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Review

Update on the laboratory investigation of dyslipidemias

I. Ramasamy

Worcester Royal Hospital, UK

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ABSTRACT

The role of the clinical laboratory is evolving to provide more information to clinicians to assess cardiovascular disease (CVD) risk and target therapy more effectively. Current routine methods to measure LDL-cholesterol (LDL-C), the Friedewald calculation, ultracentrifugation, electrophoresis and homogeneous direct methods have established limitations. Studies suggest that LDL and HDL size or particle concentration are alternative methods to predict future CVD risk. At this time there is no consensus role for lipoprotein particle or subclasses in CVD risk assessment. LDL and HDL particle concentration are measured by several methods, namely gradient gel electrophoresis, ultracentrifugation-vertical auto profile, nuclear magnetic resonance and ion mobility. It has been suggested that HDL functional assays may be better predictors of CVD risk. To assess the issue of lipoprotein subclasses/particles and HDL function as potential CVD risk markers robust, simple, validated analytical methods are required. In patients with small dense LDL particles, even a perfect measure of LDL-C will not reflect LDL particle concentration. Non-HDL-C is an alternative measurement and includes VLDL and CM remnant cholesterol and LDL-C. However, apolipoprotein B measurement may more accurately reflect LDL particle numbers. Non-fasting lipid measurements have many practical advantages. Defining thresholds for treatment with new measurements of CVD risk remain a challenge. In families with genetic variants, ApoCIII and lipoprotein (a) may be additional risk factors. Recognition of familial causes of dyslipidemias and diagnosis in childhood will result in early treatment. This review discusses the limitations in current laboratory technologies to predict CVD risk and reviews the evidence for emergent approaches using newer biomarkers in clinical practice.

1. Background

Diseases of the heart and circulatory system (atherosclerotic cardiovascular disease, CVD) are the main cause of death and in Europe accounts for over 4 million deaths each year. The main forms of CVD are coronary heart disease (CHD) and stroke. The causes of CVDs are multifactorial. Some of these factors such as lifestyle, tobacco smoking, dietary habits, dyslipidemias, elevated blood pressure, type 2 diabetes (DM) are modifiable, while age and male gender are non-modifiable [1].

Intervention studies performed with patients with (secondary) and without (primary) CHD demonstrate the efficacy of lipid lowering therapies [2, 3]. As a consequence, various organisations and agencies have issued differing recommendations for the management of dyslipidemias. The American College of Cardiology (ACC) and the American Heart Association (AHA) published a set of guidelines on the control of blood cholesterol to reduce CHD. The ACC/AHA guidelines did not identify specific LDL-C or non-HDL-C treatment goals for primary or secondary prevention of CVD [4]. The European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines address lipid clinics and metabolic units who are dealing with dyslipidemias [5]. The National Lipid Association (NLA) [6] and the National Institute for Health and Care Excellence (NICE) (https://www.nice.org.uk/guidance/cg181) have introduced guidelines for the management of dyslipidemias and risk reduction for CVD. The National Cholesterol Education Program (NCEP) updated report on the Adult Treatment Panel III recommended non-high density lipoprotein cholesterol (non-HDL-C) as a secondary target in patients with TG ≥ 200 mg/dL [7]. Though there are common modalities between them substantial differences exist between them as well. The relative merits of these guidelines have been reviewed previously [8, 9, 10, 11]. Recommendations for the treatment of dyslipidemias are based on the principles (i) an elevated level of cholesterol carried by circulating apolipoprotein B (apoB) containing lipoproteins (non-HDL-C) and low density lipoprotein cholesterol (LDL-C) are the root cause of atherosclerotic CVD (ii) reducing the elevated levels of atherogenic cholesterol will lower the risk of atherosclerotic CVD. (Table 1). Current guidelines recommend the assessment of total risk for CHD as this is the product of a number of risk factors. The commonly used risk assessment systems are the Framingham, SCORE (Systemic COronary Risk

E-mail address: indrar@ozemail.com.au.

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Table 1
Guidelines for managing dyslipidemias.

Recommending Body	Consider drug treatment Non-HDL-C (mg/dL) LDL-C (mg/dL)	Treatment goal Non-HDL-C (mg/dL) LDL-C (mg/dL)	Risk Assessment	
NLA	0–1 risk factor[a] ≥190 ≥160; 2 risk factors ≥160 ≥130; ≥3 risk factors ≥130 ≥100; Very high risk ≥100 ≥70	< 130 < 100; Very High risk patients < 100 < 70	High risk is defined as ≥10% using Adult Treatment Panel III Framingham Risk Score; ≥15% using 2013 pooled cohort equations, ≥45% using Framingham long term risk calculation If LDL-C ≥ 190 mg/dL severe hypercholesterolemia phenotype is to be considered	ApoB is a target for treatment after patient has been treated for atherogenic cholesterol. Treatment goals for apoB are < 90 mg/dL and for very high risk patients < 80 mg/dL. Elevated TG is not a target for treatment unless ≥500 mg/dL
EAS/ESC	LDL-C mg/dL (mmol/L) CVD risk < 1% > 190 (4.9) CVD risk ≥1 to < 5% 100(2.5)– < 155(4.0) CVD risk > 5 to < 10% < 70(1.8) CVD risk ≥10%[b] < 70(1.8)	LDL-C mg/dL(mmol/L) CVD risk > 1 to ≤5% < 115(3.0) CVD risk ≥5 to < 10% < 100(2.6) CVD risk ≥10% < 70(1.8) or ≥50% reduction in LDL-C if target levels not reached	Total CVD risk is assessed by SCORE	apoB treatment goals are < 100 mg/dL for high risk and < 80 mg/dL for very high risk. The specific target for non-HDL-C is 30 mg/dL (0.8 mmol/L) higher than LDL-C, the cholesterol fraction present in 150 mg/dL (1.7 mmol/l) of TG
ACC/AHA	LDL-C (mg/dL) ≥190 mg/dL; CVD risk ≥5%[c] age 45–75 years and LDL-C 70–189 mg/dL	LDL-C (mg/dL) At least 50% reduction in LDL-C using high intensity statin; and 30 to < 50% using moderate intensity statin	CVD risk assessed by the pooled cohort equation	
NICE	People (age > 40–84 years) with a formal 10 year risk assessment of CVD of 10%[d]	40% reduction in non-HDL cholesterol	QRISK2	

[a] Nonlipid risk factors are high blood pressure, cigarette smoking and diabetes mellitus. In patients with chronic kidney disease > stage 3B (estimated glomerular filtration rate eGFR 30–44 mL/min/1.73 m^2) risk calculations are not used as they underestimate risk.

[b] Or documented CVD, type 1 diabetes (with target organ damage), type 2 diabetes, and eGFR < 60 mL/min/1.73 m^2.

[c] Other factors eg family history, diabetes, abnormal coronary artery calcium, high sensitivity C-reactive protein may be considered prior to drug treatment. Drug treatment is initiated in the presence of clinical CVD.

[d] Risk assessment tool is not used to assess CVD risk in people with an eGFR < 60 mL/min/1.73 m^2 and/or albuminuria, type 1 diabetes or familial hypercholesterolemia (FH).

Estimation). ASSIGN (CV risk estimation model from the Scottish Intercollegiate Guidelines Network, http://assign-score.com/estimate-the-risk/), Q-risk (QResearch cardiovascular risk), PROCAM (Prospective Cardiovascular Munster Study), WHO (World Health organisation) [12,13] and Joint British Societies for the prevention of cardiovascular risk (JBS3, http://www.jbs3risk.com/). The accuracy of risk assessment calculations is controversial [14]. Lifestyle modification (adhering to a heart healthy diet, regular exercise, avoiding tobacco, maintaining a healthy weight) remains a major component for CVD risk reduction. After lowering of LDL–C to recommended levels there is still a substantial residual risk of CHD. Some of this risk is probably explained by elevated levels of cholesterol in other lipoprotein subclasses than LDL [15]. Measurement of lipoproteins, however, remains a primary tool for assessment and monitoring of patients with or at risk of developing cardiovascular disease.

2. Lipoprotein profiles

The major classes of lipoproteins are HDL, LDL, intermediate density lipoproteins (IDL), very low density lipoproteins (VLDL) and chylomicrons (CM). Lipoprotein metabolism is complex with many subclasses of lipoproteins and different lipid contents of subclasses. The various subclasses of lipoproteins derived from the endogenous pathway are a continuum from VLDL secreted from the liver, which is then degraded into IDL (by lipolysis and exchange of lipids and apolipoproteins in the plasma) which in turn is further degraded into LDL.

The human intestine synthesises CM from dietary fat, predominantly in the form of triacylglycerol. In the lymph and blood, CMs acquire apoCI, apoCII, apoCIII and apoE. After gaining apoCII the activator of lipoprotein lipase (LPL), CM interacts with the enzyme and triglyceride (TG) hydrolysis is initiated. The remaining relatively triglyceride-depleted chylomicron 'remnant' particles which are enriched in cholesteryl ester can interact with receptors on hepatocytes and be removed from the circulation. Each LDL particle contains a single apoB100 molecule. The major apolipoproteins of VLDL are apoB100, apoAIV, apoCI, CII, and CIII and apoE. CMs contain the same apolipoproteins as VLDL, and also contain AI, AII, AIII and apoB48 instead of apoB100. The metabolism of HDL and its role in reverse cholesterol transport is complex. ApoAI is the major HDL protein and is secreted from the liver and intestine as a lipid poor protein. It acquires phospholipids and unesterified cholesterol from peripheral tissues to form discoidal nascent HDL. The cholesterol is esterified by lecithin:cholesterol acytransferase (LCAT) to form larger and more spherical HDL. Cholesteryl ester transfer proteins (CETP) transfers cholesterol ester from HDL to other apoB containing lipoproteins. HDL facilitates reverse cholesterol transport by which cholesterol from peripheral tissues is returned to the liver for excretion [16].

Risk factor screening including lipid profiles is recommended in several groups of men and women. ESC guidelines recommend lipoprotein profile screens in men ≥ 40 years of age and women ≥ 50 years of age, as well as subjects with evidence of atherosclerosis, type II diabetes, family history of premature CVD, central obesity, autoimmune chronic inflammatory conditions, certain drug treatments which predispose to dyslipidemias and clinical manifestations of genetic dyslipidemias [17]. NLA recommends fasting or nonfasting lipoprotein profiles in all adults ≥ 20 years of age at least every 5 years. [6]. NICE guidelines recommend people older than 40 should have their estimate of CVD risk reviewed on an ongoing basis.