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Review

High density lipoproteins: Measurement techniques and potential biomarkers of cardiovascular risk

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ABSTRACT

Plasma high density lipoprotein cholesterol (HDL) comprises a heterogeneous family of lipoprotein species, differing in surface charge, size and lipid and protein compositions. While HDL cholesterol (C) mass is a strong, graded and coherent biomarker of cardiovascular risk, genetic and clinical trial data suggest that the simple measurement of HDL-C may not be causal in preventing atherosclerosis nor reflect HDL functionality. Indeed, the measurement of HDL-C may be a biomarker of cardiovascular health. To assess the issue of HDL function as a potential therapeutic target, robust and simple analytical methods are required. The complex pleiotropic effects of HDL make the development of a single measurement challenging. Development of laboratory assays that accurately HDL function must be developed validated and brought to high-throughput for clinical purposes. This review discusses the limitations of current laboratory technologies for methods that separate and quantify HDL and potential application to predict CVD, with an emphasis on emergent approaches as potential biomarkers in clinical practice.

Contents

Abbreviations: 2D-PAGGE, two dimensional polyacrylamide gradient gel electrophoresis; ApoA-I, apolipoprotein A-I; CHD, coronary heart disease; CVD, cardiovascular disease; HDL, high density lipoprotein; HPLC, High Performance Liquid Chromatography; LCAT, lecithin–cholesterol acyltransferase; LDL, low density lipoprotein; MALDI, matrix-assisted laser desorption/ionization; MOP, myeloperoxidase; MS/MS, tandem-mass spectrometry; ND-PAGGE, non-denaturant polyacrylamide gradient gel electrophoresis; NMR, nuclear magnetic resonance; PEG, polyethylene glycol; PON1, paraoxonase 1; SELDI, surface enhanced laser desorption/ionization; TOF, time-of-flight; UTC, ultracentrifugation

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1. Introduction

Plasma levels of high density lipoprotein cholesterol (HDL-C) are strongly associated with atherosclerotic cardiovascular disease, especially coronary artery disease (CAD). This observation is strong, graded and coherent across the populations studied [1]. In post-hoc analysis of clinical trials, HDL-C remains a powerful predictor of residual risk, even at low LDL-C levels [2]. In recent years, Mendelian randomization experiments have casted doubt on the causal link between HDL-C and CAD [3]. Furthermore, drugs that increase HDL-C, including fibrates, niacin and the cholesteryl ester transfer protein inhibitors torcetrapib and dalcetrapib have failed to show improved cardiovascular outcomes. One possible explanation to explain the discrepancy between the epidemiological, genetic and clinical trial data is that the measurement of the cholesterol mass within HDL fails to capture the complexity of a highly dynamic process [4,5]. HDL particles differ in size, ranging from 7 nm to 17 nm, shape (unfolded protein, discoidal and spherical), lipidome and proteome [6,7]. The measurement of HDL-C has been standardized and current precipitation techniques achieve a high degree of accuracy for clinical purposes (Table 1). However, there is no accepted "gold standard" technique for the measurement of HDL particles. More refined techniques have been developed based on the physical and functional properties of HDL (Tables 2, 3). In this review, we will address the techniques of HDL measurement, determine whether the information provided ads to our ability to predict CVD, and evaluate the limitations of these assays. The structural and composition (proteomic/lipidomic) of HDL may provide further insights on its function [8,9]. HDL particles possess many pleiotropic properties that are unrelated to their cholesterol mass or the ability to transport it in the blood. These properties, observed in vitro, may be a better metric to determine CVD risk. These effects include HDL anti-inflammatory and anti-oxidant properties, vascular endothelial cell, nitric oxide (NO) production, expressions of inflammatory mediators, and endothelial progenitor cell proliferation [5,10–13]. Further, the structure and composition analysis of HDL particles (proteomic/lipidomic), which provide additional insight into the assessment of HDL particles with specific functions, are also discussed (Tables 4, 5).

2. Controversy surrounding the relationship between HDL-cholesterol measurement and CAD

Epidemiological studies have shown a consistent inverse association between HDL-C concentration and CAD [1]. Clinical trials aimed at raising HDL-C pharmacologically have failed to show clinical benefits in terms of CAD reduction [14–17]. Moreover, Mendelian randomization studies do not support a causal role for HDL-C in the pathogenesis of CAD [3]. HDL-C level is a static measurement that likely represents a biomarker of cardiovascular health, rather than a risk factor. Recent clinical studies suggest that HDL-C is a helpful biomarker, but functional testing, such as the cholesterol efflux capacity of HDL improves discrimination, independently of HDL-C levels [18]. Despite the coherent epidemiological data suggesting a cardioprotective role for HDL-C, the antiatherogenic properties of different particles that constitute HDL are highly heterogeneous and have yet to be fully quantified and their roles properly evaluated. The cholesterol efflux capacity is likely more reflective of a biologically relevant pathway in the prevention of atherosclerosis and CAD [18].

Thus, a new paradigm states that we need to determine and measure the anti-atherogenic properties of HDL, rather than the cholesterol mass within HDL. Other methods for measuring HDL function, reflecting relevant causal pathways need to be established. Indeed, the cholesterol content of HDL does not represent many biologically important HDL properties that are relevant to CVD (Tables 2, 3). Methods for measurement of HDL sub-fractions, as well as physiochemical (Table 2) and functional (Table 3) may be more effective in predicting CAD risk than HDL-C [19]. Thus, the concept that HDL-C does not necessarily reflect HDL function, and that HDL function may be a better biomarker of cardiovascular risk must be emphasized. Recently, various alternate HDL phenotypes are being examined as surrogates for the beneficial actions of HDL [5]. The functional heterogeneity of HDL particles makes the identification of effective clinical method to quantify HDL function an ongoing challenge [5,20,21]. The pleiotropic HDL biological activities (biomarker) have immediate relevance to understanding the key mechanisms implicated in the pathophysiology of atherosclerosis and thrombosis. Even though some HDL biomarkers, such as cholesterol efflux capacity look promising, it is too early to embrace these measurements in the clinical realm [22] (Table 5).

3. Methods of HDL measurement

In clinical practice, the standard measure of HDL is the cholesterol content in HDL particles after precipitation of apoB-containing lipoproteins (Table 1). More refined techniques to determine HDL-C in serum include ultracentrifugation (UTC) [23], electrophoresis [24,25], high performance lipoprotein chromatography (HPLC) [26,27], precipitation-based methods [28], direct measuring methods [29,30], and nuclear magnetic resonance (NMR) [31] (Table 2).

3.1. Precipitation methods for the separation of HDL

HDL-C is first separated by precipitating apoB containing lipoproteins from serum by using a combination of polyanions, typically such as heparin–$MnCl_2$, dextran sulfate–$MgCl_2$ or phosphotungstate–$MgCl_2$ [32,33], and a divalent cation, such as magnesium, heparin–manganese, or calcium [34]. Subsequently, HDL is quantified as cholesterol in the supernatant [35]. Polyethylene glycol (PEG) although not a polyanion is also used to precipitate apoB-containing lipoproteins [36,37]. This method is a convenient, reproducible, and rapid way to extract HDL from patient serum or plasma [38]. Incomplete precipitation of apoB lipoproteins [35] is a major drawback of this method [33,39]. Supernatant turbidity, observed with hypertriglyceridemia, inflammatory conditions and cryopreservation [29,40,41] may lead to discordant results between methods [29,35,42]. Commercial immunoprecipitation reagent using specific antibodies directed against HDL particles could be effective in serum with elevated triglycerides [33]. Because of specificity of anti-apoB antibodies, HDL particles will not co-precipitate with apoB, which may be an issue with chemical precipitation methods [33]. Another limitation is that the