Advanced lipoprotein testing using NMR spectroscopy

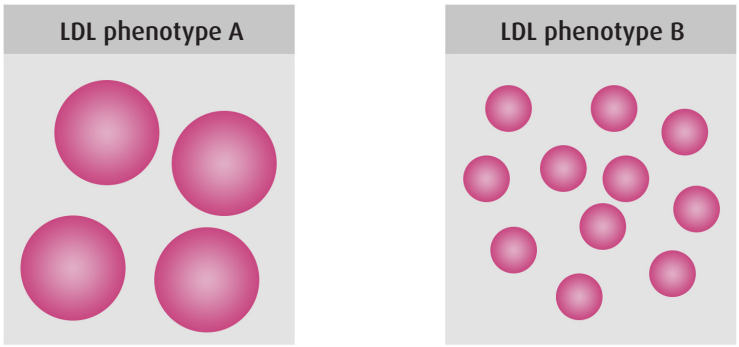
LipoComplete®

Clinical significance

Measurement of lipids in the blood is an integral part of the general preventive health examination. Screening for disorders of lipid metabolism and estimating the risk of cardiovascular disease is typically performed via measurement of triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol in the serum. Using these parameters, a suitable classification of disorders of lipid metabolism (hypertriglyceridemia, hypercholesterolemia, combined hyperlipidemia, HDL deficiency), cardiovascular risk assessment and therapy monitoring is, for the most part, possible. However, diagnostic limitations arise from the fact that lipoproteins are extremely complex particles composed of triglycerides, free and esterified cholesterol, phospholipids, apolipoproteins, enzymes and fat-soluble vitamins. Due to their varied composition, lipoproteins constitute a continuum of very large, light particles rich in lipids to very small, dense particles rich in proteins. Therefore, the main lipoprotein classes VLDL, LDL and HDL can be further divided into several subclasses which differ in size, density and particle concentration. The same amount of LDL cholesterol can thus be packed either into a smaller number of large LDL particles or into larger number of small LDL particles, which has significant consequences in terms of atherogenicity (Fig. 1). Numerous epidemiological studies have shown that small, dense LDL (sdLDL) are much more atherogenic than their larger counterparts. Thus a predominance of sdLDL has been recognized as a new and independent risk factor for atherosclerosis by National Cholesterol Education Program Adult Treatment Panel III of the US National Institutes of Health. In addition, among the major HDL subfractions, larger, lighter HDL-2 has been reported to be more atheroprotective than the smaller, denser HDL-3 subspecies.

Altogether, a growing number of scientific publications point out that the inclusion of lipoprotein subclasses and lipoprotein particle concentration in a preventive health screening can improve risk stratification and prediction of cardiovascular events. The existence of different pro- and antiatherogenic LDL and HDL subclasses may at least partly explain some seemingly paradoxical observations. For example, most patients with coronary heart disease (CHD) have only slightly increased or even `normal´ LDL cholesterol levels. On the other hand, high LDL cholesterol is not necessarily associated with a higher risk of CHD. Even high HDL cholesterol may not always be atheroprotective. Subclasses specific, mostly still unknown functional properties of HDL particles seem to be more important than total HDL cholesterol.

Fig. 1. Illustration of lipoprotein particles showing their varied composition.



The same amount of LDL cholesterol can be packed either in few large, lightweight LDL particles (left = LDL phenotype A) or in many small, dense LDL particles (right = LDL phenotype B).

LDL cholesterol	=	**LDL cholesterol**
LDL particle number	<	**LDL particle number**
LDL size (25.5 - 27.5 nm)	>	**LDL size** (24.2 - 25.5 nm)
LDL density (1.025 - 1.040 g/ml)	<	**LDL density** (1.040 - 1.064 g/ml)
atherogenicity	<<<	atherogenicity (3-7 fold)

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Laboratory diagnostics

For the reasons mentioned above, measurement of lipoprotein subclasses with respect to their particle diameter and particle concentration may be useful for advanced lipoprotein diagnostics. Unfortunately, there is currently no generally accepted reference method for the determination of LDL subclasses. The methods in use (ultracentrifugation, nuclear magnetic resonance (NMR) spectroscopy, gel electrophoresis, HPLC, homogeneous assays, precipitation methods) are based on different properties of LDL subclasses (density, size, charge) and therefore their results are not comparable with one another. Consequently, there is no consistent, standardized nomenclature in lipoprotein subclasses and terminology is largely based on the methodology used.

The MVZ Labor Ravensburg offers an advanced analytical technique for the determination of lipoprotein subclasses by NMR spectroscopy (LipoComplete®).

NMR spectroscopy is a purely physical method translating NMR signals of terminal methyl groups of lipids into lipoprotein particle concentrations, average diameters of lipoprotein particles and lipid concentrations of lipoprotein subclasses by use of patented mathematical procedures. NMR spectroscopy is highly accurate and thus highly reproducible which is important for long-term observations. A plasma sample can be analyzed within a few minutes with little personnel expenditure, allowing the fully automated high throughput of several hundred samples per day.

The following parameters are determined by means of LipoComplete® (NMR spectroscopy):

- triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol
- particle concentrations of lipoprotein subclasses (VLDL, LDL, large LDL, small LDL, HDL, large HDL, small HDL)
- average particle diameter of lipoproteins (VLDL, LDL, HDL)

Indications

Although clinical benefits of an extended lipoprotein profiling are still under discussion, some guidelines and expert committees recommend the measurement of lipoprotein subclasses and LDL particles for the risk assessment and management of cardiovascular disease.

Determination of lipoprotein subclasses is indicated in the following situations:

- Advanced risk stratification in type 2 diabetes mellitus, metabolic syndrome, insulin resistance, polycystic ovarian syndrome, in dialysis patients with chronic renal failure.
- Diagnosis of the atherogenic lipoprotein phenotype in patients with raised triglyceride, low HDL cholesterol and normal LDL cholesterol levels.
- Verification of the diagnosis of familial combined hyperlipidemia.
- Diagnostic clarification in individuals or families with prominent CHD but inconspicuous conventional lipids.
- Monitoring of pharmacotherapy and dietary and lifestyle changes.

The **LipoComplete®** method is suitable essentially for questions focusing on the particle properties of lipoproteins (particle concentration, particle size) for example, CHD risk stratification or therapy monitoring during statin therapy.

Interpretation

These LipoComplete® lipoprotein profiles comprise a detailed interpretation of the findings with graphical representation in addition to the measured values.

Material and pre-analytics

2.0 mL of fasting serum (12 h fasting period) is required for the tests. Serum can be stored at 4-10 °C for up to 5 days. For longer term storage, serum should be kept at -80 °C. A temperature of -20 °C is inadequate. EDTA and heparin plasma are likewise inadequate.

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References available on request from the author.

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