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Original Article

# Long-Term Probucol Treatment Prevents Secondary Cardiovascular Events: a Cohort Study of Patients with Heterozygous Familial Hypercholesterolemia in Japan

Shizuya Yamashita[1], Hideaki Bujo[2], Hidenori Arai[3], Mariko Harada-Shiba[4], Shigeyuki Matsui[5], Masanori Fukushima[6], Yasushi Saito[7], Toru Kita[8], and Yuji Matsuzawa[9]

[1]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan
[2]Department of Genome Research and Clinical Application, Chiba University Graduate School of Medicine, Chiba, Japan
[3]Department of Geriatric Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
[4]Department of Bioscience, National Cardiovascular Center Research Institute, Osaka, Japan
[5]Department of Pharmacoepidemiology, School of Public Health, Kyoto University, Kyoto, Japan
[6]Translational Research Center, Kyoto University Hospital, Kyoto University Graduate School of Medicine, Kyoto, Japan
[7]Department of Clinical Cell Biology, Chiba University Graduate School of Medicine, Chiba, Japan
[8]Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
[9]Department of Internal Medicine, Sumitomo Hospital, Osaka, Japan

***Aim***: The POSITIVE study assessed whether long-term treatment with probucol, a potent anti-oxidant and cholesteryl ester transfer protein (CETP) activator, is associated with a lowered risk of cardiovascular events in a very high-risk population: familial hypercholesterolemia (FH).

***Methods***: The study cohort included 410 patients with heterozygous FH, diagnosed between 1984 and 1999 by cardiovascular and metabolic experts at fifteen centers. Traceable patients were screened using predefined eligibility criteria. The primary outcome measure for comparison between probucol exposure and non-exposure was the time to the first cardiovascular event involving hospitalization.

***Results***: Analysis revealed significant differences in baseline characteristics and follow-up treatment between exposure and non-exposure. An observed indication bias was the use of probucol in more severe FH at diagnosis, both for primary and secondary prevention. When the multivariate Cox regression procedure was used after adjustment for possible confounding factors, probucol lowered the risk (hazard ratio [HR], 0.13; 95% confidence interval [CI], 0.05–0.34) in secondary prevention ($n=74$) and was statistically significant ($p<0.001$), although not significant (HR, 1.5; 95% CI, 0.48–4.67; $p=0.49$) in primary prevention ($n=233$). Safety assessment found no specific difference between exposure and non-exposure.

***Conclusion***: Long-term probucol treatment may prevent secondary attack in a higher cardiovascular risk population of heterozygous FH.

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**Key words**; Atherosclerosis, Antioxidants, CETP activator, Dyslipidemia

## Introduction

Cardiovascular (CV) diseases, including coronary heart disease and stroke, are the leading cause of death in Japan. Prevention of fatal CV events is therefore the final goal as well as the rationale of cholesterol-lowering therapy.

Probucol, a conventional cholesterol-lowering drug, originated with the report by Barnhart in 1970 [1]. The drug has been used clinically in Japan since 1985. Nearly 60,000 Japanese patients still take probucol; western countries discontinued probucol use after

Address for correspondence: Shizuya Yamashita, Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
E-mail: shizu@imed2.med.osaka-u.ac.jp
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the original manufacturer's withdrawal notice to the United States FDA in 1995 after 18 year's use of the drug. Probucol's cholesterol-lowering mechanism has not yet been clearly established, but it is thought to increase catabolic excretion of cholesterol into bile[2]. Later studies[3-5] have described new mechanisms of probucol, including anti-atherogenic and anti-oxidant actions. Another controversial and anti-atherogenic feature of probucol is its paradoxical effect of lowering high-density lipoprotein cholesterol (HDL-C). This action reflects, most likely, its molecular mechanisms: promoting cholesterol efflux, and enhancing reverse cholesterol transport by activation of cholesteryl ester transfer protein (CETP)[6-8] and class B type 1 scavenger receptor[9,10]. Matsuzawa and his colleagues reported an observed close correlation between the extent of regression in Achilles' tendon xanthoma and probucol-induced decrease in HDL-C levels in patients with familial hypercholesterolemia (FH)[11].

No large-scale, randomized, double blind comparative study has been conducted to justify the use of probucol in the prevention of CV events or diseases. however, clinical studies as well as pre-clinical data have been accumulating evidence of the clinical worth of probucol in arteriosclerotic diseases. Numerous clinical results, including a reduction in Achilles' tendon xanthoma thickness after long-term treatment for FH[12,13], reduced rates of restenosis after angioplasty[14-16], and a decrease in carotid artery intima-media thickness[17,18] support the therapeutic and preventative effects of probucol on arteriosclerotic lesions and plaque. To evaluate the risk and benefit of long-term probucol treatment, we conducted a cohort study to determine whether probucol treatment is associated with the risk reduction of CV events in patients with heterozygous FH, a very high-risk population.

## Methods

### Study Cohort

We registered patients with FH who received treatment between January 1, 1984 and December 31, 1999 at 15 centers specializing in CV and metabolic diseases, including FH, nationwide. Patients were traceable by medical recend and met the diagnostic criteria for heterozygous FH under the Japan Atherosclerosis Society Guidelines (2002) for the Diagnosis and Treatment of Atherosclerotic CV Diseases[19]. Definite heterozygous FH was defined as having at least two of the major features: total cholesterol (TC) of 260 mg/dL and above; tendon xanthoma or xanthoma tuberosum; reduced or abnormal receptor activity noted by LDL receptor analysis. Probable heterozygous FH was defined as having at least one each of the major (as above) and minor features: palpebral xanthoma; arcus juvenilis (< 50 years); juvenile (< 50 years) ischemic heart disease. For other eligibility criteria, we excluded patients with possible homozygous FH or with severe ventricular arrhythmias (polymorphic premature ventricular contractions). Possible homozygous FH was defined as having any one of the clinical features: defect of homozygous or hetero-polymeric LDL receptors confirmed by gene analysis; no LDLR activity observed by receptor analysis, severe elevation of plasma TC higher than 500 mg/dL; xanthoma or atherosclerotic vascular lesions including symptoms of juvenile ischemic heart disease; hypercholesterolemia confirmed in both parents; history of ischemic heart disease confirmed in both parents; or poor response to any 3-hydroxy-3methyl-glutaryl-coenzyme A reductase inhibitor (statin).

During the study period between June, 2004 and September, 2005, we collected anonymous case report forms with the patients' baseline data, including medical history, findings at clinical examination, medication data, and laboratory data. The investigators transcribed the data on to case report forms (identified by a code) from the stored medical charts of the patients. The observation period was the period for which each patient's clinical course could be traced. The longest observation period exceeded 20 years for patients on stable doses of probucol.

We required a sample size of 200 in both the probucol exposure and non-exposure groups, supposing a difference of 10% in the incidence of CV events for 5 years (15% in exposure and 25% in non-exposure). A least 400 subjects were needed to detect the difference with 80% power and a type I error of 5% at the 5% significance level with two-sided log-rank test based on normal approximation. The study protocol was approved through the process of ethics committee or institutional review board at each center.

### Definitions and Endpoints

The primary outcome measure was the time to the first CV event, defined as acute myocardial infarction (MI), angina pectoris (AP), heart failure (HF), stroke, transient ischemic attack (TIA) or arteriosclerotic peripheral artery diseases (PAD) leading to hospitalization or death as well as sudden death within 24 hours of an observed intrinsic event. The obtained baseline data at the first visit of each patient included demographic characteristics: sex, date of diagnosis at the participant medical center, age, height, weight, and habits of smoking and drinking. Body mass index (BMI) was calculated as weight in kilograms divided

by the square of height in meters. The other collected characteristic factors at diagnosis were the presence of xanthoma and its location, prior CV event, onset date if any prior CV event, treatment for the event, and other possible risk factors for CV events, including the presence of hypertension, diabetes, ventricular arrhythmia, and PAD. We collected data on cholesterol-lowering therapy (with or without probucol) and other concomitant therapy with anti-platelet, antihypertensive or diabetic drugs. Dates of drug initiation, discontinuation, re-administration, and termination were entered as elemental information. Treatment period was defined as the length from initiation until medication termination, or until the occurrence of the defined CV event, whichever came first. A lipid profile of TC, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and HDL-C, blood pressure, level of fasting blood sugar (FBS), hemoglobin$A_{1c}$ ($HbA_{1c}$), and thickness of tendon xanthoma in both feet were variables of interest, seen as potential predictors of CV events. We obtained measurements of those variables on a yearly basis after each patient was diagnosed. LDL-C levels were calculated from TC and HDL-C measurements with the Friedewald formula in TG $<400$ mg/dL. For TG of 400 mg/dL and more than 400 mg/dL, the expression of 0.16 X TG was applied in stead of 0.2 X TG[20]. Most patients had fasted compliantly at periodic checkups of their lipid levels. We set a follow-up period of 10 years for the measurements.

**Statistical Analyses**

The primary objective of analysis was a comparison between probucol exposure and non-exposure to evaluate whether treatment with probucol (500 mg to 1,000 mg daily) for FH provided CV benefits. The analysis was based on intent-to-treat principles. The secondary objective was to assess whether changes in the lipid profile after probucol treatment predicted CV events in the cohort. Event-free survival, defined as the time from diagnosis to the first CV event, was determined as a response variable. Statistical analysis was performed to evaluate clinical outcomes separately for secondary and primary prevention groups; that is, patients with or without a history of CV events at diagnosis.

Baseline characteristics of each group were explored to detect risk factors for CV events because potential confounders, including indication bias, were anticipated. For baseline comparison, Wilcoxon's rank sum test and Fisher's exact test were used for continuous variables and categorical variables respectively. For detection of risk factors, univariate Cox proportional hazards regression with a baseline variable as covariate was used as a screening step to determine the relationship with CV events. Variables that achieved significance at the level of 20% in univariate analysis were subsequently included in a multivariate Cox proportional hazards regression using backward variable selection. Variables proving significant at the 10% significance level were selected as risk factors to be adjusted. Consequently, probucol treatment effect was evaluated using the multivariate Cox model with adjustment for the selected baseline variables. Finally, the other observed treatment factors: cholesterol-lowering drugs other than probucol, LDL-apheresis, anti-platelet drugs, anti-hypertensive drugs, and diabetic drugs were entered into that model to assess their effects.

For the association between changes in lipid profile after probucol treatment and the risk of CV events, pre-treatment values of TG, LDL-C, HDL-C as well as TC, and each lipid reduction ratio after treatment were used as covariates. Multivariate analyses of time from probucol start to the first CV event used multivariate Cox's proportional hazards models. Statistical analysis was peerformed with SAS version 8.2.

## Results

**Patient Characteristics**

We collected data from the medical records of 541 patients, and excluded the data of 131 patients that did not meet eligibility predefined in the protocol.

The flow diagram (**Fig. 1**) gives reasons for the exclusion. A substantial fraction of probucol-exposed patients, 80.0% and 93.2%, took probucol within two years after diagnosis for in primary and secondary prevention groups, respectively. Baseline characteristics at diagnosis are given for each group (**Table 1**, 2). The secondary prevention group (**Table 2**) had prior diseases of AP, MI, stroke, HF, and TIA. This group was found to have significant higher proportions of men (60.2%, $p<0.01$), smokers (50.0%, $p<0.01$), hypertension (40.9%, $p<0.001$) diabetes (15.9%, $p=0.02$), and older median age (52 years, $p=0.01$) than the primary prevention group. Moreover, the group tended to have hypo-HDL cholesterolemia of median 42 (20–90) mg/dL, and to receive combined treatments with anti-platelet drugs (56.8%), anti-hypertensive drugs (53.4%), and LDL-apheresis (14.8%).

Comparison between probucol-exposed and non-exposed groups revealed significant differences in some baseline characteristics and treatments, which showed a confounding indication that patients with more severe FH took probucol. For baseline characteristics, the exposed group for primary prevention had more

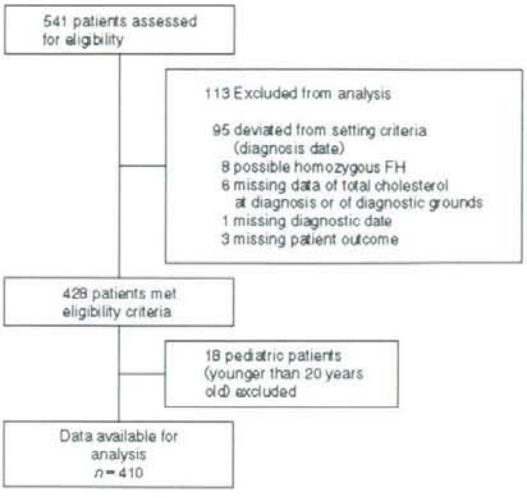



**Fig. 1.** Patient Flowchart.

We collected data from the medical records of 541 patients, and excluded the data of 131 patients who did not meet the eligibility predefined in the protocol. The flow diagram gives reasons for the exclusion.

palpebral xanthoma (13.4%, $p=0.05$), thicker median measurement of tendon xanthoma (12.5 mm, $p<0.01$), higher median $HbA_{1c}$ (5.8%, $p=0.03$), and more use of antihypertensive drugs (25.3%, $p<0.01$). Their lipid profile was more severe with a higher median baseline TC (325 mg/dL, $p=0.001$), a higher median LDL-C level (253 mg/dL, $p<0.001$), and a lower HDL-C level (47 mg/dL, $p<0.001$) than the unexposed group. The exposed group for secondary prevention had a higher prevalence of post-MI (44.6%, $p<0.01$) than the unexposed group. Observed medications were also significantly different between the exposed and unexposed groups. The exposed group used anti-hypertensive drugs concomitantly at a higher rate (25.3% vs. 11.2%, $p<0.01$) for primary prevention.

Descriptive analysis of baseline characteristics and treatments during observation implies that in both primary and secondary prevention, the exposed groups tended to include patients with more severe FH at diagnosis. Arguably, patients considered more severe at diagnosis would receive more intensive treatment, including probucol.

### Outcomes

We present the absolute number of CV events requiring hospitalization by prevention group with details of the events (**Table 3**). The incidence of CV events without consideration of confounding factors was 11.6% in the exposed group and 4.5% in the unexposed group for primary prevention. For secondary prevention, the incidence was 27.0% in the exposed group and 64.3% in the unexposed group. The event-free survival curve of the secondary prevention group is given (**Fig. 2**).

To identify risk factors for CV events, we determined the relationship between the incidence and every baseline variable using univariate Cox regression at a significant level of 20%. Variables proving significant at the 10% significance level in multivariate Cox regression were selected as risk factors to be adjusted. We estimated the effect of treatment after adjusting the selected risk factors. We calculated hazard ratios (HRs) with 95% confidence interval (CI) for binary variables, BMI ≥25 vs BMI <25, drinking vs no drinking, for example, and the indicated HRs corresponded to a 1 standard deviation increase for continuous variables, including TC. Estimated results are given (**Table 4**).

In the primary prevention group, significant variables were BMI ≥25 (HR 1.86, 95% CI 0.87–3.98; $p=0.11$), drinking (HR 2.17, 95% CI 1.02–4.63; $p=0.05$), tendon xanthoma (HR 2.17, 95% CI 0.76–6.23; $p=0.15$), prior diseases other than CV events (HR 1.87, 95% CI 0.87–3.99; $p=0.11$), PAD (HR 5.23, 95% CI 0.70–39.2; $p=0.11$), diabetes (HR 2.27, 95% CI 0.79–6.50; $p=0.13$), TC (HR 1.37, 95% CI 0.99–1.89; $p=0.06$), HDL-C (HR 0.75, 95% CI 0.50–1.12, $p=0.16$), SBP (HR 1.48, 95% CI 1.00–2.18; $p=0.05$), and the thickness of tendon xanthoma (HR 1.50, 95% CI 1.06–2.14; $p=0.02$). Three of these variables, drinking, TC, and PAD were selected for adjustment at the 10% significance level as a result of a multivariate Cox regression with backward variable selection. After adjustment for these three baseline variables, we found no significant effect by probucol at the 5% significant level. The estimated hazard ratio of probucol use for CV events was 1.50 (95% CI 0.48–4.67; $p=0.49$).

In the secondary prevention group, significance variables were drinking (HR 1.74, 95% CI 0.80–3.79; $p=0.17$), presence of palpebral xanthoma (HR 5.34, 95% CI 2.26–12.61, $p<0.001$), TIA (HR 4.16, 95% CI 0.54–32.21; $p=0.17$), history of coronary artery bypass graft (HR 0.31, 95% CI 0.11–0.90; $p=0.03$), hypertension (HR 0.58, 95% CI 0.26–1.28; $p=0.18$), diabetes (HR 2.89, 95% CI 1.30–6.42; $p<0.01$), and fasting blood sugar (HR 1.31, 95% CI 0.91–1.89; $p=0.15$). Two of these variables, palpebral xanthoma and diabetes, were selected for adjustment at the 10% sig-

**Table 1.** Baseline characteristics of patients in primary prevention group[†]

| Characteristics | Primary prevention No. (%) of patients | | | $p$ |
|---|---|---|---|---|
| | All $n=322$ | Exposed $n=233$ (72.4) | Unexposed $n=89$ (27.6) | |
| Age, mean (range) | 49 (27-74) | 50 (20-74) | 47 (20-72) | 0.18 |
| Men, No. (%) | 134 (41.6%) | 96 (41.2%) | 38 (42.7%) | 0.90 |
| BMI ≥25 | 71 (22.5%) | 49 (21.4%) | 22 (25.6%) | 0.45 |
| Smoker | 99 (33.2%) | 74 (34.1%) | 25 (30.9%) | 0.68 |
| Drinker | 124 (42.2%) | 93 (43.7%) | 31 (38.3%) | 0.43 |
| Xanthoma | 259 (80.7%) | 190 (81.9%) | 69 (77.5%) | 0.43 |
| Tendon xanthoma | 245 (76.3%) | 181 (78.0%) | 64 (71.9%) | 0.30 |
| Nodular xanthoma | 28 (8.7%) | 22 (9.5%) | 6 (6.7%) | 0.51 |
| Palpebral xanthoma | 36 (11.2%) | 31 (13.4%) | 5 (5.6%) | 0.05 |
| PAD | 4 (1.2%) | 1 (0.4%) | 3 (3.4%) | 0.07 |
| Hypertension | 54 (16.8%) | 40 (17.2%) | 14 (15.7%) | 0.87 |
| Diabetes | 22 (6.9%) | 17 (7.3%) | 5 (5.6%) | 0.81 |
| Lipid profile, mg/dL | | | | |
| TC[§] | 320 (188-493) | 325 (188-493) | 307 (194-464) | 0.001 |
| TG[§] | 120 (28-1289) | 121 (34-1068) | 120 (28-1289) | 0.96 |
| HDL-C[§] | 49 (20-108) | 47 (20-90) | 52 (27-108) | <0.001 |
| LDL-C[§] | 244 (45-425) | 253 (98-425) | 223 (45-403) | <0.001 |
| Blood Pressure, mmHg | | | | |
| SBP[§] | 129 (82-190) | 128 (82-190) | 131 (90-190) | 0.57 |
| DBP[§] | 0 (48-120) | 80 (48-120) | 80 (56-120) | 0.91 |
| FBS (mg/dL)[§] | 95 (63-276) | 94 (63-140) | 95 (81-276) | 0.41 |
| $HbA_{1c}$ (%)[§] | 5.7 (4.1-12.4) | 5.8 (4.1-9.7) | 5.3 (4.3-12.4) | 0.03 |
| Tendon xanthoma thickness (mm)[§] | 12.1 (7.5-49.0) | 12.5 (7.5-49.0) | 10.5 (8.0-20.0) | <0.01 |
| Treatment | | | | |
| Cholesterol-lowering drugs (non-probucol) | 302 (93.8%) | 219 (94.0%) | 83 (93.3%) | 0.80 |
| LDL-apheresis | 7 (2.2%) | 6 (2.6%) | 1 (1.1%) | 0.68 |
| Anti-platelet drugs | 49 (15.2%) | 41 (17.6%) | 8 (9.0%) | 0.06 |
| Anti-hypertensive drugs | 69 (21.4%) | 59 (25.3%) | 10 (11.2%) | <0.01 |
| Diabetic drugs | 15 (4.7%) | 12 (5.2%) | 3 (3.4%) | 0.37 |

[†]Continuous variables compared by Wilcoxon's rank sum test, distribution of categorical variables by Fisher's exact test. [§]Data are median (range). All data are number (%) unless otherwise indicated. Each percentage shown is related to the total number with measurement data. BMI, body mass index; PAD, peripheral artery disease; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; $HbA_{1c}$, hemoglobin $A_{1c}$. LDL-C was calculated with the Friedewald formula.

nificance level as a result of multivariate Cox regression analysis using a backward variable selection. After adjustment for these two baseline variables, the hazard ratio of probucol use for CV events was estimated to be 0.13 (95% CI 0.05-0.34) and significant ($p<0.001$). In sensitivity analyses, we also obtained similar estimation results on probucol for various sets of baseline covariates for adjustment.

The lipid levels of TC, LDL-C and HDL-C were lowered after probucol treatment both in primary and secondary prevention. In the primary prevention group, the median (range) levels of TC, TG, LDL-C and HDL-C closest to before treatment were respectively 305 (165-493), 119 (35-1068), 228 (107-425) and 48 (25-96) mg/dL, and those at 10-year treatment were, respectively, 222 (141-371), 94 (43-335), 157 (91-311) and 39 (17-81) mg/dL. In the secondary prevention, the median levels of TC, TG, LDL-C and HDL-C closest to before atment were, respectively, 320 (191-469), 129 (37-636), 240 (117-381) and 44 (24-90) mg/dL, and those at 10-year treatment were, respectively, 211(135-305), 71 (48-475),

**Table 2.** Baseline characteristics of patients in secondary prevention group

| Characteristics | Secondary prevention No. (%) of patients | | | *p* |
|---|---|---|---|---|
| | All $n=88$ | Exposed $n=74$ (84.1) | Unexposed $n=14$ (15.9) | |
| Age, mean (range) | 52 (23-71) | 51 (29-70) | 53 (23-71) | 0.62 |
| Men, No. (%) | 53 (60.2%) | 46 (62.2%) | 7 (50.0%) | 0.55 |
| BMI ≥25 | 21 (25.3%) | 17 (24.3%) | 4 (30.8%) | 0.73 |
| Smoker | 42 (50.0%) | 38 (53.5%) | 4 (30.8%) | 0.23 |
| Drinker | 39 (46.4%) | 33 (46.5%) | 6 (46.2%) | 1.00 |
| Xanthoma | 75 (85.2%) | 63 (85.1%) | 12 (85.7%) | 1.00 |
| Tendon xanthoma | 71 (80.7%) | 61 (82.4%) | 10 (71.4%) | 0.46 |
| Nodular xanthoma | 7 (8.0%) | 6 (8.1%) | 1 (7.1%) | 1.00 |
| Palpebral xanthoma | 8 (9.1%) | 5 (6.8%) | 3 (21.4%) | 0.11 |
| PAD | 2 (2.3%) | 2 (2.7%) | 0 (0.0%) | 1.00 |
| Hypertension | 36 (40.9%) | 30 (40.5%) | 6 (42.9%) | 1.00 |
| Diabetes | 14 (15.9%) | 9 (12.2%) | 5 (35.7%) | 0.04 |
| Lipid profile, (mg/dL) | | | | |
| TC† | 332 (191-469) | 334 (191-469) | 322 (229-444) | 0.41 |
| TG† | 128 (37-636) | 128 (37-636) | 136 (63-318) | 0.85 |
| HDL-C† | 42 (20-90) | 42 (20-90) | 39 (26-73) | 0.91 |
| LDL-C† | 249 (117-381) | 256 (117-381) | 245 (138-354) | 0.57 |
| Blood Pressure, mmHg | | | | |
| SBP† | 129 (90-180) | 128 (96-180) | 136 (90-166) | 0.97 |
| DBP (mmHg)† | 80 (52-114) | 80 (52-114) | 78 (60-104) | 0.33 |
| FBS (mg/dL)† | 96 (72-252) | 97 (72-197) | 94 (79-252) | 0.96 |
| HbA1c (%)† | 5.8 (4.1-10.6) | 5.5 (4.1-8.1) | 6.4 (5.3-10.6) | 0.06 |
| Tendon xanthoma thickness (mm)† | 14.5 (5.8-25.0) | 15.0 (5.8-25.0) | 10.0 (8.5-18.8) | 0.09 |
| Prior CV events | | | | |
| Angina Pectoris | 45 (51.1%) | 36 (48.6%) | 9 (64.3%) | 0.39 |
| Myocardial Infarction | 34 (38.6%) | 33 (44.6%) | 1 (7.1%) | <0.01 |
| Stroke | 7 (8.0%) | 4 (5.4%) | 3 (21.4%) | 0.08 |
| Heart failure | 2 (2.3%) | 2 (2.7%) | 0 (0.0) | 1.00 |
| TIA | 2 (2.3%) | 1 (1.4%) | 1 (7.1%) | 0.29 |
| Treatment | | | | 0.08 |
| Cholesterol-lowering drugs (non-probucol) | 81 (92.0%) | 70 (94.6%) | 11 (78.6%) | |
| LDL-apheresis | 13 (14.8%) | 11 (14.9%) | 2 (14.3%) | 1.00 |
| Anti-platelet drugs | 50 (56.8%) | 44 (59.5%) | 6 (42.9%) | 0.38 |
| Anti-hypertensive drugs | 47 (53.4%) | 42 (56.8%) | 5 (35.7%) | 0.24 |
| Diabetic drugs | 6 (6.8%) | 3 (4.1%) | 3 (21.4%) | 0.05 |

†Data are the median (range). All data are numbers (%) unless otherwise indicated. Each percentage is related to the total number with measurement data. TIA indicates transient ischemic attack.

147 (124-197) and 33 (17-70) mg/dL. Sub-analysis of changes in the lipid profile after probucol treatment detected significant three predictors of CV event risk: higher baseline TC (HR 2.74, 95% CI 1.05-7.16; $p=0.04$) in the primary prevention group; reduction in TG (HR 0.22, 95% CI 0.06-0.86; $p=0.03$); and reduction in LDL-C (HR 0.17,95% CI 0.03-0.90; $p=0.04$) after treatment in the subset of the secondary prevention group on stable doses of probucol. Neither TC nor HDL-C after treatment was associated with CV event risk in the probucol-exposed group, which indicates that reduction of the HDL-C level after probucol treatment is not related to CV event risk for probucol-exposed patients.

We evaluated the safety of probucol for all collected data from 541 patients, and found 56 adverse

**Table 3.** Incidence of cardiovascular events

| | | | Cardiovascular Event | No event | Total | $p$ |
|---|---|---|---|---|---|---|
| Primary prevention ($n$=322) | Exposed ($n$=233) | | 27 (11.6%) | 206 | 233 | |
| | | MI | 4 | | | |
| | | AP | 18 | | | |
| | | Str. | 3 | | | |
| | | TIA | 1 | | | |
| | | PAD | 1 | | | 0.058 |
| | Unexposed ($n$=89) | | 4 (4.5%) | 85 | 89 | |
| | | AP | 1 | | | |
| | | Str. | 2 | | | |
| | | TIA | 1 | | | |
| Secondary prevention ($n$=88) | Exposed ($n$=74) | | 20 (27.0%) | 54 | 74 | |
| | | MI | 6 | | | |
| | | AP | 12 | | | |
| | | HF | 1 | | | |
| | | Str. | 1 | | | 0.012 |
| | Unexposed ($n$=14) | | 9 (64.3%) | 5 | 14 | |
| | | MI | 2 | | | |
| | | AP | 6 | | | |
| | | Str. | 1 | | | |

MI, myocardial infarction; AP, angina pectoris; HF, heart failure; Str., stroke; TIA, transient ischemic attack; PAD, peripheral artery disease.
†One of the 4 patients died after 12 months of probucol termination.

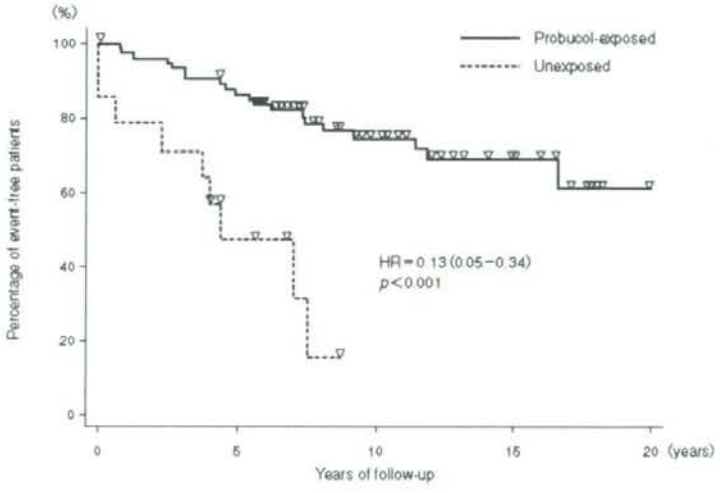
events in 18 patients. Malaise, pruritus, macrocytic anemia and pain in the extremities were recorded as adverse drug reactions associated with probucol. We noted and reported gastric cancer stage III immediately to the Ministry of Health and Welfare as an unexpected serious event, because of an unknown drug relation due to many concomitant drugs, although probucol was found to be non-carcinogenic alone[21]. Six deaths were observed in the population not taking probucol or stopping probucol. There was no other difference in the incidence of adverse events, including serious events, between probucol exposure and non-exposure.

## Discussion

Many data from large-scale randomized controlled trials have overwhelmingly demonstrated the clinical benefits of lowering cholesterol with statins[22, 23], yet the rapid and extensive prophylactic use of cholesterol-lowering drugs remains controversial. Few studies have addressed the clinical risks and benefits of long-term treatment of hyperlipidemia among women[24] or elderly patients[25]. The safety of long-term cholesterol-lowering therapy, including the issue of associated cancer risk or benefit, remains inconclusive because of conflicting clinical evidence[26]. More importantly, conclusions from the results of randomized controlled trials are limited by their relatively short follow-up periods (generally less than 5 years) in the analyzed studies.

In long-term treatment for FH, probucol was used with other cholesterol lowering drugs in over 80% of the secondary prevention group-those with a more severe clinical outlook than the primary prevention group: a higher prevalence of hypertension and diabetes, significant thicker tendon xanthoma, more combined therapy with LDL-apheresis, anti-platelet drugs, and anti-hypertensive drugs. The high rate of probucol use in FH was surprising, different from expected. This might partly reflect the prescription behavior of experts with the result that intractable patients responded to the regimen.

In the secondary prevention, the higher-risk group, probucol exposure was associated with a reduction in the risk of cardiovascular events (HR 0.13; 95% CI 0.05-0.34) with high significance ($p<0.001$), while it was not significant in the primary prevention group. This result was also contrary to our expectation that probucol exposure would likely be associated with increased event risk due to a confounding indication-that patients considered more severe at diagnosis would receive more treatment, including probucol. We did not collect the details of non-probucol drugs




Number at risk

| Years | 0 | | | | | 5 | | | | | 10 | | | | | 15 | | | | | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exposed | 74 | 71 | 70 | 68 | 66 | 62 | 54 | 50 | 42 | 38 | 34 | 30 | 25 | 19 | 17 | 13 | 12 | 8 | 3 | 1 | 0 |
| Unexposed | 14 | 11 | 11 | 10 | 9 | 5 | 4 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Estimates of event-free rates are according to whether patients received probucol. The cumulative probability of remaining without events was higher in patients treated with probucol ($p<0.001$; log-rank test).

**Fig. 2.** Kaplan-Meier Estimates of Event-free Rate.

For secondary prevention, the incidence of cardiovascular events was 27.0% in the exposed group and 64.3% in the unexposed group. An event-free survival curve for the secondary prevention group is given.

**Table 4.** The results of multivariate analysis using Cox regression procedure

| Factor | Primary prevention | | | Secondary prevention | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | $p$ | HR | 95% CI | $p$ |
| Baseline variables | | | | | | |
| Total cholesterol | 1.58 | 1.06-2.33 | 0.02 | - | - | - |
| Drinking | 2.43 | 1.09-5.44 | 0.03 | - | - | - |
| Peripheral artery disease | 5.27 | 0.51-54.63 | 0.16 | - | - | - |
| Palpebral xanthoma | - | - | - | 2.94 | 1.02-8.47 | 0.05 |
| Diabetes | - | - | - | 2.58 | 0.76-8.76 | 0.13 |
| Treatment in follow-up | | | | | | |
| Probucol use | 1.50 | 0.48-4.67 | 0.49 | 0.13 | 0.05-0.34 | <0.001 |
| Anti-platelet drug use | - | - | - | 2.48 | 1.00-6.17 | 0.05 |

to simplify the study procedure. However, we would likely exclude underused statins because of the reduced use of non-probucol drugs from the possible factors of the higher event rate in the unexposed group, because statins were available when all of the 9 recurrent patients (**Table 3**) started and the patients continued on cholesterol-lowering drugs. We suppose, therefore, that the reasons for this unanticipated great risk reduction include some antioxidant and anti-atherogenic actions[3, 4, 27] of probucol. The finding in second prevention may be suggested by the report[27] that probucol significantly decreased *in vitro* LDL oxidizability measured under typically strong oxidative conditions, and that long-term treatment with probucol had an

anti-atherogenic effect in Watanabe Heritable Hyperlipidemic rabbits. From the observation that the baseline lipid profile was not different between the two groups of exposure and non-exposure in secondary prevention, the drug might exhibit greates effectiveness in post-cardiovascular disease patients, in possibly advanced lipid accumulation and inflammation, which are associated with the circulation of oxidized LDL[28].

In primary prevention, we observed an almost significant increase of events in the exposed group (**Table 3**), and an apparently increased risk (HR 1.5), although not statistically significant after adjustment (**Table 4**). We suppose, however, that the ideal effects of probucol might be concealed by the following factors noted in primary prevention. The exposed group had a worse lipid profile (TC, LDL-C and HDL-C levels), higher $HbA_{1c}$, and thus definitely a higher risk than the unexposed group. Furthermore, 8 (nearly 30%) of the 27 patients experiencing cardiovascular events in the exposed group discontinued probucol when they had events. This was consistent with the different finding between primary and secondary preventions in the exposed group: less than half of the patients (113 of 233) in primary prevention continued on probucol, while 53 (72%) of 74 patients continued in secondary prevention. This estimation might be conservative.

The controversial and paradoxical action of probucol– lowering HDL-C– level was not associated with the risk of CV events in the cohort, therefore, the association between low levels of HDL-C and an increased risk for CV events or death indicated by the early Framingham Heart Study[29] may not be extrapolated to probucol-treated patients. This proposition is consistent with recent findings that a lowered HDL-C level is not always atherogenic, but that the quality or function of HDL-C is more important than the HDL-C levels[30]. In fact, increased levels of HDL-C with torcetrapib, a CETP inhibitor, were not associated with a significant clinical benefit in patients with coronary disease[31], FH[32] or mixed dyslipidemia[33].

We speculate that enhanced reverse cholesterol transport by CETP activation as a result of probucol treatment also contributed to the detected risk reduction in the cohort. The observed positive outcome of probucol, a CETP activator, might be a mirror image of the negative clinical trial results for the CETP inhibitor[34]. Reports[35, 36] of increased coronary heart disease in CETP deficiency despite increased HDL-C levels, and the molecular approach to review CETP deficiency[37] support our hypothesis, at least in Japanese genealogy. Interestingly, a recent basic research reports that human CETP expression enhances the mouse survival rate in an experimental systemic inflammation model[38], indicating for the first time a role for CETP in the defense against the exacerbated production of proinflammatory mediators.

For the safety evaluation, we found no cardiotoxic adverse drug reaction including QT/QTc prolongation or torsade de pointes, in this study, although probucol can cause them[16, 39, 40].

We obtained these results from an observational study with no control for inaccuracy, unexpected bias or confounding factors. We could not assure the precision of the baseline measurements due to unrecorded data. The participant centers were major hospitals for FH, but not all hospitals in Japan, because the study was conducted as part of a post-marketing study by a pharmaceutical manufacturer within the framework of the Japanese government regulations. Some restrictions on collecting data might have resulted in unexpected small numbers in the unexposed group in secondary prevention, although we think that the study cohort represents nearly a nationwide population of heterozygous FH in Japan. The results derived from patient data in Japan can not necessarily be generalized to patients in western countries.

Despite these limitations of the study, however, we could evaluate the outcome of long-term probucol treatment in the medical practice setting for FH, a high-risk population, for as long as 20 years in Japan. The significant risk reduction of CV events observed in the secondary prevention group holds clinical significance and suggests some beneficial therapeutic actions of this drug in arteriosclerotic diseases. The hypothesis from the findings warrants a randomized controlled trial for verification of the secondary prevention, and needs further research into the molecular mechanisms or roles of CETP in pathogenesis.

## Author Contributions

Dr. Yamashita had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Matsuzawa, Kita, Saito, Fukushima, Matsui. Acquisition of data: Yamashita, Bujo, Arai, Harada-Shiba, Saito, Kita, Matsuzawa. Analysis and interpretation of data: Yamashita, Bujo, Arai, Harada-Shiba, Matsui, Saito, Fukushima, Kita, Matsuzawa.

Drafting of the manuscript: Yamashita, Bujo, Arai, Harada-Shiba, Matsui, and Fukushima. Critical revision of the manuscript for important intellectual content: Yamashita, Matsui, Fukushima, Kita, Saito,

and Matsuzawa. Statistical analysis: Matsui and Fukushima. Administrative, technical, or material support: Fukushima, Matsui, Kita, Saito, and Matsuzawa. Study supervision: Yamashita, Fukushima, Matsui, Kita, Saito, and Matsuzawa.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## Disclosures

From the formerly Daiichi and Otsuka, Dr. Matsui, Dr. Fukushima, Dr. Matsuzawa, and Dr. Kita received fees and expenses for meetings related to protocol design, statistical and clinical interpretation of the data; Dr. Bujo, Dr. Arai, Dr. Harada-Shiba received honoraria and travel expenses for lectures, Dr.Yamashita, Dr. Bujo, Dr. Arai received fees and travel expenses for a meeting related to clinical interpretation of the data. Dr. Yamashita received consultancy fees from Otsuka. Dr. Matsuzawa is contracted as a short-term adviser to Otsuka in medical science. Dr. Saito received travel expenses only.

## POSITIVE Investigators

Osaka University Hospital, Suita (S. Yamashita, T. Maruyama); National Cardiovascular Center, Suita (M. Harada-Shiba); Sumitomo Hospital, Osaka (Y. Minami); Chiba University Hospital, Chiba (H. Bujo); Asahi General Hospital, Asahi (N. Hashimoto); Kawatetsu Chiba Hospital, Chiba (M. Takahashi); Nishifuna Naika, Funabashi (M. Shinomiya); Kashiwado Hospital, Chiba (K. Kosuge); Numazu City Hospital, Numazu (Y. Hayashi); Toho University Sakura Medical Center, Sakura (K. Shirai, Y. Miyashita); Matsudo City Hospital, Matsudo (T. Oeda); Kyoto University Hospital, Kyoto (M.Yokode, H. Arai); Hiroshima General Hospital of West Japan Railway Company, Hiroshima (K. Takata); Maizuru Kyosai Hospital, Maizuru (R. Tatami); Kido Hospital, Niigata (T. Miida)

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Opinion

# Proposed Guidelines for Hypertriglyceridemia in Japan with Non-HDL Cholesterol as the Second Target

Hitoshi Shimano, Hidenori Arai, Mariko Harada-Shiba, Hirotsugu Ueshima, Takao Ohta, Shizuya Yamashita, Takanari Gotoda, Yutaka Kiyohara, Toshio Hayashi, Junji Kobayashi, Kazuaki Shimamoto, Hideaki Bujo, Shun Ishibashi, Koji Shirai, Shinichi Oikawa, Yasushi Saito, and Nobuhiro Yamada

The Research Committee for Primary Hyperlipidemia, Research on Measures for Intractable Diseases by the Ministry for Health, Labor, and Welfare in Japan.

The Japan Atherosclerosis Society (JAS) guidelines for the prevention of atherosclerotic diseases, proposing management for LDL cholesterol as the primary target, have successfully contributed to the prevention of cardiovascular events; however, recently, the impact of hypertriglyceridemia as an additional cardiovascular risk has become understood, especially in light of the rise in obesity, metabolic syndrome, and diabetes in the Japanese population. Rather than waiting to obtain conclusive domestic data confirming that hypertriglyceridemia is a cardiovascular risk factor and that its management is efficacious, we propose guidelines for hypertriglyceridemia using non-HDL cholesterol as a second target.

*J Atheroscler Thromb, 2008; 15:116-121.*

**Key words;** Hyperlipidemia, Dyslipidemia, Triglycerides, HDL cholesterol, LDL cholesterol

## Introduction

Many prospective epidemiological studies have indicated a positive relationship between serum triglyceride (TG) levels and the incidence of coronary heart disease (CHD)[1, 2]. TG-rich lipoproteins such as remnant lipoproteins and small dense LDL particles are increased in hypertriglyceridemia and have been established to be atherogenic by numerous clinical and experimental studies[3-6]; however, classification of the plasma TG level as an independent risk factor for atherosclerosis has been controversial. This is partly because plasma TG levels are inversely intercorrelated by other well-established risk factors, such as low HDL cholesterol. To date, large scale trials for intervention targeting plasma TGs with TG reducing agents such as fibrates have not reached definitive conclusions about their effectiveness on primary endpoints, although fibrates have some impact on both primary and secondary prevention in small scale studies[7-9].

The precise estimation of plasma TGs as a cardiovascular risk is confounded by other risk factors, such as obesity, diabetes, hypertension and smoking. In addition, a cluster of metabolic risk factors, such as visceral obesity and insulin resistance with hypertriglyceridemia, referred to as metabolic syndrome, indicates that plasma TG concentrations are tightly linked to other strong risk factors for CHD. Thus, patients with elevated TGs are at increased risk for CHD, although greater risk cannot be independently explained by TGs. Meanwhile, recent meta-analyses suggested that plasma TGs could be an independent factor for CHD[1, 2]. Supportively, many experimental studies indicated that triglyceride-rich lipoproteins as well as LDL are atherogenic. Taken together, these data suggest that hypertriglyceridemia should be regarded as a semi-independent risk factor and should be included as a clinical target for the prevention of CHD. Considering the increasing prevalence of obesity, metabolic syndrome, and diabetes in this country, guidelines specialized for patients with hypertriglyceridemia need to be immediately established. In this study, we propose new guidelines for Japanese patients with hypertriglyceridemia

Address for correspondence: Hitoshi Shimano, Department of Internal Medicine (Endocrinology and Metabolism) Graduate School of Comprehensive Human Sciences University of Tsukuba, 1-1-1Tennodai, Tsukuba Ibaraki 305-8575, Japan.
E-mail: shimano-tky@umin.ac.jp
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**Table 1.** Plasma lipid profile of severe and mild type IIb hyperlipidemic patients sub-grouped by non-HDL cholesterol level

| Male | severe type IIb non-HDLc >190 mg/dL | mild type IIb non-HDLc <190 mg/dL | p |
|---|---|---|---|
| n | 51 | 54 | |
| Total Cholesterol | 270±41.8 | 234±40.3 | 0.001 |
| Triglycerides | 347±286 | 236±110 | 0.031 |
| HDL Cholesterol | 42.4±8.0 | 54.9±15.2 | 0.000 |
| LDL Cholesterol | 159±51.6 | 135±38.1 | 0.029 |
| non-HDL Cholesterol | 228±41.6 | 182±39.1 | 0.000 |

| Female | severe type IIb non-HDLc >180 mg/dL | mild type IIb non-HDLc <180 mg/dL | p |
|---|---|---|---|
| n | 52 | 48 | |
| Total Cholesterol | 265±29.6 | 231±20.2 | 0.000 |
| Triglycerides | 242±120 | 218±56 | 0.1 |
| HDL Cholesterol | 47.3±14.1 | 63.2±19.5 | 0.000 |
| LDL Cholesterol | 175±40.4 | 125±17.9 | 0.000 |
| non-HDL Cholesterol | 224±30.2 | 168±14.9 | 0.000 |

Subjects were patients who visited the outpatient clinic of the Endocrinology and Metabolism Unit of Tsukuba University Hospital on a regular basis (monthly or bimonthly) as described in Materials and Methods. Data are the means±SD (mg/dL).

using non-HDL as a secondary target after the goal for LDL cholesterol as the primary target is achieved.

## Materials and Methods

A total of 1,124 patients in Tsukuba University hospital in 2006 were consecutively included in the study (**Table 1**). Patients with severe illness were excluded. Plasma total cholesterol (TC), LDL-C, TG, HDL-C, glucose and HbA1c in either the fasted or fed state were determined enzymatically with the Hitachi 7070. Plasma HDL-C concentration was measured by a direct method using polyethylene-glycoso-pretreated enzymes. We calculated LDL-C concentration with Friedewald's formula (TC-TG/5-HDL-C) when TG was less than 400 mg/dL. Plasma non-HDL-C concentration was calculated as TC − HDL-C. One hundred and five male and 100 female patients were diagnosed with Type IIb hyperlipidemia (TC>220 mg/dL and TG>150 mg/dL). They were subcategorized into two groups according to their non-HDL cholesterol level (**Table 1**).

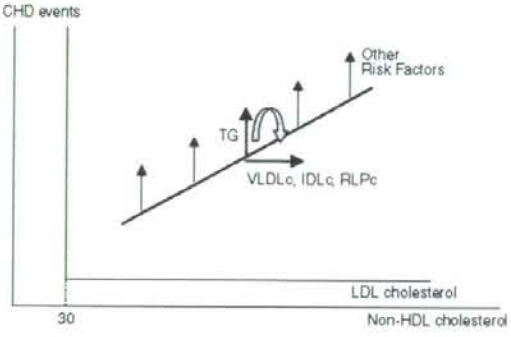


**Fig. 1.** Rationale for usage of non-HDL cholesterol: impact of TG and other risk factors on correlation between LDL-cholesterol CHD event.

nonHDL cholesterol=Total cholesterol − HDL cholesterol=VLDL cholesterol + IDL cholesterol (remnant lipoprotein cholesterol) + LDL cholesterol (Friedewald formula).
VLDL cholesterol + IDL cholesterol (RLP cholesterol) =TG/5
The risk of hypertriglyceridemia is approximated to VLDL, IDL, and RLP cholesterol estimated as TG/5, and incorporated into non-HDLC. The difference between non-HDL cholesterol and LDL cholesterol on X-axis was set up at 30 mg/dL based upon the data from Fig. 2.

## Results and Discussion

### Advantage of Non-HDL Cholesterol as a Marker for Hypertriglyceridemia

LDL cholesterol has been established as the most potent predictor of CHD and is currently the primary target for treatment and prevention. Other risk factors, including TG, diabetes, obesity, and metabolic syndrome, do not directly elevate plasma LDL cholesterol, but could enhance the risk of LDL cholesterol by shifting up the curve, as depicted in **Fig. 1**. To evaluate and manage the risk of hypertriglyceridemia, the TG level must be interpolated into the risk of plasma cholesterol. In patients with high TGs, most VLDL cholesterol resides in the smaller (remnant) VLDL fraction. Cholesterol of remnant lipoproteins (VLDL and IDL), which is concomitantly increased by elevation of plasma TG is an appropriate surrogate marker of hypertriglyceridemia. TG-rich remnant lipoproteins have been established as atherogenic lipoproteins[4, 5]. Thus, RLPc, a commercially available laboratory test for remnant lipoprotein cholesterol, could be a suitable marker for the atherogenicity of hypertriglyceridemia; however, this test is expensive and is not practical for use as a routine parameter. In contrast, non-HDL cholesterol, defined as total cholesterol − HDL cholesterol, is easily calculated, and represents the sum-

mation of VLDL/IDL (remnant) cholesterol and LDL cholesterol. It reflects the risks for all apoB-containing lipoproteins and could be an excellent marker for atherogenic lipoproteins. Plasma TG itself is not an appropriate marker for CHD risk due to its internal and dietary variability. In contrast, non-HDL cholesterol is not affected by dietary states and has much less daily variability than TG.

**Predictive Power of Non-HDL Cholesterol**

Non-HDL cholesterol reflects the risks of both hypertrigyceridemia and LDL-cholesterol[10, 11]. Several studies have indicated that non-HDL cholesterol is better than LDL cholesterol in its predictive power of cardiovascular diseases, indicating that VLDL cholesterol could contribute to CVD[12]. Non-HDL cholesterol is also a useful marker in a variety of subpopulations: men, the elderly, and patients with high-risk diseases such as diabetes and end-stage renal disease[13-16]. Our current clinical data from patients with type Ⅱb hyperlipidemia also support the usefulness of non-HDL cholesterol (**Table 1**). In our outpatient clinic, 70% of patients had diabetes and roughly 10% were type Ⅱb hyperlipidemia (cholesterol>220 mg/dL and TG>150 mg/dL). These type Ⅱb hyperlipidemic patients were equally divided into two sub-groups: severe (non-HDL cholesterol levels ≥ 190 mg/dL for male patients and 180 mg/dL for female patients) and mild <190 mg/dL for male patients and 180 mg/dL for female patients. When the severe and mild Ⅱb groups were compared, total, LDL, HDL cholesterol, and TG levels were significantly different among these two groups for both genders, except for serum triglyceride in females (**Table 1**). These data indicate that non-HDL cholesterol is an excellent marker representing all the components of dyslipidemia. The usefulness of non-HDL cholesterol rather than low-density lipoprotein cholesterol as a tool for lipoprotein cholesterol screening and assessment of risk and therapy has been already recognized in the USA[17, 18]. Another candidate marker for both remnant and LDL cholesterol is plasma apoB level[19]. ApoB is a direct marker for the particle number of apoB-containing lipoproteins and reflects risks of both remnants and LDL. Non-HDL cholesterol is highly correlated with apoB, and should replace this specialized and expensive laboratory test despite some reports indicating that apoB is better than non-HDL cholesterol for the predictive power of CHD[13, 20].

However, according to the Friedewald formula, the TG risk in non-HDL cholesterol represents only one fifth of TG levels as remnant cholesterol, and thus, the contribution of the risk is relatively weak compared to that of LDL cholesterol. Our previous data indicated that the correlation of non-HDL cholesterol to LDL cholesterol was much stronger than that to the TG level (**Fig. 2**)[21]. It should be noted that non-HDL cholesterol is not a specific marker for hypertriglyceridemia. Rather, non-HDL cholesterol should be regarded as a general single marker for both hypercholesterolemia and/or hypertriglyceridemia.

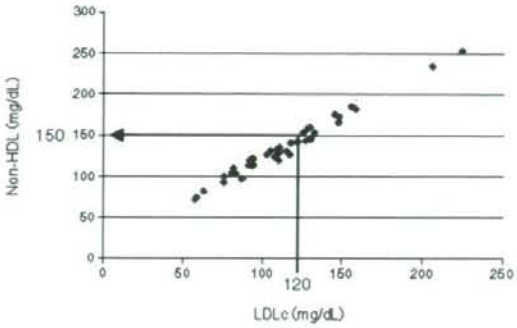


**Fig. 2.** Distribution of non-HDL cholesterol vs. calculated LDL cholesterol in normolipidemic patients.

Non-HDL cholesterol and LDL cholesterol calculated from Friedewald formula were highly correlated. Subjects were from the outpatient clinic of Tsukuba University Hospital[21].

**Proposed Guidelines for Hypertriglyceridemia**

Based upon these considerations, we propose guidelines for hypertriglyceridemia in Japanese patients using non-HDL cholesterol as a secondary target, as shown in **Table 2**. This is an extended version of the 2007 edition of the Japan Atherosclerosis Society (JAS) guidelines for the prevention of atherosclerotic diseases in which LDL cholesterol is the primary marker and target. It is essentially similar to the AHA-ATPⅢ guidelines for hyperTG in USA[22]. ATPⅢ recommends using non-HDL cholesterol as a secondary target when plasma TG is greater than 200 mg/dL because VLDL cholesterol is not significantly accumulated if TG is less than 200 mg/dL[23]. We do not have enough clinical data for Japanese on the relationship between TG and VLDL cholesterol to provide the appropriate TG level where the use of a non-HDL marker should be considered. Currently, we recommend using non-HDL for patients with hypertriglyceridemia (TG>than 150 mg/dL). Even for patients with hypertriglyceridemia, the primary target is still LDL cholesterol. In the 2007 JAS guidelines, goals of LDL for the secondary prevention group and the primary prevention group with category Ⅰ, Ⅱ, and Ⅲ are 100, 120, 140, and 160 mg/

**Table 2.** Proposed Japanese Guidelines for Hypertriglyceridemia

| Treatment | Categories | Coronary Risk Factors other than LCL-C | Goal for plasma lipids (mg/dL) Primary LDL-C | Secondary nonHDL-C | HDL-C |
|---|---|---|---|---|---|
| Primary Prevention Improving lifestyle as the first line, followed by medication | I (Low Risk Group) | 0 | <160 | <190 | ≥ 40 |
| | II (Intermediate) | 1~2 | <140 | <170 | |
| | III (High) | ≥ 3 | <120 | <150 | |
| Secondary Prevention Improving lifestyle & medication | Past History of CHD | | <100 | <130 | |

Goals for control depend upon categories of LDL cholesterol and non-HDL cholesterol. The primary target in hypertriglyceridemia is LDL-cholesterol. If the goal for LDL-cholesterol in the Japanese Guidelines for Atherosclerosis 2007 is already achieved, nonHDL-C is the secondary target. For the patients with TG > 500 mg/dL, potential genetic disorders and the prevention of acute pancreatitis should be considered. Coronary risk factors other than LDL-cholesterol include low HDL cholesterol, aging, diabetes, hypertenstion, smoking, past history of CHD, and obesity (visceral obesity).

dL, respectively. Goals for non-HDL cholesterol in each group are those for LDL cholesterol plus 30 mg/dL. This is based upon our outpatient clinic data that non-HDL cholesterol was 30 mg/dL higher than LDL cholesterol (**Fig. 2**)[21]. ATP III also recommends using LDL cholesterol goal + 30 mg/dL[24]. This also corresponds to the calculated VLDL cholesterol of the cut-off point of normal TGs (150/5 mg/dL). This goal is arbitrarily set and could be modified in the future, especially when the relative atherogenicity of remnants and LDL cholesterol are more precisely determined. In the case of TGs of greater than 500 mg/dL, the risk of pancreatitis should be carefully considered as a potential acute complication.

### Treatment of Hypertriglyceridemia Based upon Non-HDL Cholesterol Level

Treatment of patients with hypertriglyceridemia for primary prevention should be initiated with lifestyle modifications, especially reducing weight and increasing physical activity. Lifestyles exacerbating hypertrigylyceridemia, such as overweight, obesity, physical inactivity, cigarette smoking, excess alcohol intake, and very high carbohydrate diets, need to be improved. Other disorders and drugs that cause secondary hypertriglyceridemia, including diabetes, chronic renal filure, nephrotic syndrome, and steroid therapy, should also be treated first. In the event that lifestyle modification for at least three months is not effective to achieve the goal of non-HDL cholesterol, medication should be considered. Currently, due to lack of evidence to fully justify the use of fibrates for high TGs prior to statins, it is recommended to use a statin as the first line choice for high non-HDL cholesterol. If statin therapy is already used to control LDL cholesterol, management of non-HDL should be targeted by increasing the dose of the statin or switching to a stronger form. This is based upon the notion that remnant lipoproteins, as well as LDL, are taken up through LDL receptors that are up-regulated by statins. In the case of type III hyperlipidemia, or if high non-HDL cholesterol is much more prominent than LDL cholesterol because of hypertriglyceridemia, fibrates could be considered as they specifically reduce plasma TGs and are effective against type III hyperlipidemia. However, LDL cholesterol should be carefully monitored since fibrates occasionally raise LDL cholesterol following a decrease in TGs (VLDL cholesterol). In case the goal for LDL cholesterol is not attainable, the addition of cholestimide and/or ezetimibe to statin could be considered, whereas EPA could be considered for hypertriglyceridemia. A positive result from a recent large scale Japanese study using both EPA and pravastatin to estimate the prevention of atherosclerotic events, justifies superimposing EPA on statin therapy, although the contribution of the plasma TG-lowering effect of EPA to the prevention of cardiovascular events is not yet determined[25]. The complexity of the choice of medication for high non-HDL cholesterol is currently inevitable because no agents specifically decrease non-HDL cholesterol. Drug information strongly warns against the use of both statins and fibrates because of increasing the risk of the life-threatening side effect of rhabdomyolysis. Joint use is justified only when the benefit exceeds the risk, which requires expertise in this field; however, considering the very few reports of rhabdomyolysis as a severe side effect in recent post-market studies in Japan, carefully prescribing both agents for high-risk patients such as those with type IIb hyperlipidemia could be re-considered. Joint use might be restricted in the elderly or renal compromised patients. In addition, monitoring mus-

cle symptoms and plasma creatine phosphokinase is necessary in patients prescribed either statins or fibrates.

**Conclusions and Future Prospect of the Guidelines**

Non-HDL cholesterol containing both LDL cholesterol and remnant cholesterol, is an excellent predictor of atherosclerotic risk, and should be a treatment target. Non-HDL cholesterol is simple, convenient, and free from dietary variations. These advantages are crucial for nation-wide use of the guidelines and health check activity. This simple measurement could also make it possible to re-evaluate previous clinical studies using this parameter to offer a good chance of estimating the usefulness and importance of this marker in a large meta-analytical scale.

In the current study, we propose that LDL cholesterol is the primary target and non-HDL cholesterol should be the secondary target for elevated TG. Considering that non-HDL and LDL cholesterol are partially redundant, non-HDL could replace LDL as the primary target and as a general marker for both elevated cholesterol and TG. As **Table 1** shows, non-HDL cholesterol could be used as a general and convenient lipid marker for type Ⅱb hyperlipidemia.

This proposal still faces the recent problem of selecting lipid markers for the initial assessment for dyslipidemia. The recent GL focus has been on LDL cholesterol rather than TC, while LDL cholesterol has a problem the lower reliability for direct measurement. In addition, a considerable portion of hypertriglyceridemia is not applicable to this equation. For subjects with hypertriglyceridemia, application of this new GL eventually requires all TC, TG, HDL, and LDL cholesterol measurements to assess both LDL and non-HDL cholesterol. Currently, however, the Japanese medical system covers only three out of four lipid measurements as healthcare services provided by health insurance. Further Japanese clinical studies and careful evaluation of the data, as well as technical improvements of reliable LDL cholesterol measurements, are required to determine the most efficient protocol to select lipid measurements as the initial assessment of dyslipidemia to prevent CVD in Japan. Furthermore, guidelines for HDL cholesterol should also be established, although the relative importance and positioning of non-HDL and HDL is yet to be determined.

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Yoshiyuki Watanabe
Kensuke Uotani
Tetsuro Nakazawa
Masahiro Higashi
Naoaki Yamada
Yoshiro Hori
Suzu Kanzaki
Tetsuya Fukuda
Toshihide Itoh
Hiroaki Naito

# Dual-energy direct bone removal CT angiography for evaluation of intracranial aneurysm or stenosis: comparison with conventional digital subtraction angiography

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Y. Watanabe · K. Uotani · T. Nakazawa · M. Higashi · N. Yamada · Y. Hori · S. Kanzaki · T. Fukuda · H. Naito
Department of Radiology,
National Cardiovascular Center,
Osaka, Japan

Y. Watanabe (✉)
Department of Radiology,
St. Luke's International Hospital,
9-1 Akashicho, Chuo-ku,
Tokyo, 104-8565, Japan
e-mail: yowatana@luke.or.jp
Tel.: +81-6-68335012
Fax: +81-6-68727486

T. Itoh
Siemens Asahi Medical Technologies,
Tokyo, Japan

**Abstract** Dual energy CT can be applied to bone elimination for cerebral CT angiography (CTA). The aim of this study was to compare the results of dual energy direct bone removal CTA (DE-BR-CTA). To those of DSA. Twelve patients with intracranial aneurysms and/or ICA stenosis were performed on a dual-source CT in dual energy mode. A post-processing software selectively remove bone structures using the two energy data sets. 3D-images with and without bone removal were reviewed and compared to DSA. Dual energy bone removal was successful in all patients. For 10 patients, bone removal was good and CTA MIP images could be used for vessel evaluation. For 2 patients, bone removal was moderate with some bone remnants but this did not disturb the 3D visualization. Three aneurysms adjacent to the skull base were only partially visible in conventional CTA but were fully visible in DE-BR-CTA. In 5 patients with ICA stenosis, DE-BR-CTA revealed the stenotic lesions on the MIP images. The correlation between DSA and DE-BR-CTA was good (r2=0.822), but DE-BR-CTA lead to an overestimation of stenosis. DE-BR-CTA is able to eliminate bone structure using only a single CT data acquisition and is useful to evaluate intracranial aneurysms and stenosis.

**Keyword** Cerebral CTA · Dual-energy CT · Dual-source CT · Bone elimination · Brain

## Introduction

Cerebral computed tomography angiography (CTA) has become a powerful, noninvasive diagnostic tool for evaluating cerebrovascular disease [1–3]. However, single-source CTA still has drawbacks compared to digital subtraction angiography (DSA), in particular for the evaluation of arteries with calcified plaque or vessels located next to the skull bone, as these vasculatures cannot be unambiguously distinguished from surrounding bony or calcified structures. This problem can be solved by applying subtracting CTA to a noncontrast and a contrast CT data set to eliminate bones [4–8]. Dual-source, dual-energy CT has the potential to distinguish iodine from bone or calcifications using the attenuation difference between the two energies [9].

Here, we evaluated the performance of dual-energy direct bone removal CTA (DE-BR-CTA) for diagnosing