Heart and Vessels (2019) 34:227–236
https://doi.org/10.1007/s00380-018-1247-9

ORIGINAL ARTICLE

Increased triglyceride/high-density lipoprotein cholesterol ratio may be associated with reduction in the low-density lipoprotein particle size: assessment of atherosclerotic cardiovascular disease risk

Katsuaki Yokoyama[2] · Shigemasa Tani[1,2] · Rei Matsuo[2] · Naoya Matsumoto[2]

Received: 11 June 2018 / Accepted: 17 August 2018 / Published online: 23 August 2018

Abstract
Hypertriglyceridemia, which often leads to both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) metabolic disorders, is a strong risk factor for the development of atherosclerotic cardiovascular disease (ASCVD). We hypothesized that the triglyceride (TG)/HDL cholesterol (TG/HDL-C) ratio may be more useful for estimation of the LDL-particle size, as a well-known risk factor for ASCVD, as compared to the serum TG level per se. Polyacrylamide gel electrophoresis was used in this study to estimate the LDL-particle size [relative LDL migration (LDL-Rm value)] in 649 consecutive patients with one additional risk factor for ASCVD. Multivariable regression analysis identified both serum TG ($\beta = 0.556$, $p < 0.0001$) and the serum TG/HDL-C ratio ($\beta = 0.607$, $p < 0.0001$) as independent indicators of the LDL-particle size. In terms of evaluation of the accuracy of indicators of LDL-Rm values equal to or greater than 0.40, which are suggestive of the presence of large amounts of small-dense LDL and represent the upper limit (mean + 2 standard deviation) of the normal range in this population, both the serum TG level and serum TG/HDL-C ratio showed high accurate areas under the receiver-operating characteristic curve (0.900 vs. 0.914), but with a negative likelihood ratio of 0.506 vs. 0.039, indicating that the TG/HDL-C ratio model is superior for excluding patients with values below the cutoff value and with LDL-Rm values ≥ 0.40. Furthermore, in 456 patients followed up for at least 1 year, multivariable regression analysis identified increased serum TG/HDL-C ratio as an independent predictor of a decreased LDL-particle size. These results suggest that the serum TG/HDL-C ratio may be more useful for assessing the risk of ASCVD as compared to the serum TG level per se. To reduce the risk of ASCVD, it may be important to focus not only on changes of the serum LDL-C, but also on those of the serum TG/HDL-C ratio.

Keywords TG/HDL-C ratio · LDL-Rm value · LDL-particle size · ASCVD

Introduction

Usefulness of low-density lipoprotein cholesterol (LDL-C)-lowering therapy has been established on the ground of the results of large-scale clinical studies conducted to verify the effects of statins [1, 2], statins plus concomitant ezetimibe therapy [3], and treatment with a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor in preventing the development of atherosclerotic cardiovascular disease (ASCVD) [4]. It is considered inevitable, therefore, to also emphasize countermeasures against residual independent risk factors, such as hypertriglyceridemia and depressed serum high-density lipoprotein cholesterol (HDL-C) [5].

However, large-scale clinical studies have so far failed to demonstrate the usefulness of reducing the serum triglyceride levels with fibrates, or elevating the serum HDL-C levels using cholesteryl transfer protein (CETP) inhibitors or niacin for preventing ASCVD [6].

Thus, a number of problems remain to be resolved in regard to countermeasures against elevated serum triglyceride (TG) levels and reduced serum HDL-C, which give rise to downsizing of the LDL-particle size (LDL particle size has been shown as an atherogenic risk factor for

Clinical Trial Registration: UMIN (http://www.umin.ac.jp/) Study ID: UMIN000032934 retrospectively registered 11 Jun 2017.

✉ Shigemasa Tani
tani.shigemasa@nihon-u.ac.jp

[1] Department of Health Planning Center, Nihon University Hospital, 1-6 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8309, Japan

[2] Department of Cardiology, Nihon University Hospital, Tokyo, Japan

ASCVD, independent of the serum LDL-C); management of these factors in the clinical setting entails difficulties [5]. Hypertriglyceridemia is the primary factor that facilitates downsizing of LDL particles and has a profound impact on HDL metabolism and is thereby associated with depressed serum HDL-C. Hypertriglyceridemia and depressed serum HDL-C are closely related disorders of lipid metabolism, and epidemiologic studies have led to recognition of the serum TG/HDL-C ratio as a more reliable risk marker of ASCVD [7, 8].

The methods usually employed to measure the LDL-particle size are density gradient ultracentrifugation, non-denaturing gradient gel electrophoresis, and nuclear magnetic resonance spectroscopy; however, these are difficult to apply in clinical settings, due to their cost and complexity [9]. The only procedurally simple method for assessing the LDL-particle size that is currently practicable on a commercial basis in Japan is polyacrylamide gel electrophoresis (PAGE) [10, 11].

On the grounds of the above mentioned, we hypothesized that the TG/HDL-C ratio, which is a straightforward marker of the serum levels of TG level and HDL-C is a more reliable predictor of the LDL-particle size than the serum TG level per se, and may be applicable as a new tool for assessing the risk of ASCVD when used in combination with PAGE, which enables simple estimation of the LDL-particle size in the clinical practice setting.

The objective of this study was to attempt evaluation of the risk of ASCVD using PAGE-assessed LDL-particle size and TG/HDL-C ratio using cross-sectional and longitudinal research designs.

Methods

Study design and study populations

This study was designed as a hospital-based cross-sectional and longitudinal study to investigate the relationships between the serum TG/HDL-C ratio and LDL-heterogeneity, as assessed by PAGE, in patients with one or more risk factors for ACVD. Furthermore, we examined the relationship between the changes in the TG/HDL-C ratio and the change in the LDL-particle size in cases in which additional measurements could also be conducted 1 year later. This study is a subanalysis of our previous study [10]. The study was conducted on a sample of 700 consecutive outpatients who had undergone regular examinations and blood examinations at the Cardiovascular Center, Surugadai Nihon University Hospital, between April 2009 and October 2009.

The criterion for patient registration in the cross-sectional study was the presence of one or more risk factors for ASCVD. The ASCVD risk factors and criteria for their diagnosis used in this study were hypertension (systolic pressure $\geq$ 140 mm Hg and/or diastolic blood pressure $\geq$ 90 mm Hg, or taking antihypertensive medication); diabetes mellitus (fasting plasma glucose $\geq$ 126 mg/dL and/or HbA1c $\geq$ 6.5%, or current treatment with anti-diabetic agents); dyslipidemia (serum LDL-C $\geq$ 140 mg/dL, serum TG $\geq$ 150 mg/dL and/or serum HDL-C $<$ 40 mg/dL, or taking lipid-lowering medication); chronic kidney disease [CKD; the severity of the CKD was determined on the basis of the estimated glomerular filtration rate (GFR) using the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation modified by a Japanese coefficient] [12].

Patients were not enrolled if they met any of the following exclusion criteria: presence of hepatic dysfunction (serum alanine aminotransferase and aspartate aminotransferase levels $\geq$ 2 times the upper limit of normal), known malignant disease, diagnosis of acute coronary syndrome within 3 months prior to the study, and/or serum TG $\geq$ 400 mg/dL.

The study designs and objective were approved by the Surugadai Nihon University Hospital Ethics Committee.

Measurement of laboratory parameters

Fasting blood samples were collected in the early morning after the subjects had fasted for 12 h. The serum levels of total cholesterol (TC), HDL-C and TG were measured by the standard methods. Serum LDL-C was estimated using the Friedewald formula [13]. The remnant-like particles' cholesterol (RLP-C) level was measured by an immunoadsorption assay (SRL Co., Ltd., Tokyo, Japan). The serum apolipoprotein (apo) level was determined by turbidimetric latex agglutination assays (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The malondialdehyde-modified LDL (MDA-LDL) level was measured by an enzyme-linked immunosorbent assay (SRL). The high-sensitivity C-reactive protein (hs-CRP) level was measured by a nephelometric assay (Behring Diagnostic Marburg, Germany).

Measurement of the LDL-Rm values

Relative migration LDL (LDL-Rm), an indicator of the LDL-particle size, was measured relative to the mobility value of LDL by performing PAGE with the LipoPhor system (Joko, Tokyo, Japan). The LDL-Rm value was calculated as the distance between the very low density lipoprotein (VLDL) peak and the LDL peak divided by the distance between the VLDL peak and the HDL peak (Fig. 1). Several studies have reported that LDL-Rm values $\geq$ 0.40 suggest the presence of a large amount of small-dense (sd)-LDL in the LDL fraction [9, 14, 15]. In particular, Hirano, et al. demonstrated that LDL-Rm = 0.40 corresponded to an LDL diameter of 25.5 nm as determined by PAGE, which is the cutoff value to distinguish LDL size phenotype A (large

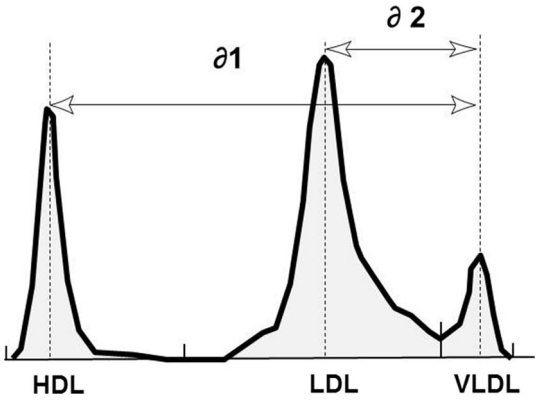

Fig. 1 Measurement of LDL-Rm value by lipoprotein polyacrylamide gel disc electrophoresis. LDL-Rm value calculated from densitometer analysis of polyacrylamide disc gel electrophoresis; *LDL-Rm value* ∂2/∂1; *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very LDL

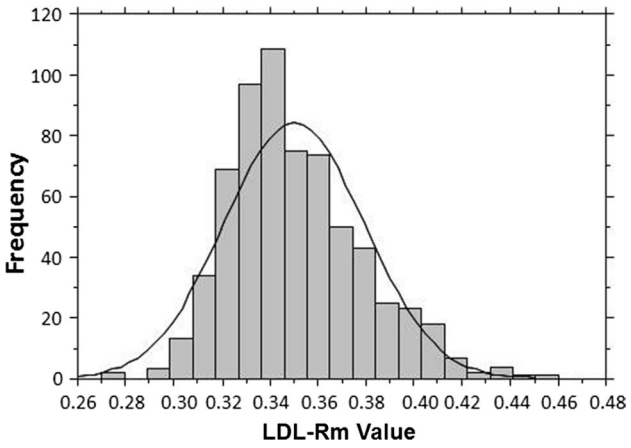

Fig. 2 Frequency distribution curve of LDL-Rm value. *LDL* low-density lipoprotein, *LDL-Rm* relative LDL migration

buoyant LDL) from LDL phenotype B (sd-LDL) [9]. The subjects of this study were not healthy persons in the general population, but the upper limit of the reference interval of the subjects' LDL-Rm values (mean ± 2 standard deviations (SDs) [covering 95‰ of the population: 0.350 ± 0.058]) was 0.408, and approximately the same as the value of 0.40 reported above. Accordingly, we conducted this study on the assumption that a large amount of sd-LDL was present in the LDL fraction when the LDL-Rm value was ≥ 0.40.

As described above, LDL-Rm is often used for qualitative evaluation of LDL-particle size in the clinical practice setting. Namely, LDL-Rm represents the average size of all LDL particles, which are aggregates of heterogeneously sized particles, and not the absolute amount of sd-LDL. However, until now, since LDL-Rm has been handled as a continuous variable when evaluating changes in the LDL-particle size in drug interventional and observational studies [16–18], in the present study also, we handled this parameter as a continuous variable. The distribution of the LDL-Rm values is shown in Fig. 2.

Statistical analysis

Data are expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables. For variables with a significantly skewed distribution, the data were expressed as the interquartile range (IQR).

In a subset analysis performed according to quartiles of the TG/HDL-C ratio, we used analysis of variance (ANOVA) followed by Bonferroni's adjustment for covariates if differences had been detected in the patient characteristics or ASCVD-related markers. Univariable and multivariable regression analyses were performed to identify independent predictors of the LDL-Rm value. All variables that showed correlation with the LDL-Rm values at p values of < 0.05 in the univariable regression analysis were entered into the multivariable model. In this study, we created the following two univariable and multivariable analysis models using the TG/HDL-C ratio and serum TG level as independent variables: the serum TG model (model 1) and the TG/HDL-C ratio model (model 2). Receiver-operating characteristic (ROC) analyses were performed to determine the cutoff values of the serum TG and the TG/HDL-C ratio for predicting LDL-Rm values ≥ 0.40. Furthermore, the diagnostic accuracy parameters calculated based on the results of the ROC analyses [area under the curve (AUC), sensitivity, specificity, and likelihood ratio: positive likelihood ratio, LR + = sensitivity/(1 – specificity), negative likelihood ratio LR − = (1 – sensitivity)/specificity] were also compared. In the 456 patients who could be followed up for at least 1 year, multivariable regression analyses were performed to identify independent variables associated with the absolute changes (Δ) in the LDL-Rm values. $p < 0.05$ was considered to be indicative of statistical significance. All statistical analyses were performed with the SPSS software (SPSS Inc., Chicago, Illinois, USA) for Windows (version 12.0.1).

Results

Patients

We excluded 51 subjects from the analysis because of missing laboratory data. Eventually, 649 subjects (438 male, 211 female) were included in the analysis. The LDL-Rm values ranged from 0.270 to 0.460 (mean ± SD: 0.350 ± 0.029). The TG/HDL-C ratio ranged from 0.364 to 10.633 (median, interquartile; 2.56 [1.39–3.44]). The patients were divided into quartiles of the TG/HDL-C ratio. In the subjects, the

quartile ranges were as follows: 0.364–1.373 (quartile 1: $n = 162$), 1.395–2.241 (quartile 2: $n = 162$), 2.255–3.429 (quartile 3: $n = 163$), and 3.455–10.633 (quartile 4: $n = 162$). In addition, there were no cardiovascular events during the 1-year follow-up period in the 456 subjects (414 male, 142 female).

Comparison of the LDL-particle size according to the TG/HDL-C quartiles

The LDL-Rm value, which reflects the estimated LDL-particle size, increased significantly as the quartile of the TG/HDL-C ratio increased (Fig. 3).

Comparison of the patient characteristics and laboratory profile according to the TG/HDL-C ratio quartiles

The proportion of males increased, and that of females decreased, significantly with increasing quartile of the TG/HDL-C ratio. The body mass index (BMI) increased significantly as the TG/HDL-C ratio quartile increased. The fasting blood glucose (FBG) and HbA1c increased significantly with increase of the TG/HDL-C ratio quartile. Hypertension, cigarette smoking, dyslipidemia, hyperuricemia, and diabetes mellitus were associated with high TG/HDL-C ratios. The percentage of prescriptions for antiplatelet, β-blockers, statins, and fibrates increased significantly with increasing TG/HDL-C ratio quartile.

The serum HDL-C and apo A-1 levels decreased significantly as the TG/HDL-C ratio increased. The serum levels of LDL-C, TG, non-HDL-C, apo B, apo C-II, apo C-III, apo E, RLP-C, MDA-LDL, and the white blood cell count increased significantly with increasing TG/HDL-C ratios (Tables 1, 2).

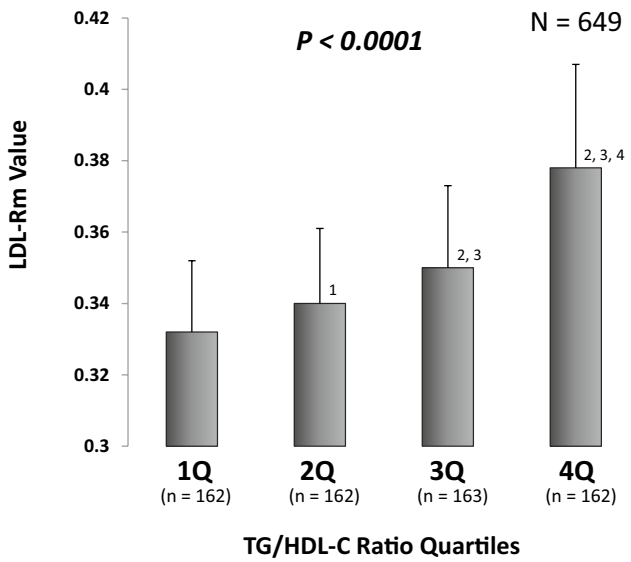

Fig. 3 Comparison of LDL-Rm value, estimated LDL-particle size according to the TG/HDL-C ratio quartiles. *LDL* low-density lipoprotein, *LDL-Rm* relative low-density lipoprotein migration, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol. ANOVA and post hoc tests with Bonferroni correction were performed to evaluate the between-group differences. $^{1}p < 0.01$, $^{2}p < 0.0001$, vs. 1Q. $^{3}p < 0.0001$, vs. 2Q. $^{4}p < 0.0001$, vs. 3Q

Univariable and multivariable regression analyses to identify factors associated with the LDL-Rm value, which reflects the LDL-particle size

The BMI was positively correlated with the LDL-Rm values. Male gender, cigarette smoking, hypertension, diabetes mellitus and dyslipidemia were associated with high LDL-Rm values. The serum LDL-C, TG, and the TG/HDL-C ratio levels were significantly and positively correlated with the LDL-Rm values. Therefore, these variables were entered into a multivariable regression model. The multivariable regression analysis identified the serum LDL-C and TG levels as being independently and significantly associated with the LDL-Rm values (model 1). Subsequent univariable and multivariable analyses performed using the TG/HDL-C ratio as an independent variable in place of the serum TG level revealed that the serum LDL-C and the TG/HDL-C ratio were also independently and significantly associated with the LDL-Rm values (model 2) (Table 3). The relationships among the serum TG levels, the TG/HDL-C ratio, and LDL-Rm values are shown in Fig. 4.

A comparison between the serum TG level and the TG/HDL-C ratio, as indicators of LDL-particle size

Since both the serum TG level and the TG/HDL-C ratio have been shown as independent predictors of the LDL-Rm, we compared the diagnostic accuracy of the serum TG per se and that of the TG/HDL-C ratio for predicting the LDL-particle size. ROC analyses were performed to determine the cutoff values of the serum TG and the TG/HDL-C ratio to predict LDL-Rm values ≥ 0.40. Indicators of the diagnostic accuracy obtained by the ROC analyses are shown in Fig. 5. Both models showed highly accurate AUCs. However, the LR− was significantly lower in the TG/HDL-C ratio model than in the TG model.

Multivariable regression analysis to identify factors correlated with absolute changes in the LDL-Rm value

In this cross-sectional study, we confirmed that an elevated TG/HDL ratio was an important factor of a decrease of the

Table 1 Patient characteristics according to the TG/HDL-C ratio quartiles

	All cases ($n=649$)	1Q ($n=162$)	2Q ($n=162$)	3Q ($n=163$)	4Q ($n=162$)	p value
Age (years)	62 ± 14	61 ± 16	62 ± 14	64 ± 14	63 ± 13	0.398
Male/female, *n* (%)	438 (67)/211 (33)	85 (52)/77 (48)	104 (64)/58 (37)	120 (74)/43 (26)	124 (77)/38 (23)	< 0.0001
BMI (kg/m^2)	24.0 ± 3.9	21.9 ± 3.4	24.4 ± 4.3[b]	24.2 ± 3.4[b]	25.4 ± 3.8[b]	< 0.0001
Hypertension, *n* (%)	450 (69)	93 (57)	116 (72)	114 (70)	127 (78)	0.001
Cigarette smoking, *n* (%)	91 (14)	15 (9.3)	26 (16)	16 (9.8)	34 (21)	0.010
Dyslipidemia, *n* (%)	429 (66)	78 (48)	109 (67)	110 (67)	132 (81)	< 0.0001
Hyperuricemia	112(17)	13 (8.0)	24 (15)	24 (15)	46 (28)	< 0.0001
Diabetes mellitus, *n* (%)	178 (27)	28 (17)	37 (23)	50 (31)	63 (39)	< 0.0001
FBG (mg/dL)	114 ± 36	108 ± 24	110 ± 35	114 ± 42	125 ± 40[a,b,c]	< 0.001
HbA1c (%)	5.93 ± 0.77	5.75 ± 0.59	5.90 ± 0.84	5.99 ± 0.85[a]	6.08 ± 0.75[a,d]	0.001
CKD stage 3 ≥, *n* (%)	181 (28)	48 (30)	41 (25)	47 (29)	45 (28)	0.880
eGFR (ml/min/1.73 m^2)	70.4 ± 18.6	70.4 ± 17.8	71.3 ± 19.1	71.0 ± 18.3	68.5 ± 19.4	0.517
CVD, *n* (%)	159 (24)	29 (18)	39 (24)	48 (29)	43 (27)	0.092
CAD, *n* (%)	142 (22)	25 (15)	37 (23)	44 (27)	36 (22)	0.083
Cerebral infarction, *n* (%)	19 (2.9)	4 (2.5)	3 (1.9)	4 (2.5)	8 (4.9)	0.369
Peripheral arterial disease, *n* (%)	10 (1.5)	1 (0.6)	3 (1.9)	3 (1.8)	3 (1.9)	0.691
Concomitant drugs, *n* (%)						
Antiplatelet	180 (28)	35 (22)	36 (22)	57 (35)	52 (32)	0.001
ACE inhibitors/ARBs	255 (39)	51 (31)	66 (41)	68 (42)	70 (43)	0.128
β-Blockers	135 (21)	26 (16)	32 (20)	40 (25)	47 (29)	0.026
Calcium channel blockers	295 (45)	61 (38)	80 (49)	75 (46)	79 (49)	0.131
Lipid-modifying drugs	319 (49)	58 (36)	84 (52)	84 (52)	93 (57)	< 0.001
Statins	305 (47)	58 (36)	79 (49)	79 (48)	89 (55)	0.006
Fibrates	10 (1.5)	0 (0)	0 (0)	6 (3.7)	5 (3.1)	0.018
Others	15 (2.3)	1 (0.6)	8 (4.9)	2 (1.2)	4 (2.5)	0.054

BMI body mass index, *FBG* fasting blood glucose, *Hb* hemoglobin, *CKD* chronic kidney disease, *eGFR* estimate glomerular filtration rate, *CVD* cardiovascular disease, *CAD* coronary artery disease, *ACE* angiotensin-converting enzyme, *ARB* angiotensin receptor blocker

[a] $p<0.01$, [b] $p<0.0001$, vs. 1Q

[c] $p<0.001$, vs. 2Q

[d] $p<0.01$, vs. 3Q

LDL-particle size. Therefore, we investigated the relationship between the absolute changes (Δ) in the TG/HDL-C ratio and the Δ LDL-Rm value using a longitudinal method. To investigate the effects of increase and decrease of the TG/HDL-C ratio on the Δ LDL-Rm values, we carried out multivariable regression analyses using the Δ LDL-Rm value as a dependent variable and the Δ TG/HDL-C ratio as an independent variable, with adjustments for the risk factors for ASCVD, lipid-modifying use, and Δ LDL-C in 456 patients who could be followed up for at least 1 year after this cross-sectional study. This analysis revealed that an elevated Δ TG/HDL-C ratio was an independent predictor of a decrease of Δ LDL-Rm value. Furthermore, statistical analyses of data from patients receiving or not receiving lipid-modifying drugs revealed similar findings (Table 4). However, the value of β (standard partial regression coefficient) of the Δ TG/HDL-C ratio as an independent variable in the multivariable analysis model was lower in the group receiving lipid-modifying drugs (statins in most cases) than in the group not receiving lipid-modifying drugs.

Discussion

The present study yielded the following findings: the serum TG/HDL ratio is a more reliable determinant of the LDL-particle size and a more straightforward marker of the cardiometabolic risk than the serum TG level per se; the higher the TG/HDL ratio, the smaller the size of the atherogenic LDL particles. Furthermore, combined cross-sectional and longitudinal studies have also confirmed the relationship between increased TG/HDL-C ratio and decreased LDL-particle size.

In the present study, the AUCs obtained from the ROC analyses to determine the cutoff serum TG levels and the TG/HDL-C ratios for predicting LDL-Rm values ≥ 4.0

Table 2 Laboratory profiles according to the TG/HDL-C quartiles

	All cases ($n=649$)	1Q ($n=162$)	2Q ($n=162$)	3Q ($n=163$)	4Q ($n=162$)	p value
TG/HDL-C ratio*	2.26 (1.39/3.44)	0.98 (0.75/1.22)	1.80 (1.63/2.92)[b]	1.65 (1.04/2.37)[b,d]	4.79 (3.84/6.11)[b,d,f]	< 0.0001
Lipids (mg/dL)						
TC	195 ± 37	197 ± 40	196 ± 35	188 ± 33	198 ± 40	0.044
LDL-C	110 ± 30	102 ± 31	114 ± 30[a]	108 ± 26	116 ± 33[b]	0.001
HDL-C	58 ± 17	75 ± 18	60 ± 11[b]	53 ± 12[b,d]	45 ± 9[b,d,f]	< 0.0001
TG*	120 (87/176)	68 (53/84)	108 (92/121)[b]	98 (69/131)[b,d]	217 (184/265)[b,d,f]	< 0.0001
Non-HDL-C	136 ± 34	122 ± 34	136 ± 31[a]	135 ± 28[a]	153 ± 36[b,d,f]	< 0.0001
Apolipoproteins (mg/dL)						
apo A-I	146 ± 30	167 ± 31	149 ± 25[b]	140 ± 28[b,c]	131 ± 22[b,d,e]	< 0.0001
apo B	90 ± 21	79 ± 19	89 ± 19[b]	89 ± 18[b]	101 ± 23[b,d,f]	< 0.0001
apo C-II	4.5 ± 1.8	3.7 ± 1.3	4.0 ± 1.5	4.5 ± 1.6[b]	5.7 ± 2.2[b,d,f]	< 0.0001
apo C-III	9.8 ± 3.2	9.9 ± 2.6	9.1 ± 2.8	9.6 ± 2.8	12 ± 3.5[b,d,f]	< 0.0001
apo E	4.1 ± 1.1	4.0 ± 1.2	3.8 ± 1.0	3.8 ± 0.9	4.6 ± 1.2[b,d,f]	< 0.0001
Other lipid markers						
RLP-C (mg/dL)*	5.3 (3.9/7.4)	3.7 (3.0/4.8)	4.4 (3.5/5.4)	4.2 (3.2/5.7)[b,d]	8.8 (7.3/11.6)[b,d,f]	< 0.0001
MDA-LDL (U/L)	110 ± 44	92 ± 35	103 ± 35	111 ± 43[b]	132 ± 52[b,d,f]	< 0.0001
Inflammatory markers						
WBC count (mm^{-3})	6065 ± 1622	5453 ± 1355	6042 ± 1503[a]	6151 ± 1862[b]	6604 ± 1538[b,c]	< 0.0001
hs-CRP (mg/L)*	0.50 (0.30/3.5)	0.30 (0.20/0.70)	0.40 (0.20/1.17)	0.40 (0.30/1.05)	0.80 (0.50/1.60)	0.131

TG triglyceride, *HDL* high-density lipoprotein, *TC* total cholesterol, *LDL* low-density lipoprotein, *apo* apolipoprotein, *RLP* remnant-like particle, *MDA* malondialdehyde, *WBC* white blood cell, *hs-CRP* hyper-sensitivity C-reactive protein

*Median; IQR in parentheses

[a]$p<0.001$, [b]$p<0.0001$, vs. 1Q

[c]$p<0.01$, [d]$p<0.0001$, vs. 2Q

[e]$p<0.01$, [f]$p<0.0001$, vs. 3Q

Table 3 Univariable and multivariable regression analyses to identify factors associated with the LDL-Rm value, which reflects the LDL-particle size

Dependent variables	Univariate		Multivariate Model 1		Multivariate Model 2	
	r	p value	β	p value	β	p value
Age	0.024	0.538	0.059	0.104	0.037	0.284
Male gender	0.126	0.001	0.080	0.018	0.046	0.162
BMI	0.190	< 0.0001	0.041	0.243	0.004	0.911
Cigarette smoking	0.078	0.048	0.008	0.813	− 0.004	0.902
Hypertension	0.102	0.010	− 0.0003	0.993	0.008	0.810
Diabetes mellitus	0.081	0.039	− 0.023	0.525	− 0.025	0.456
Dyslipidemia	0.168	< 0.0001	0.038	0.387	0.018	0.663
Lipid-modifying drug	0.090	0.021	− 0.005	0.898	0.020	0.613
LDL-C	0.195	< 0.0001	0.117	0.0007	0.147	< 0.0001
TG	0.592	< 0.0001	0.556	< 0.0001	–	–
TG/HDL-C ratio	0.633	< 0.0001	–	–	0.607	< 0.0001

The abbreviations are the same as in Tables 1 and 2, r = correlation coefficient, β = standard partial regression coefficient

were high, indicating that the prediction models were excellent. However, the LR + was low in both models that is, the detection rate is relatively low to make a definitive diagnosis of LDL-Rm values ≥ 0.40, even if the serum TG level and/or the TG/HDL-C ratio are above the cut-off. This is likely because the LDL-Rm value, i.e., LDL-particle size, is determined not only by the serum TG and HDL-C levels, but also by other factors, including insulin

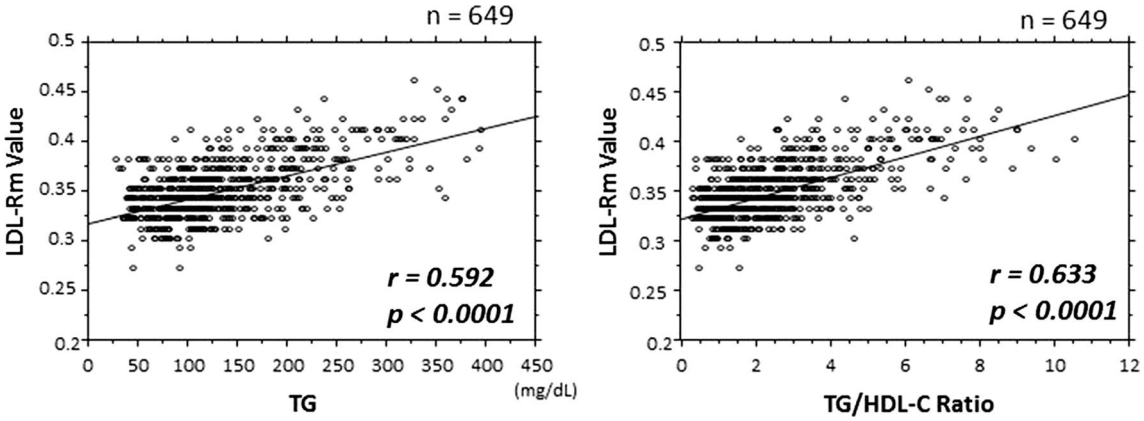

Fig. 4 Relationship between serum TG level, TG/HDL-C ratio, and LDL-RM value. *LDL* low-density lipoprotein, *LDL-Rm* relative low-density lipoprotein migration, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol

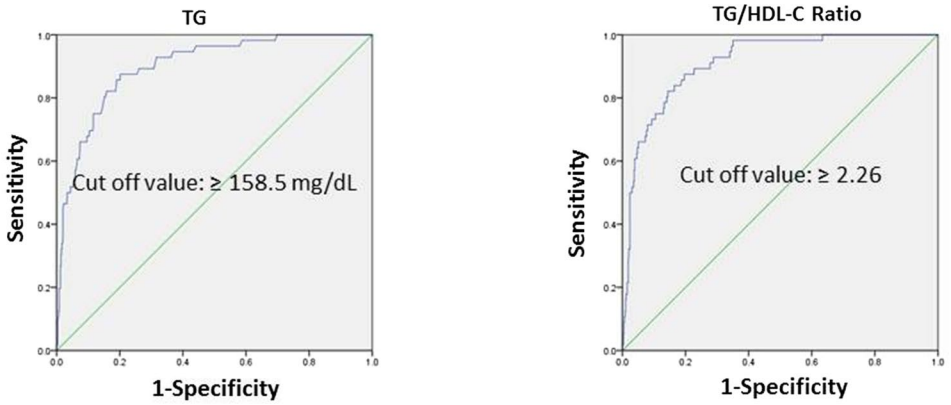

Fig. 5 Receiving-operating characteristic analysis of predictors of LDL-Rm value ≥ 0.40—comparison of diagnostic accuracy. *LDL-Rm* relative low-density lipoprotein migration, *AUC* area under the curve, *CI* confidence interval, *PPV* positive predictive value, *NNP* negative predictive value, *LR* + positive likelihood ratio, *LR* − negative likelihood ratio

	AUC (CI)	Sensitivity	Specificity	LR+	LR-
TG model	0.900 (0.860-0.939)	0.875	0.247	1.162	0.506
TGHDL-C ratio model	0.914 (0.881-0.948)	0.982	0.451	1.789	**0.039**

Table 4 Multivariable regression analysis to identify factors correlated with absolute changes in the LDL-Rm value

Dependent variables	β	p value	Dependent variables	β	p value	Dependent variables	β	p value
Age	− 0.538	0.273	Age	0.008	0.901	Age	− 0.080	0.298
Male gender	0.030	0.503	Male gender	− 0.030	0.633	Male gender	0.116	94.000
BMI	− 0.004	0.938	BMI	0.037	0.574	BMI	− 0.044	0.537
Cigarette smoking	− 0.007	0.869	Cigarette smoking	0.126	0.038	Cigarette smoking	− 0.161	0.020
Hypertension	− 0.040	0.332	Hypertension	− 0.027	0.664	Hypertension	− 0.099	0.149
Diabetes mellitus	− 0.004	0.932	Diabetes mellitus	0.031	0.603	Diabetes mellitus	− 0.087	0.265
Dyslipidemia	− 0.060	0.198	Dyslipidemia	0.036	0.549	Dyslipidemia	− 0.040	0.598
Lipid-modifying drug	0.052	0.244	Δ LDL-C	0.057	0.347	Δ LDL-C	0.012	0.868
Δ LDL-C	0.052	0.244	ΔT G/HDL-C ratio	0.275	< 0.0001	ΔT G/HDL-C ratio	0.388	< 0.0001
ΔT G/HDL-C ratio	0.338	< 0.0001	Multiple $r = 0.315$, $F = 3.223$, $p = 0.001$			Multiple $r = 0.495$, $F = 6.233$, $p < 0.0001$		
Multiple $r = 0.362$, $F = 7.486$, $p < 0.0001$								

All cases ($n = 456$); lipid-modifying drug use ($n = 273$); no lipid-modifying drug use ($n = 183$)

The abbreviations are the same as in Tables 1 and 2, Δ = absolute change from baseline, β = standard partial regression coefficient

resistance, visceral fat mass and overall TG metabolism [5]. However, the results of the present study showed that the LR− to evaluate the diagnostic accuracy was significantly lower in the TG/HDL-C ratio model than in the TG model. Therefore, if the TG/HDL-C ratio is lower than the cutoff, the LDL-Rm value is highly unlikely to be ≥ 0.40, and we think that this model is excellent for exclusion diagnosis as an indicator of the diagnostic accuracy [19, 20]. These results suggest that the TG/HDL-C ratio was the superior variable as an independent predictor of the LDL-particle size as compared to the serum TG level per se.

Thus, the present results show the possibility that the TG/HDL-C ratio may be applicable as a new management target for the prevention of ASCVD, i.e., towards the objective of minimizing any residual risk for ASCVD, in the current scenario, where the validity of control of the serum LDL-C, as one of the strongest risk factors for ASCVD, has become relatively well established.

Prospective cohort studies conducted to investigate the association of the TG/HDL-C ratio with the risk of development of coronary artery disease have demonstrated that the higher the TG/HDL-C ratio, the poorer the prognosis related to coronary artery disease [7, 8]. In regard to the underlying mechanisms, findings have suggested that a higher TG/HDL-C ratio is not only associated with downsizing of the LDL-particle size and consequent progression of atherosclerosis, but also with worsening of the insulin resistance [21, 22] and increased visceral fat deposition [23]. In the present study, we also demonstrated that the FBG and the BMI, a marker of visceral fat which is the basic abnormality in metabolic syndrome (Mets), increased with increasing TG/HDL-C ratios.

In this study, increase of the TG/HDL-C ratio was also shown to be associated with increase in the levels of atherogenic lipid markers, and similar findings were obtained in the very large database of lipids-4 (VLDL-4) study. The elevations in the lipid markers are considered to result from metabolic abnormalities of TG and HDL associated with increase of the TG/HDL-C ratios [24]. Namely, increased levels of highly atherogenic triglyceride-rich proteins (TRLs) and apolipoproteins, which play key roles in the metabolism of TRLs [5], in particular, of apoC3, have attracted attention as new risk factors for coronary artery disease [25]. It has been reported that hypertriglyceridemia and hypo-HDL cholesterolemia lead to an increase in MDA-LDL, an indicator of oxidative stress, that is closely involved in the progression of atherosclerosis [26]. In contrast, antiatherogenic serum HDL-C and apoA-1 levels tended to significantly decrease [27]. Interestingly, white blood cell counts, which are associated with predictive markers of Mets and coronary artery disease [28, 29], increased with increasing TG/HDL-C ratios.

It has been shown that the LDL-particle size, as measured by nuclear magnetic resonance or non-denaturing gradient gel electrophoresis, is better correlated with the TG/HDL-C ratio than with the serum TG level [21, 30]. The possibility of risk assessment of ASCVD using the LDL-Rm value, i.e., the estimated LDL-particle size calculated from PAGE data, readily practicable in the clinical practice setting, and of the TG/HDL-C ratio is considered truly relevant. The results of the ROC analysis in the VLDL-4 study lend support to the present finding in terms of the cutoff value of the TG/HDL-C ratio for predicting sd-LDL [24]. On the other hand, since the cutoff value of the LDL-particle size for the TG/HDL-C ratio is subject to the influence of the obesity, which differs depending on the gender and race, further investigation is warranted.

Hypertriglyceridemia is liable to be complicated by depressed serum HDL-C, and although both of these parameters are mutually independent ASCVD risk factors, the most relevant cases are those with concurrent hypertriglyceridemia and depressed serum HDL-C, which have a potent atherogenic effect. However, it is necessary to include other factors as well to set the TG/HDL-C ratio, an indicator of the risk of ASCVD, as a clinical tool in terms of a concrete numerical value. This is an important task that needs to be accomplished in the future [31].

The concept of the TG/HDL-C ratio, however, may be of great help in facilitating interpretation of the results of an intervention study being conducted to verify the effect of a new fibrate agent endowed with more potent TG-lowering and HDL-C-increasing effects in suppressing cardiovascular events as compared to conventional fibrate drugs currently in use [32]. It would appear that TG, LDL and HDL metabolisms interact to form a network, and that HDL per se also has a role as an antiatherogenic factor. It would be reasonable to speculate, therefore, that the use of the TG/HDL-C ratio corrected by the serum HDL-C rather than the serum TG alone is more rational as a method for estimating the LDL-particle size.

Interestingly, the multivariable analysis in the longitudinal study revealed that the influence of changes of the serum TG/HDL-C ratio on the changes in the LDL-particle size was smaller in the group receiving lipid-modifying drugs than in the group not receiving lipid-modifying drugs. Namely, an increase of the TG/HDL ratio was not readily associated with a decrease of the LDL-particle size in the group receiving lipid-modifying drugs. Looked at from another point of view, it could represent the influence of the antiatherogenic effects of lipid-modifying drugs.

Study limitations and clinical implications

First, the data of subjects with risk factors for ASCVD were analyzed in this study; more detailed results could be

expected from analyses of data from homogeneous patient groups, such as patients with Mets [33] and insulin-resistant patients [34]. Second, this study did not include assessment of other LDL-particle sizes or comparison with the TG/HDL-C ratio. Third, it is essential to correlate imaging diagnosis-verified progress of atherosclerosis with changes in the TG/HDL-C ratio. Finally, we have to consider improving the diagnostic accuracy of LDL-Rm values, to enable simple estimation of the LDL-particle size in the clinical practice setting, in particular, creation of an LDL-particle size prediction model with a higher positive likelihood ratio.

Conclusion

The results support the hypothesis that TG/HDL-C ratio may be useful for assessing the risk of ASCVD compared with serum TG level per se. To reduce the risk of ASCVD, it may be of particular importance to pay attention not only to the serum level of LDL-C, but also to the serum TG/HDL-C ratio, which is associated with LDL heterogeneity. However, for clinical application of the TG/HDL-C ratio as an indicator of the LDL-particle size based on the LDL-Rm value, further improvement of the diagnostic accuracy is required.

Acknowledgements The authors would like to thank International Medical Information Center (www.imic.or.jp/services/translation.html) for the English language review, and Dr. Yasuyuki Suzuki for helpful statistical analysis. The authors would also like to thank Mr. Gary Cooper for his help in editing the English manuscript.

Funding No funding was obtained for this study

Compliance with ethical standards

Conflict of interest The author declares that he/she has no competing interests.

References

1. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R, Cholesterol Treatment Trialists' (CTT) Collaborators (2005) Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 366:1267–1278
2. Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, Barnes EH, Voysey M, Gray A, Collins R, Baigent C, Cholesterol Treatment Trialists' (CTT) Collaborators (2012) The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet 380:581–590
3. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM, IMPROVE-IT Investigators (2015) Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med 372:2387–2397
4. Bohula EA, Giugliano RP, Leiter LA, Verma S, Park JG, Sever PS, Pineda AL, Honarpour N, Wang H, Murphy SA, Keech A, Pedersen TR, Sabatine MS (2018) Inflammatory and cholesterol risk in the FOURIER Trial (further cardiovascular outcomes research with PCSK9 inhibition in patients with elevated risk). Circulation. https://doi.org/10.1161/CIRCULATIONAHA.118.034032
5. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, Goldberg AC, Howard WJ, Jacobson MS, Kris-Etherton PM, Lennie TA, Levi M, Mazzone T, Pennathur S (2011) American Heart Association Clinical Lipidology, Thrombosis, and Prevention Committee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 123:2292–2333
6. Ip CK, Jin DM, Gao JJ, Meng Z, Meng J, Tan Z, Wang JF, Geng DF (2015) Effects of add-on lipid-modifying therapy on top of background statin treatment on major cardiovascular events: a meta-analysis of randomized controlled trials. Int J Cardiol 191:138–148
7. Bittner V, Johnson BD, Zineh I, Rogers WJ, Vido D, Marroquin OC, Bairey-Merz CN, Sopko G (2009) The triglyceride/high-density lipoprotein cholesterol ratio predicts all-cause mortality in women with suspected myocardial ischemia: a report from the Women's Ischemia Syndrome Evaluation (WISE). Am Heart J 157:548–555
8. Wan K, Zhao J, Huang H, Zhang Q, Chen X, Zeng Z, Zhang L, Chen Y (2015) The association between triglyceride/high-density lipoprotein cholesterol ratio and all-cause mortality in acute coronary syndrome after coronary revascularization. PLoS ONE 10(4):e0123521
9. Hirano T, Ito Y, Yoshino G (2005) Measurement of small dense low-density lipoprotein particles. J Atheroscler Thromb 12:67–72
10. Tani S, Matsumoto M, Nagao K, Hirayama A (2014) Association of triglyceride-rich lipoproteins-related markers and low-density lipoprotein heterogeneity with cardiovascular risk: effectiveness of polyacrylamide-gel electrophoresis as a method of determining low-density lipoprotein particle size. J Cardiol 63:60–68
11. Tani S, Takahashi A, Nagao K, Hirayama A (2015) Effect of dipeptidyl peptidase-4 inhibitor, vildagliptin on plasminogen activator inhibitor-1 in patients with diabetes mellitus. Am J Cardiol 115:454–460
12. Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Hara S, Ura N, Kiyohara Y, Hirakata H, Watanabe T, Moriyama T, Ando Y, Inaguma D, Narita I, Iso H, Wakai K, Yasuda Y, Tsukamoto Y, Ito S, Makino H, Hishida A, Matsuo S (2007) Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. Clin Exp Nephrol 11:41–50
13. Sniderman AD, Blank D, Zakarian R, Bergeron J, Frohlich J (2003) Triglycerides and small dense LDL: the twin Achilles heels of the Friedewald formula. Clin Biochem 36:499–504
14. Nakano T, Inoue I, Seo M, Takahashi S, Awata T, Komoda T, Katayama S (2009) Rapid and simple profiling of lipoproteins by polyacrylamide-gel disc electrophoresis to determine the heterogeneity of low-density lipoproteins (LDLs) including small, dense LDL. Recent Pat Cardiovasc Drug Discov 4:31–36
15. Yoshino G, Hirano T, Kazumi T (2002) Treatment of small dense LDL. J Atheroscler Thromb 9:266–275
16. Tani S, Nagao K, Matsumoto M, Hirayama A (2013) Highly purified eicosapentaenoic acid may increase low-density lipoprotein particle size by improving triglyceride metabolism in patients with hypertriglyceridemia. Circ J 77:2349–2357

17. Tani S, Matsuo R, Kawauchi K, Yagi T, Atsumi W, Hirayama A (2018) A cross-sectional and longitudinal study between association of n-3 polyunsaturated fatty acids derived from fish consumption and high-density lipoprotein heterogeneity. Heart Vessels 33:470–480
18. Tani S, Takahashi A, Nagao K, Hirayama A (2016) Association of lecithin-cholesterol acyltransferase activity measured as a serum cholesterol esterification rate and low-density lipoprotein heterogeneity with cardiovascular risk: a cross-sectional study. Heart Vessels 31:831–840
19. Grimes DA, Schulz KF (2005) Refining clinical diagnosis with likelihood ratios. Lancet 365:1500–1505
20. Davidson M (2002) The interpretation of diagnostic test: a primer for physiotherapists. Aust J Physiother 48:227–232
21. McLaughlin T, Reaven G, Abbasi F, Lamendola C, Saad M, Waters D, Simon J, Krauss RM (2005) Is there a simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? Am J Cardiol 96:399–404
22. Salazar MR, Carbajal HA, Espeche WG, Leiva Sisnieguez CE, Balbín E, Dulbecco CA, Aizpurúa M, Marillet AG, Reaven GM (2012) Relation among the plasma triglyceride/high-density lipoprotein cholesterol concentration ratio, insulin resistance, and associated cardio-metabolic risk factors in men and women. Am J Cardiol 109:1749–1753
23. Salazar MR, Carbajal HA, Espeche WG, Aizpurúa M, Maciel PM, Reaven GM (2014) Identification of cardiometabolic risk: visceral adiposity index versus triglyceride/HDL cholesterol ratio. Am J Med 127:152–157
24. Quispe R, Manalac RJ, Faridi KF, Blaha MJ, Toth PP, Kulkarni KR, Nasir K, Virani SS, Banach M, Blumenthal RS, Martin SS, Jones SR (2015) Relationship of the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio to the remainder of the lipid profile: the very large database of lipids-4 (VLDL-4) study. Atherosclerosis 242:243–250
25. TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute, Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, Konig IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissino D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E (2014) Kathiresan S (2014) Loss-of-function mutations in APOC3, triglycerides, and coronary disease. N Engl J Med. 71:22–31
26. Kondo A, Li J, Manabe M, Saito K, Kanno T, Maekawa M (2003) Relationship between high-density lipoprotein-cholesterol and malondialdehyde-modified low-density lipoprotein concentrations. J Atheroscler Thromb 10:72–78
27. Shah PK, Kaul S, Nilsson J, Cercek B (2001) Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming, part I. Circulation 104:2376–2383
28. Oda E (2013) High-sensitivity C-reactive protein and white blood cell count equally predict development of the metabolic syndrome in a Japanese health screening population. Acta Diabetol 50:633–638
29. Madjid M, Awan I, Willerson JT, Casscells SW (2004) Leukocyte count and coronary heart disease: implications for risk assessment. J Am Coll Cardiol 44:1945–1956
30. Maruyama C, Imamura K, Teramoto T (2003) Assessment of LDL particle size by triglyceride/HDL-cholesterol ratio in non-diabetic, healthy subjects without prominent hyperlipidemia. J Atheroscler Thromb 10:186–191
31. Kiyosue A (2018) Nonfasting TG/HDL-C ratio seems a good predictor of MACE in CAD patients with statin therapy. Could it be a treatment target? J Cardiol 71:8–9
32. Pemafibrate to reduce cardiovascular OutcoMes by reducing triglycerides IN patiENts With diabeTes (PROMINENT) (ClinicalTrials.gov Identifier: NCT03071692
33. Cuschieri S, Vassallo J, Calleja N, Pace N, Mamo J (2017) The effect of age, gender, TG/HDL-C ratio and behavioral lifestyles on the metabolic syndrome in the high risk Mediterranean Island population of Malta. Diabetes Metab Syndr. Suppl 1:S321–S327
34. Murguía-Romero M, Jiménez-Flores JR, Sigrist-Flores SC, Espinoza-Camacho MA, Jiménez-Morales M, Piña E, Méndez-Cruz AR, Villalobos-Molina R, Reaven GM (2013) Plasma triglyceride/HDL-cholesterol ratio, insulin resistance, and cardiometabolic risk in young adults. J Lipid Res 54:2795–2799