesterol levels can cause premature atherosclerosis, and high triglyceride levels can result in pancreatitis. The laboratory investigations that are useful in the diagnosis and management of dyslipidemia include measurement of cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, both directly and through calculation. Useful tests that can be done in the laboratory to exclude secondary causes of dyslipidemia are measurement of thyroid-stimulating hormone, blood glucose, liver enzymes, creatinine, and plasma and urine protein.

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In the past decade, the lowering of elevated blood cholesterol levels by diet and drug therapy has significantly reduced cardiovascular morbidity and mortality.1 The transfer of this concept to clinical practice, however, involves several discrete steps and a fund of knowledge that may not be available to all health care professionals. In this review article, we focus on the appropriate laboratory testing for dyslipidemia.

Lipoprotein Composition and Metabolism

In humans, the three major lipid moieties other than fatty acids are phospholipids, cholesterol, and triglycerides. In clinical medicine, phospholipids are primarily important for fetal lung maturity. Thus, we focus only on cholesterol and triglycerides. Cholesterol is an important precursor of bile acids, a precursor for steroidogenesis in the adrenals and gonads, and an important constituent of cell membranes. Triglycerides are an important substrate for energy use. Neither cholesterol nor triglycerides are soluble in water; they must be packaged in lipoprotein particles to be transported to various tissues. These lipoprotein particles are composed of a hydrophilic coat that consists of apoproteins, free cholesterol and phospholipid, and a hydrophobic core made up of cholesterol ester and triglycerides.

The lipoproteins can be classified by their density on ultracentrifugation or by their electrophoretic mobility (Table 1). Chylomicrons and very-low-density lipoprotein (VLDL) are the main triglyceride-carrying particles. Approximately two thirds of plasma cholesterol is carried in low-density lipoprotein (LDL); the remaining is carried in high-density lipoprotein (HDL).

After the ingestion of a fatty meal and micellar formation (a step crucial in fat absorption), the lipid is packaged into chylomicrons in the enterocytes and secreted into the circulation by way of the lymphatic system. In the circulation, lipoprotein lipase, an enzyme that requires apoprotein C11 as a cofactor, acts on the lipid. This action rapidly produces remnant particles, which are removed by endocytosis in the liver. Originating primarily from the liver, VLDL carries endogenous triglyceride and contains apoprotein B, apoprotein E, and apoprotein C. It is also hydrolyzed by lipoprotein lipase-producing remnants. After hydrolysis, the remnants are subjected to endocytosis by the liver or undergo further metabolism (by the action of hepatic lipase) to yield LDL, a cholesterol-rich lipoprotein that contains only apoprotein B-100. The remaining lipoprotein, HDL, is synthesized in the liver and intestine and transports 20% to 30% of plasma cholesterol. This lipoprotein is believed to participate in reverse cholesterol transport: it picks up unesterified cholesterol in peripheral tissues and esterifies it by the action of lecithin cholesterol acyl

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transfase (the cofactor of which is apoprotein A1, the major apoprotein in HDL). High-density lipoprotein shuttles the cholesterol back to the liver for excretion as bile acid and neutral sterols.

**Dyslipidemias**

Disorders of lipoprotein metabolism can be broadly classified as hypercholesterolemia, hypertriglyceridemia, hypoalphalipoproteinemia, and combined hyperlipidemia.1

**Hypercholesterolemia**

The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) has classified hypercholesterolemia as follows:2 An elevated serum cholesterol level is defined as 240 mg/dL (6.20 mmol/L) or higher, a borderline cholesterol level is 200 to 239 mg/dL (5.17-6.18 mmol/L), and a desirable cholesterol level is less than 200 mg/dL (<5.17 mmol/L). An important point is that an HDL cholesterol level less than 35 mg/dL (0.90 mmol/L) is considered low and constitutes an independent risk factor for coronary artery disease. A lipoprotein analysis should be done in anyone with an elevated total cholesterol level, a borderline cholesterol level with an HDL cholesterol level less than 35 mg/dL (0.90 mmol/L), or two additional risk factors. A lipoprotein profile is also necessary in patients with a cholesterol level less than 200 mg/dL (5.17 mmol/L) and an HDL cholesterol level less than 35 mg/dL (0.90 mmol/L). The various risk factors used to determine whether lipoprotein analysis is necessary in a patient with a borderline cholesterol level include age (men > 45 years; women > 55 years or premature menopause without estrogen replacement therapy), family history of coronary heart disease, smoking, hypertension, HDL cholesterol level less than 35 mg/dL (0.90 mmol/L), diabetes, and cerebrovascular or peripheral vascular disease. In the borderline group, one risk factor is subtracted from the risk profile if the HDL cholesterol level is at least 60 mg/dL (1.55 mmol/L).

After hypercholesterolemia has been established, secondary causes must be ruled out (Table 2). The classic gene disorder that results in very high cholesterol levels is familial hypercholesterolemia,3 an autosomal dominant disorder characterized by high LDL cholesterol levels due to a genetic defect that results in decreased LDL receptor activity. Familial hypercholesterolemia can be classified into two types: type II-A (high LDL cholesterol and normal plasma triglyceride levels) and type II-B (high LDL cholesterol and high plasma triglyceride levels).

The most common cause of the type II-A phenotype is not familial hypercholesterolemia but rather primary moderate hypercholesterolemia or polygenic hypercholesterolemia. Although the exact cause of this disorder is unknown, it is believed to be due to a complex interaction of genetic and environmental factors and predisposes patients to premature atherosclerosis.

**Hypertriglyceridemia**

The link between high triglyceride levels and coronary artery disease is complex and can be explained by association with low HDL cholesterol levels; predominance of small, dense LDL particles; and increased levels of remnant lipoproteins and procoagulant factors.4 However, the NCEP has not identified triglycerides as an independent risk
Table 2. Common Causes of Secondary Hyperlipidemia

<table>
<thead>
<tr>
<th>Predominantly hypertriglyceridemia</th>
<th>Alcoholism</th>
<th>Diabetes mellitus</th>
<th>Drugs, such as estrogen, steroids, or β-blockers</th>
<th>Obesity</th>
<th>Renal failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominantly hypercholesterolemia</td>
<td>Cholestasis</td>
<td>Drugs, such as diuretics and cyclosporine</td>
<td>Hypothyroidism</td>
<td>Nephrotic syndrome</td>
<td></td>
</tr>
</tbody>
</table>

factor for coronary artery disease. With regard to triglycerides, the major problem arises with the chylomicronemia syndrome, in which triglyceride levels are 1,000 mg/dL (11.3 mmol/L) or greater. Patients with this syndrome are at increased risk for pancreatitis. Chylomicronemia results from deficiency in lipoprotein lipase or its cofactor, apoprotein C-II.5 A very high plasma triglyceride level can result from the type V disorder, which occurs in adults and presents with fasting chylomicronemia and elevated VLDL levels. In these patients, a secondary factor usually increases plasma triglycerides (Table 2).

**Hypoalphalipoproteinemia**

Low levels of HDL cholesterol (hypoalphalipoproteinemia) are an independent risk factor for coronary artery disease, as clearly shown by the Framingham Heart Study.6 Common causes of low HDL cholesterol level include smoking, renal disease, obesity, and use of beta-blockers. The familial form of hypoalphalipoproteinemia is associated with premature atherosclerosis. Thus, the Adult Treatment Panel of the NCEP has determined that an HDL cholesterol level less than 35 mg/dL (<0.90 mmol/L) is an inverse risk factor for atherosclerosis. However, certain persons with very low HDL cholesterol levels as a result of mutations in apoprotein A-I do not have an increased propensity to premature atherosclerosis.7

**Combined Hyperlipidemia**

Combined hyperlipidemia results in elevation in both cholesterol and triglyceride levels. The common causes of combined hyperlipidemia are familial combined hyperlipidemia and type III dyslipidemia. In the former, patients have hypercholesterolemia, hypertriglyceridemia, or both. This condition appears to be inherited as an autosomal dominant trait8-11 and to result in hepatic overproduction of lipoproteins that contain apoprotein B-100. These patients have an increased propensity for premature atherosclerosis; their apoprotein B levels are elevated and their HDL levels are low. Diagnosis depends on demonstration of several lipoprotein phenotypes in the patient’s first-degree relatives or a changing lipoprotein profile in the index patient.

Type III dyslipidemia (also called familial dysbetalipoproteinemia, remnant removal disease, or broad beta disease) usually involves an abnormality in apoprotein E and homozygosity for apoprotein E2 (rather than for apoprotein E3, which is predominant in the normal population).12 Patients usually present with combined hyperlipidemia, similar elevations in cholesterol and triglyceride levels, and an increased accumulation of remnant lipoproteins. Type III dyslipidemia predisposes patients to both premature coronary artery disease and peripheral vascular disease. Electrophoresis of serum yields a broad beta band. This is one of the rare disorders in which a basic lipoprotein profile is not sufficient; further testing, such as ultracentrifugation to quantitate VLDL cholesterol, is necessary. In these patients, the ratio of VLDL cholesterol to total triglycerides should exceed 0.30.1

**Laboratory Investigation**

The first step in the laboratory investigation of dyslipidemias is the measurement of total plasma cholesterol and triglyceride levels. Plasma or serum can be used to measure the lipid profile. Plasma is usually collected in tubes that contain EDTA as an anticoagulant. Values obtained in plasma for both cholesterol and triglyceride are approximately 3% lower than values in serum. Because the lipid measurements exhibit a large biological variability, at least two lipid estimates should be done, preferably 1 week apart.2 The reported biological variability for total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol is 6.1%, 25%, 8.2%, and 7.5%, respectively.13 According to the Adult Treatment Panel of the NCEP, serum cholesterol in adults should be measured at least once every 5 years.

Necessary precautions for obtaining a blood sample for a lipoprotein profile include a 10- to 12-hour fast, avoidance of alcohol or exercise the evening before blood sampling, no sampling...
for up to 2 to 3 weeks after a minor illness and up to 3 months after a major illness; and discontinuation of therapy with drugs that affect lipid metabolism for at least 4 to 6 weeks before the first blood sampling.

The new guidelines state that classification of dyslipidemia begins with the measurement of total and HDL cholesterol. Levels of HDL cholesterol are primarily measured to assess atherosclerotic risk and should be obtained in persons meeting one of the following criteria: total cholesterol level less than 200 mg/dL (<5.17 mmol/L) and HDL cholesterol level less than 35 mg/dL (<0.90 mmol/L); borderline cholesterol level (200–239 mg/dL [5.17–6.18 mmol/L]) and HDL cholesterol level less than 35 mg/dL (<0.90 mmol/L) or at least two risk factors; and a total cholesterol level greater than 240 mg/dL (>6.20 mmol/L). The LDL cholesterol level can be obtained from the measurement of total cholesterol, total triglycerides, and HDL cholesterol by using the Friedewald formula:

\[
LDL = \frac{\text{total cholesterol} - \text{HDL cholesterol} - \text{triglycerides}}{5}
\]

This formula is not valid, however, if the triglyceride level exceeds 400 mg/dL (4.52 mmol/L) or if the patient has chylomicronemia or type III dyslipidemia.

To rule out type III dyslipidemia, β-quantification by ultracentrifugation and lipoprotein electrophoresis must be done. β-Quantification allows measurement of VLDL cholesterol, but the process is laborious and time-consuming and involves expensive instruments that are not routinely available in most laboratories.

Although primary investigation of dyslipidemia does not warrant lipoprotein electrophoresis, this procedure has two useful applications. It can be used to identify the broad beta-band of type III dyslipidemia and differentiate it from the type II-B pattern (which is characterized by distinct beta and pre-β bands). Lipoprotein electrophoresis can also be used to diagnose chylomicronemia because chylomicrons remain at the origin on serum electrophoresis. In patients with high fasting triglyceride levels, the refrigerator test may be useful to determine the presence of high VLDL or chylomicron levels. In a plasma sample stored in the refrigerator for 18 hours, a turbid infranate indicates an increased VLDL level; a creamy layer on the top indicates the presence of chylomicrons.

Although an increased apoprotein B level, decreased apoprotein A-1 level, and decreased apoprotein A-1/apoprotein B ratio have been shown to be good markers for identifying coronary artery disease, no prospective studies have confirmed these observations. Thus, the addition of these assays to the current lipoprotein repertoire is not uniformly accepted. However, measurement of apoprotein B in patients with combined hyperlipidemia and a suspected diagnosis of familial combined hypercholesterolemia is useful, especially in patients with a normal LDL cholesterol level and clinical atherosclerosis, who may have an elevated apolipoprotein B level (hyperapobetalipoproteinemia).

Lipoprotein(a), a circulating lipoprotein, has recently been associated with an increased coronary risk. Lipoprotein(a) is similar to LDL in protein and lipid composition but also contains a highly glycosylated apoprotein(a) that is linked through a disulfide bridge to apoprotein B-100. Numerous epidemiologic studies have suggested that lipoprotein(a) is an important risk factor for cerebrovascular as well as cardiovascular disease. In vitro evidence also supports a prothrombotic activity for lipoprotein(a). In addition, lipoprotein(a) has been shown in atherosclerotic plaques. Although much evidence suggests that lipoprotein(a) measurement may be useful in assessing coronary risk, there is little uniformity in the measurement of these levels in different laboratories. Thus, this test must be standardized before it can be added to the tests used in clinical practice to assess risk for coronary artery disease. Other laboratory tests that are available in the clinical laboratory and are useful in ruling out secondary hyperlipidemia include urinalysis (to identify proteinuria of the nephrotic syndrome), plasma glucose (diabetes mellitus), urea and creatinine (chronic renal failure), thyroid-stimulating hormone (hypothyroidism), liver function tests (cholestasis), and serum protein electrophoresis (paraproteinemia and the nephrotic syndrome).

Thus, in most patients, measurement of cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol by use of the Friedewald formula is sufficient in the workup. Although cholesterol and triglycerides can be measured enzymatically or chemically, most laboratories use rapid automated enzymatic analysis. Cholesterol is measured enzymatically after the
addition of cholesterol esterase and oxidase. Hydrogen peroxide release is coupled to a peroxidase reaction with a chromophore or can be measured by a coupled system that produces the reduced form of nicotinamide adenine dinucleotide phosphate. Triglycerides are routinely measured by using a bacterial lipase to hydrolyze triglycerides into glycerol and fatty acids; glycerol kinase then converts glycerol to glycerol-3-phosphate and adenosine diphosphate. If glycerol blanking is not offered (by incorporating pre-treatment with glycerol kinase), triglyceride levels may be overestimated because of glycerol in cases of diabetes, hemodialysis, liver disease, and contamination of phlebotomy devices.

Levels of HDL cholesterol are usually measured with a precipitation method in which apoprotein B-containing lipoproteins such as LDL, VLDL, and lipoprotein lipase-remnants are precipitated by using polyanions in the presence of divalent cations (such as heparin-manganese chloride, phosphotungstate-magnesium chloride, or dextran sulfate-magnesium chloride). However, direct methods are being evaluated for the measurement of HDL. In most patients, except those with hypertriglyceridemia (triglyceride levels of 400 mg/dL [4.52 mmol/L] or more) or type III dyslipidemia, the LDL cholesterol level is calculated by the Friedewald equation. A direct immunoseparation method for LDL cholesterol has recently become available; in this assay, VLDL, lipoprotein lipase-producing remnants, and HDL are sequestered on a filter after aggregation to latex beads that contain antibodies to apoprotein A-1 and apoprotein E. The LDL cholesterol level is then measured in the filtrate by using a cholesterol assay. This method is useful for patients with hypertriglyceridemia and those with type III dyslipidemia. Other experimental tests of interest include determination of remnant lipoproteins, measurement of LDL size by gradient gel electrophoresis, determination of plasma homocysteine levels, and measurement of LDL oxidizability and antioxidant status. However, much research is needed before these tests become part of the routine laboratory repertoire for atherosclerosis screening.

The NCEP has provided guidelines for measurement of LDL, HDL, and triglycerides. In addition to providing a goal for bias and coefficient of variation, the NCEP has reported goals for total error (for cholesterol, 8.9%; for triglycerides, < 15%; for HDL cholesterol, < 22%; for LDL cholesterol, < 12%). To attain these goals, laboratories therefore need to participate in standardization programs, such as a College of American Pathologists (Northfield, Ill) survey and the Alert Proficiency Program from Pacific Biometrics (Irvine, Calif) (which also includes assessment of LDL cholesterol by β-quantification and the direct LDL assay on fresh samples).

**Conclusion**

Most dyslipidemias can be monitored by the measurement of cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol by the Friedewald equation (or the direct LDL cholesterol assay when the Friedewald equation is unreliable). There is no doubt that additional markers to assess the risk for premature atherosclerosis will continue to emerge and will be incorporated into the routine lipoprotein repertoire.

**References**