LIPOPROTEIN PROFILING

in CLINICAL DIAGNOSTICS
and LIFE SCIENCE RESEARCH
LIPOPROTEINS AND CARDIOVASCULAR DISEASE

High blood cholesterol is a well-known risk factor for cardiovascular disease (CVD), in particular coronary artery disease (CAD), myocardial infarction and other consequences of atherosclerosis. For transport in the blood, cholesterol and other lipids are packaged into lipoprotein particles, which are classified according to composition, size, density and physiological functions into major classes: VLDL (very low density lipoprotein), IDL (intermediate density lipoprotein), LDL (low density lipoprotein) and HDL (high density lipoprotein). Because of their prognostic significance for CVD, LDL- and HDL-cholesterol are widely known as standard laboratory parameters. However, half of the individuals who are hospitalized due to CAD have normal LDL-cholesterol levels [1]. It is now established that, as opposed to putting them in homogeneous classes, lipoproteins can, as a continuum ranging from small dense to large buoyant particles, be grouped more precisely into subclasses with varying atherogenicity.

WHY SHOULD LIPOPROTEIN SUBCLASSES BE MEASURED?

Certain lipoprotein subclasses are widely acknowledged as emerging risk factors for CVD [2-5]. The association between lipoprotein subclass cholesterol, particle concentration, particle size and CVD outcomes has been demonstrated by a number of studies [6-27]. Within the LDL fraction, especially small LDL particles are positively associated with CVD risk [4,10,12-22]. While high levels of large HDL particles seem to have a protective effect, this point needs more extensive investigation because of varying study results [19,23-26]. In a large-scale prospective study of more than 20,000 healthy women, NMR (nuclear magnetic resonance) determined lipoprotein particle concentrations and sizes predicted incident CVD events independently of classical risk factors [21]. Adjusted Hazard Ratios (HRs) of 2.51 (95% confidence interval (CI) 1.91 - 3.30) for LDL particle concentration (LDL-P), 0.91 (95% CI 0.75–1.12) for HDL particle concentration (HDL-P), 0.64 (95% CI 0.52–0.79) for LDL size and 0.65 (95% CI 0.51–0.81) for HDL size [20] were observed ranking them in the top group of risk factors. The association of NMR-measured LDL particle concentration and future CVD events was confirmed in the large ‘Multi-Ethnic Study of Atherosclerosis’ (MESA) with a HR of 1.32 (95% CI 1.19–1.47) [11]. Furthermore, the two nested case-control studies by Blake et al. [14] and El Harchaoui et al. [10] found that LDL-P was a strong predictor of future cardiovascular risk. In addition, a matched case-control study within the ‘Multiple Risk Factor Intervention Trial’ showed that higher levels of HDL-P were associated with a lowered risk of CVD death in men with metabolic syndrome (HR 0.50, fourth quartile compared to first quartile (95% CI 0.26-0.96)) [26]. Another nested case-control analysis by Kuller et al. showed significant differences between incident CVD cases and controls in LDL-P in women after bivariate analysis including LDL-C (odds ratio (OR) 1.11 per 100 nM (95% CI 1.03-1.09)) [27].

In addition, the concentrations of cholesterol in large HDL particles were significantly higher in controls compared to concentrations in women with myocardial infarction and angina (OR 0.73, fourth quartile compared to first quartile) [7]. In 2072 men from the ‘Québec Cardiovascular study’ population, a strong and independent association between cholesterol in small dense LDL (sdLDL) particles and the risk of long-term ischemic heart disease in the first 7 years of follow-up was observed [27]. Taken together, a growing weight of evidence supports the fact that lipoprotein subclass cholesterol, particle concentration and size are significantly and independently associated with CVD.

LIPOPROTEIN SUBCLASSES IN CURRENT GUIDELINES & POSITION STATEMENTS

Although the clinical utility of advanced lipoprotein subclass testing is still under debate, some current guidelines and expert panels recommend lipoprotein subclass measures, especially LDL-P for CVD risk assessment and patient management [28-35];

2008 Lipoprotein management in patients with cardiometabolic risk: Consensus statement from the American Diabetes Association and the American College of Cardiology Foundation

The ADA/ACC states that in comparison to LDL-C, NMR-measured LDL-P may be “a more accurate way to capture the risk posed by LDL” and appears to be a more discriminating measure of the adequacy of LDL lowering therapy [28].

2009 Apolipoprotein B and Cardiovascular Disease Risk: Position Statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices

The American Association of Clinical Chemists (AACC) states that LDL-P is “consistently more predictive of cardiovascular disease than is LDL-C” and appears to “provide a better assessment of on-treatment residual risk than LDL-C measurement”. It suggested a treatment target for LDL-P of <1100 nmol/L, similar to LDL-C in terms of population percentiles [29].

2011 Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists

The National Lipid Association (NL A) recommends the evaluation of LDL-P at the time of initial clinical
assessment and on-treatment management decisions for intermediate and high-risk patients [30].

2011 European Panel On Low Density Lipoprotein (LDL) Subclasses: A Statement on the Pathophysiology, Atherogenicity and Clinical Significance of LDL Subclasses

This European expert panel states that there is a “significant association of sdLDL with increased CVD risk” and that “evidence from angiographic clinical trials indicates that treatment benefit is related to a decrease in sdLDL particles” [31].

2012 American Association of Clinical Endocrinologists’ Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis

These AACE guidelines list LDL-P and sdLDL as risk factors to be considered. They recommend advanced lipoprotein testing for patients that fail to reach optimal lipid targets or show disease progression while at optimal guideline targets [32].

2013 AACE comprehensive diabetes management algorithm 2013

The AACE incorporated LDL-P measures into a diabetes management algorithm. Treatment should be intensified to reach treatment targets for LDL-P of <1200 nmol/L for patients with moderate risk and <1000 nmol/L for patients with high risk [33].

2013 Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies: assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices

The AACC concludes that “Apo B and LDL-P have consistently been shown to be stronger risk factors than LDL-C”. They recommend that “the measurement of particle number […] should be incorporated into the guidelines for the assessment of CVD risk” [34].

Recent evidence [36] confirms the recommendation that patients should be treated in such a way as to reach target LDL-P concentrations [11,28,29,37]. Treatment targets for LDL-P can be reached by therapeutic lifestyle intervention or by several classes of drugs, e.g. statins, fibrates, niacin, some glitazones and combination therapies that have been shown to have a beneficial effect on lipoprotein subclass distribution [11,32,34,37,38].

METHODS FOR DETERMINING LIPOPROTEIN SUBCLASSES

Several methods are available for analyzing lipoprotein subclasses. Nuclear magnetic resonance (NMR) spectroscopy is based on the mathematical deconvolution of lipid methyl group NMR signals. Each lipoprotein particle of a given size has a characteristic signal. The integrals of the signal are directly proportional to the numbers of subclass particles.

Ultracentrifugation methods fractionate lipoproteins by density. During polyacrylamide (gradient) gel electrophoresis, lipoproteins are separated by size and charge. Densitometric evaluation of the individual bands provides the percent distribution of lipids in different subclasses. An enzymatic assay for the quantitation of sdLDL cholesterol is based on selective surfactants and enzymes. Additional methods such as chromatographic methods, ion mobility, precipitation methods and others are rarely used.

<table>
<thead>
<tr>
<th>Method</th>
<th>Nuclear Magnetic Resonance (NMR)</th>
<th>Density Gradient Ultracentrifugation (UC)</th>
<th>Polyacrylamide Gel Electrophoresis (GE)</th>
<th>Direct method - Enzymatic reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major classes</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>Only sdLDL</td>
</tr>
<tr>
<td>VLDL subclasses</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>LDL subclasses</td>
<td>•</td>
<td>•</td>
<td>Only sdLDL</td>
<td>•</td>
</tr>
<tr>
<td>HDL subclasses</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Particle size</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Particle concentration</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Subclass cholesterol</td>
<td>•</td>
<td>•</td>
<td>Only sdLDL</td>
<td>•</td>
</tr>
<tr>
<td>Inter-lab. precision</td>
<td>(Very) high</td>
<td>Low</td>
<td>Moderate</td>
<td>Very high</td>
</tr>
<tr>
<td>Throughput</td>
<td>High</td>
<td>Low</td>
<td>Moderate</td>
<td>Very high</td>
</tr>
<tr>
<td>Hands-on time</td>
<td>Very short</td>
<td>Moderate</td>
<td>Short</td>
<td>Very short</td>
</tr>
<tr>
<td>Automation</td>
<td>High</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Very high</td>
</tr>
</tbody>
</table>

1Lipoprotein Profiling by numares
2HDL/LDL/VLDL/IDL
LIPOPROTEIN PROFILING BY NUMARES

numares offers a system for analyzing lipoprotein subclasses based on a patented method in combination with NMR for use in clinical laboratories and life science research institutions worldwide. Thanks to a minimal sample preparation and processing on a fully automated platform, the system delivers highly reproducible results for hundreds of samples per day in a cost-effective manner.

**Serum parameters provided:**
- Particle concentrations* in lipoprotein fractions (LDL-P, HDL-P) and subfractions
- Mean particle sizes* in VLDL, LDL and HDL fractions
- Cholesterol concentrations* in lipoprotein fractions and subfractions
- Standard lipid panel (total cholesterol, triglycerides, LDL-C, HDL-C)
- Metabolic parameters (glucose, lactate, alanine, valine, leucine, isoleucine)

*These parameters have been calibrated against other NMR and gel electrophoresis methods.

**Applications**

**The results of the test system can be used in clinical diagnostics to**
- Identify patients at risk for CVD to allow for effective prevention and timely initiation of treatment
- Predict and monitor the course of the disease to support individualized therapy decisions
- Monitor treatment efficacy to improve patient management and quality of life

**The results of the test system can be used in life science research for**
- Basic and applied research, e.g. the evaluation of lipoprotein subclasses as a cardiovascular risk factor in different populations; their response to nutrition and lifestyle interventions or their role in other disorders
- Pharmaceutical development, e.g. better characterization of the therapeutic profile of new lipid-lowering drugs
- Personalized medicine, e.g. biomarker discovery, validation and development leading to routine tests

**Successful routine use**

numares lipoprotein profiling test systems have been in routine use in the USA since 2013. Thousands of blood samples are measured and evaluated on a weekly basis.

**Product pipeline**

numares continuously strives to develop novel tests addressing unmet diagnostic needs with a focus on CVD, metabolic diseases, oncology, transplantation and nephrology.

**One contact for all your questions**

numares offers comprehensive support for system installation and validation, technical service for software and instrumentation as well as operator trainings and on site service.

**REFERENCES**


CONTACT DETAILS

numares AG
Josef-Engert-Strasse 9
93053 Regensburg
Germany
tel: +49 941 69 809 100
fax: +49 941 69 809 101
www.numares.com
info@numares.com

numares GROUP Corporation
737 N. Fifth Street
Richmond, VA 23219
USA
tel: +1-804-554-0870 Ext. 3001
fax: +1-888-705-0340
mail@numares.com
www.numares.com

This paper is intended only for a non-United States audience. It is for information purposes only. It should not be seen as medical or diagnostic advice and does not replace the services, advice or counsel of a doctor or other health care professional. numares makes every effort to provide information that is accurate, but makes no guarantee in this regard.