[A technical review]

The NMR LipoProfile® Test

Introduction

Heart disease is the leading cause of death in the US and is "expected to be the number one cause of death worldwide by 2020."[1] Approximately 60 million lipid panels are performed annually in the US, making it one of the most frequently ordered lab tests.[2]

In clinical practice, the standard lipid panel[3] is well established as a standard of care to identify patients at risk for coronary heart disease (CHD), yet one million patients die each year of cardiovascular disease. LDL-C or "bad cholesterol" is used as the surrogate measure for the LDL-related risk; however, LDL is a particle. The higher the number of LDL particles (LDL-P), the greater the risk for CHD.[4,5] It is the LDL particle that interacts with the arterial wall as the beginning of the oxidation process that forms atherosclerotic plaque.

Since the National Heart Institute's publication, "Fat Transport in Lipoproteins—An Integrated Approach to Mechanisms and Disorders" in the *New England Journal of Medicine* in 1967,[6] it has been understood that atherosclerosis is caused by high concentrations of lipoprotein particles. The ability to quantify the number of particles had not been discovered, so cholesterol was adopted as a surrogate to estimate a patient's lipid-related CHD risk; however, due to the variable size and cholesterol content of LDL particles, LDL-C alone may not be a reliable predictor of LDL-P and the risk for CHD.[4,5]

This is illustrated in the findings from the Framingham Heart Study: 50% of the people who suffered myocardial infarction (MI) had normal, or near normal, cholesterol.[5,7] Standard lipids alone may not identify everyone at lipid-related risk for heart disease. The NMR LipoProfile test provides both calculated lipid values and the number of LDL particles to help identify and manage patients at risk for CHD.

The NMR LipoProfile Test

The NMR LipoProfile test is a blood test that helps to identify patients at risk for lipid-related CHD by measuring both the number of LDL particles (LDL-P) and standard lipids. Using nuclear magnetic resonance (NMR) spectroscopy, the NMR LipoProfile test directly measures individual lipoprotein particle subfractions (VLDL, LDL [including one IDL], and HDL) in addition to calculating the cholesterol contained in lipoproteins.[8]

What makes it possible to count the number of particles in various subclasses (without separating the subclasses first) is a magnetic property specific to lipoproteins that causes the lipids to broadcast characteristic signals for each of the 15 lipoprotein subclasses.[8,10]

The process begins with the measurement of a patient's serum or plasma NMR spectra. Each lipoprotein subclass signal emanates from the total number of terminal methyl groups on the lipids contained in the particle.[8,10] The amplitude of each lipoprotein particle signal serves as a measure of the concentration of that lipoprotein.[8,10]

NMR LipoProfile Report

The NMR LipoProfile report provides the following three sections:

LDL Particle Number. The total LDL particle number (LDL-P) and small LDL-P values are reported, as well as potential goals for these values in high-risk and moderately high-risk patient populations (Table 1). Results that are greater than optimal will be flagged. The LDL-P goals align with those determined by the National Cholesterol Education Program Expert Panel (NCEP) by using the 5th, 20th, 50th, and 80th percentile of more than 6900 patients from the MESA trial as the reference group.[11]

Table 1.—Treatment Goals for LDL-C and LDL-P[11]

Patient Category	LDL-C Goal	LDL-P Goal	Population Percentile Cut Point
Moderately High Risk	<130 mg/dL	<1300 nmol/L	50th Percentile
High Risk	<100 mg/dL	<1000 nmol/L	20th Percentile
High Risk (Optional goal based on clinical judgment)	<70 mg/dL	<1000 nmol/L	

Lipids. Calculated lipid results include total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides;

Metabolic Syndrome Markers. Three parameters (average LDL size and pattern, large HDL particle number [large HDL-P], and large VLDL particle number [large VLDL-P]) are reported that are closely associated with insulin resistance and increased risk of developing type 2 diabetes mellitus.

The clinical value of the additional particle information is critical to managing CHD risk, as traditional lipids alone may not show the full effects of familiar therapies. Using this information, clinicians can make individual determinations of a patient's lipid-related risk and determine treatment plans based on a more complete and accurate measure of their LDL concentration.

For example, in the recently published Veterans Affairs HDL Intervention Trial (VA-HIT), gemfibrozil was shown to have dramatic effects at a particle level that were indistinguishable when looking at standard lipids alone.[12] Even statins, commonly used to lower LDL, work by lowering the number of LDL particles, which in turn lower cholesterol.[13]

Table 2 shows the expected particle and cholesterol effects of familiar therapies.[13] In order to minimize CHD risk, combination therapy may be necessary in some patients.

Outcomes Data

The NMR LipoProfile test is a diagnostic tool used to identify and manage risk for lipid-related heart disease. In numerous clinical trials, the NMR LipoProfile test has proved to be a superior predictor of CHD outcomes than standard lipids.[12,14-18] Table 3 features seven studies documenting improved CHD risk prediction by NMR lipoprotein particle measurement.[12,14-19] In all of these studies, prediction by the number of LDL particles (LDL-P) was independent of traditional lipids.

In one of the most recent publications, results from VA-HIT were published. This study showed "NMR measured LDL and HDL particles numbers were significant, independent predictors of incident CHD events, whereas LDL and HDL cholesterol (or ApoB and Apo A-1) were not."[12]

To date, more than 100 peer-reviewed articles and studies have been published. A complete listing of publications regarding lipoprotein particle measurement via NMR can be found at www.lipoprofile.com/control.cfm?id=63.

The National Cholesterol Education Program

NCEP ATP III guidelines outline the established treatments and goals for lipid-related heart disease[20]; however, these represent a basic standard of care for treating patient populations and may fall short when it comes to treating the individual patients.[20]

Data from the Framingham Heart Study reveal that cholesterol levels were the same in 80% of patients who experienced a MI versus those who did not experience an event. In fact, 50% of people who had an MI in this study had normal cholesterol.[7]

The limitations of cholesterol are well understood, and most clinicians recognize the need to look for additional sources of CHD risk. D.J. Rader wrote in the *Textbook of Cardiovascular Medicine*, ". . . although traditional risk factors are reasonably good at predicting excess risk above the baseline for particular populations, they allow clinicians to predict only approximately 50% to 60% of the variation in absolute risk in individual patients. . . ." and that factors that provide additional information to traditional risk factors would be beneficial in clinical practice.[21] A better tool to identify and manage patients at lipid-related risk is needed.

In 1995, the NCEP published *Recommendations on Lipoprotein Measurement*. In this report, the authors state that high levels of LDL particles increase the risk for CHD and lowering LDL concentrations reduces the risk for CHD, MI, and CHD-related death,[22] but the availability of lipoprotein particle measurement was not widespread.

The NMR LipoProfile test provides both standard lipid information as well as the number of lipoprotein particles to iden-

Table 2.—LDL Particle Number and the Lipid-altering Efficacy of Common Lipid-altering Agents[13]

Agent	Change in LDL Particle (%)	Change in LDL Cholesterol (%)	Change in Triglycerides (%)	Change in HDL Cholesterol (%)
Statins	↓ 18-55*	↓ 18-55	↓ 7-30	↑ 5-15
Nicotinic acid (niacin)**	↓ 10-25	↓ 5-25	↓ 20-50	↑ 15-35
Fibric acids (fibrates)**	↓ 5-20*	↓ 5-20***	↓ 20-50	↑ 10-20
Ezetimibe	↓ 15-25*	↓ 17-22	↓ 4-11	↑ 2-5
Bile acid sequestrants	↓ 15-30*	↓ 15-30	No change or increase	↑ 3-5
Fish oils****	Trials in progress	No change or increase	↓ 20-50	No change or increase
Phytosterols/phytostanols	Trials in progress	↓ 10-15	No change or decrease	No change or increase

*Combination of NMR LDL-P and ApoB data

**In patients with elevated numbers of small LDL particles, combination with statins usually decreases triglycerides, raises HDL cholesterol and increases LDL size–causing LDL-P to be decreased more than LDL-C.

***Fibrates may increase LDL-C blood levels in some patients with hypertriglyceridemia. This is the so-called "beta-effect" of fibrates and can occur secondary to a large increase in the conversion of VLDL to LDL as lipoprotein lipase is activated.

****The lipid-altering effects of oil listed are with administration of ~5-9 g omega-3 fatty acids per day.

Table 3.—Associations of NMR-measured Lipoproteins in Recent Cardiovascular Outcome Trials[12,14-18]

Study	CHD Status	Atherogenic Endpoint	NMR Particle Concentration Association*
Cardiovascular Health Study[14(p1179)]	Primary Prevention	Incident MI or Angina	↑ LDL-P ↑ Small LDL-P
Women's Health Study[15(p1930)]	Primary Prevention	Incident MI, Stroke, CHD Death	↑ LDL-P ↑ Small LDL-P
Framingham Heart Study[16(p1)]	Primary Prevention	Incident MI or Angina	↑ LDL-P ↑ Small LDL-P
Veterans Affairs HDL Intervention Trial (VA-HIT)[12(p1559)]	Secondary Prevention	Nonfatal MI or CHD Death	↑ LDL-P ↑ Small LDL-P ↓ HDL-P ↓ Small HDL-P
PLAC-I[17(p92)]	Secondary Prevention	Angiographic Minimum Lumen Diameter (MLD)	↑ LDL-P ↑ Small LDL-P
Healthy Women Study[18(p71i,73i)]	Primary Prevention	EBCT*** Coronary Calcification Score	↑ LDL-P ↑ Small LDL-P
Multi-Ethnic Study of Atherosclerosis (MESA)[19(p5-6)]	Primary Prevention	Carotid Intima Media Thickness (IMT)	↑ LDL-P ↑ Large LDL-P ↓ HDL-P ↓ Large HDL-P ↑ Small LDL-P ↓ Small HDL-P ↓ Medium HDL-P

*Significant and independent after multivariate analysis. ***EBCT, electron beam computed tomography.

tify and manage patients at lipid-related risk for CHD. The measurement of LDL particles (LDL-P) has been recognized with a unique category I CPT code (83704).[23]

Moving Beyond Standard Lipids Alone

In 2004, NCEP provided an "optional target" of LDL-C < 70 mg/dL for patients at high risk for CHD.[24] High-risk patients are defined as those with existing CHD, diabetes or risk factors that confer a 10-year risk for CHD >20%.

A recent study of 2355 type 2 diabetes mellitus (T2DM) patients, all with a LDL-C <100 mg/dL shows that strategy has its own limitations.[25] The findings showed that lipoprotein particle number is highly variable, even at low and very low (<70 mg/dL) levels of LDL cholesterol.[11,25]

- 61% of T2DM patients had higher risk of CHD based on LDL-P; identified despite having LDL-C levels < 100 mg/dL[25]
- 40% of T2DM patients with LDL-C < 70 mg/dL still had residual CHD risk based on elevated LDL-P.[25]

For these patients, more aggressive treatment would be recommended. Although not a focus of the study, 26% of patients with LDL-C between 70 and 100 mg/dL were at optimal levels of LDL (as determined by LDL-P <1000 nmol/L).

Summary

LDL is a particle, and LDL particles contribute to atherosclerosis. The higher the number of particles (LDL-P), the greater the risk for CHD.[4,5] Traditionally, levels of LDL are assessed not by measuring particles directly but by measuring the amount of cholesterol the particles contain (ie, LDL-C). Due to the variable size and cholesterol content of LDL particles, even the most accurate LDL cholesterol measurements will, for many individuals, provide an inaccurate measure of the number of circulating LDL particles and thus their complete LDL-related risk.[4]

Several prospective epidemiologic and clinical intervention trials demonstrate a strong association of cardiovascular events with increased LDL particle number (LDL-P) versus LDL cholesterol (LDL-C). In all of these trials, CHD associations with LDL-P were independent of standard lipids; therefore, it is advisable to minimize lipid-related CHD risk by treating both LDL particle number (LDL-P) and LDL cholesterol.

NMR LipoProfile 884247

CPT 80061; 83704

Synonyms Cholesterol; Expanded Lipoprotein Analysis (LDL-P, HDL-P, VLDL-P, LDL Size)

Test Includes Lipoprotein particle number, lipoprotein subfractions; standard lipid panel (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides); metabolic syndrome markers

Specimen Serum in NMR LipoTube (black-and-yellow-top tube) or plain red-top tube

Volume 2 mL

Minimum Volume 1 mL

Container NMR LipoTube (black-and-yellow-top tube) or plain red-top tube

Collection

1. 12 to 14 hours of fasting is recommended
2. Collect specimen in NMR LipoTube (black-and-yellow-top tube) or plain red-top tube.
3. Allow specimen to clot for 30 minutes before centrifugation.
4. Specimen should be centrifuged within 24 hours of collection.
5. Do **not** open NMR LipoTube. Pipette separated serum from plain red-top tube to a plastic transport tube.

Note: Specimens must be kept refrigerated and shipped with frozen cool packs within four days of draw to ensure integrity. Do not freeze sample and do not store at room temperature.

Storage Instructions Sample **must** be kept refrigerated. Do **not** freeze sample and do **not** store at room temperature.

Patient Preparation Patient should be fasting for 12-14 hours.

Causes for Rejection Unspun LipoTube or unseperated plain red tube; gross hemolysis; serum more than 10 days old; specimen in inappropriate container; frozen specimen; specimen stored at room temperature.

Limitations If triglyceride level is >400 mg/dL, LDL will not be calculated.

Methodology Nuclear magnetic resonance (NMR)

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