Atherosclerosis 233 (2014) 253–259

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Validation of homogeneous assays for HDL-cholesterol using fresh samples from healthy and diseased subjects

Takashi Miida[a,*], Kunihiro Nishimura[b], Tomonori Okamura[c], Satoshi Hirayama[a], Hirotoshi Ohmura[d], Hiroshi Yoshida[e], Yoh Miyashita[f], Masumi Ai[g], Akira Tanaka[h], Hiroyuki Sumino[i], Masami Murakami[i], Ikuo Inoue[j], Yuzo Kayamori[k], Masakazu Nakamura[l], Tsutomu Nobori[m], Yukihisa Miyazawa[n], Tamio Teramoto[o], Shinji Yokoyama[p]

[a] *Department of Clinical Laboratory Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[b] *National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan*
[c] *Department of Preventive Medicine and Public Health, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*
[d] *Department of Cardiology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[e] *Department of Laboratory Medicine, Jikei University Kashiwa Hospital, 163-1 Kashiwashita, Kashiwa, Chiba 277-8567, Japan*
[f] *Center of Diabetes, Endocrine and Metabolism, Toho University Sakura Hospital Medical Center, 564-1 Shimoshizu, Sakura, Chiba 285-0841, Japan*
[g] *Department of Insured Medical Care Management, Tokyo Medical and Dental University Hospital, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan*
[h] *Laboratory of Clinical Nutrition and Medicine, Kagawa Nutrition University, 3-9-21 Chiyoda, Sakado, Saitama 350-0288, Japan*
[i] *Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, 3-39-22 Showa, Maebashi, Gunma 371-8511, Japan*
[j] *Department of Endcrinology and Diabetics, Saitama Medical University, 38 Morohongo Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan*
[k] *Department of Health Sciences, Faculty of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[l] *National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan*
[m] *Department of Molecular and Laboratory Medicine, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507 Japan*
[n] *Department of Laboratory Science, Faculty of Medical Technology, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan*
[o] *Department of Internal Medicine, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan*
[p] *Nutritional Health Science Research Center and Department of Food and Nutritional Sciences, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan*

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form
9 December 2013
Accepted 13 December 2013
Available online 8 January 2014

Keywords:
Direct HDL-C assay
Designated comparison method
Standardization

ABSTRACT

Background: High-density lipoprotein-cholesterol (HDL-C) is a negative risk factor for cardiovascular events. Although several homogeneous HDL-C assays are available, their accuracy has not been validated, particularly in subjects with disease. We aimed to clarify whether HDL-C concentrations measured by homogeneous assays [HDL-C (H)] agree with those determined by the reference measurement procedures [HDL-C (RMP)] using ultracentrifugation and precipitation with heparin-manganese reagent in fresh clinical samples.

Methods: HDL-C concentrations in samples from 48 healthy subjects and 119 subjects with disease were determined using 12 homogeneous assays and RMPs.

Results: All reagents showed excellent intra- and inter-assay CVs ($<2.23\%$) for two pooled sera. Furthermore, the mean bias was within $\pm 1.0\%$ in nine reagents using samples from healthy subjects and in eight reagents using samples from subjects with disease. In a single HDL-C (H) determination, the total error requirement of the National Cholesterol Education Program (95% of results $< 13\%$) was fulfilled in nine reagents using samples from healthy subjects and six reagents in those from subjects with disease. Error component analysis revealed that only one reagent exceeded $\pm 10\%$ total error in samples from healthy subjects, whereas four reagents exceeded this error in samples from subjects with disease. Correlations between HDL-C (H) and HDL-C (RMP) revealed that the slopes were within 1.00 ± 0.06 in six reagents in healthy subjects, and eight reagents in subjects with disease.

---

[*] Corresponding author. Tel.: +81 3 5802 1104; fax: +81 3 5684 1609.
E-mail address: tmiida@juntendo.ac.jp (T. Miida).

http://dx.doi.org/10.1016/j.atherosclerosis.2013.12.033

Conclusions: Except for three reagents, HDL-C (H) agrees well with HDL-C (RMP) in subjects with common disease, but not in those with extremely low HDL-C or abnormal HDL composition.

1. Introduction

Lipoprotein profiles are closely associated with atherosclerotic disorders such as coronary artery disease and cerebrovascular disease, the major causes of death in industrialized countries [1,2]. Functionally, serum lipoproteins are divided into two groups: atherogenic and anti-atherogenic lipoproteins. Atherogenic lipoproteins consist of intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and remnant lipoproteins. Over a prolonged period of time, these lipoproteins accumulate in macrophages in the vascular walls; macrophages turn into foam cells, the main component of lipid-rich plaques. These are called “vulnerable plaques” because pro-inflammatory cells—including macrophages—degrade the fibrous cap of plaques using proteolytic enzymes, causing sudden rupture [3].

High-density lipoprotein (HDL) is the only circulating anti-atherogenic lipoprotein. HDL removes free cholesterol from somatic cells by accepting cell cholesterol via an exchange with the help of ATP-binding cassette transporter A1 (ABCA1) [4] and G1 (ABCG1) [5]. This reverse cholesterol transport pathway regresses vascular atheromatous plaques [6]. In clinical practice, the amount of HDL is expressed as the cholesterol concentration (HDL-C). Numerous cross-sectional and longitudinal epidemiological studies have shown that HDL-C is a strong negative risk factor for cardiovascular events [7,8]. In both primary and secondary prevention studies using statins, each 0.0259 mmol/L (1 mg/dL) increase in HDL-C level decreased the risk of coronary artery disease by 2–3% [9,10]. Since precipitation methods for HDL-C measurement are cumbersome and time consuming, HDL-C is measured by homogenous assays (so-called “direct assays”) using a variety of principles in almost all clinical laboratories [11]. In general, homogeneous assays eliminate or inhibit non-HDL-C with the first reagents, and then solubilize HDL particles for measuring cholesterol with the second reagents. Although the performance of seven HDL-C homogeneous assays was investigated recently, the study subjects included a considerable number of patients with rare dyslipidemia and extremely low HDL-C [12]. Furthermore, there are additional novel assays based on different principles.

We examined the precision and accuracy of the HDL-C homogeneous assays that are commercially available at present. Using fresh blood samples from healthy subjects and subjects with disease, we compared the HDL-C concentrations determined using 12 homogeneous assays [HDL-C (H)] with those determined by the reference measurement procedures [HDL-C (RMP)].

2. Methods

2.1. Study subjects

This study was planned and carried out concurrently with the multi-center study of the precision and accuracy of homogeneous assays for LDL-cholesterol (LDL-C) published previously [13]. Initially, 173 subjects consisting of volunteers and patients with disease were recruited at the participating institutions. We excluded dyslipidemic patients with extremely low or high lipoprotein concentrations [triglyceride (TG) $>$ 11.29 mmol/L (1000 mg/dL), LDL-C $<$ 0.52 mmol/L (20 mg/dL), HLD-C $<$ 0.52 mmol/L (20 mg/dL), and HDL-C $>$ 2.59 mmol/L (100 mg/dL). In addition, we excluded patients with severe systemic infections, decompensated liver cirrhosis or cholestatic liver disease [13]. Healthy subjects ($n = 48$) were defined as normolipidemic healthy volunteers who had no abnormal laboratory tests or documented diseases. The remaining 119 persons were classified as subjects with disease, according to their medical history and lipoprotein profiles. At recruitment, written informed consent was obtained from all subjects.

The study protocol was reviewed and approved by the ethics committees of all participating institutions. This study was conducted according to the latest version of the Declaration of Helsinki.

2.2. Blood sampling and delivery

Fresh venous blood was drawn into vacuum tubes (Venoject II, VP-AS109K50, Terumo, Tokyo, Japan) from each subject regardless of time lapsed since the last meal. After the blood was allowed to clot, the serum was separated within an hour and poured into 50-mL plastic tubes (430290, Corning Japan, Tokyo) to equalize the blood components. Aliquots of samples were dispensed into screw-capped tubes and delivered to either SRL (Hachioji, Japan) for homogenous assays or Osaka Medical Center for Health Science and Promotion (OMC-HSP) (Osaka, Japan) for RMP [12]. Samples were placed in a cooling box containing refrigerant, and carried in a van equipped with a refrigerator. Temperatures were monitored continuously at two sites inside the box; temperatures were maintained between 2 and 4 °C within 24 h (Supplemental Fig. 1S).

2.3. HDL-C measurement

Within 24 h after blood collection, we measured HDL-C concentrations using 12 homogeneous assays, as well as RMP—described below. At SRL, the precision and accuracy of 12 homogeneous assays were evaluated (Reagent-A, Denka Seiken; Reagent-B, Wako; Reagent-C, Sysmex; Reagent-D, Serotec; Reagent-E, Fureiya; Reagent-F, Kyowa Medex; Reagent-G, Toyobo; Reagent-H, Shino-Test; Reagent-I, Sekisui Medical; Reagent-J, Ortho Clinical Diagnostics; Reagent-K, Siemens Healthcare, and Reagent-L, Beckman Coulter). Reagents-A–G, and Reagent-I were original homogenous assays, whereas Regent-H, Reagent-J, Reagent-K and Reagent-L were introduced products from other manufacturers (Supplemental Table S1). Reagents-A to -I were run on the same automated analyzer (Hitachi-917, also called Hitachi-7170 in Japan) that was used in the preceding studies [12,13]. The other three reagents were run on three different instruments manufactured by the distributors that produced the individual reagents. All of the reagents, calibrators and controls were supplied by the respective manufacturers and distributors. Under conditions of anonymity, the operators of SRL measured HDL-C (H) in triplicate as described previously [13]. In a preliminary study, we confirmed no cross contamination between cells, and no condensation during measurements using the Hitachi-910 instrument.

HDL-C was measured by RMP at OMC-HSP. First, two tubes prepared from each sample were ultracentrifuged at 18 °C, 105,000× g for 18.5 h. The bottom fraction was recovered by discarding the floating fraction using a tube slicer. After adding heparin-manganese solution to the bottom fraction, we removed the precipitate by centrifugation (1500× g for 30 min), and obtained the HDL fraction [14]. Finally, we measured cholesterol levels