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Small Dense Low-Density Lipoprotein Cholesterol is a Potential Marker for Predicting Laser Treatment for Retinopathy in Diabetic Patients

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Aim: We explored the superiority of small dense low-density lipoprotein cholesterol (sdLDL-C) as a marker for predicting not only the occurrence of cardiovascular (CV) events but also the need for laser treatment in patients with hypercholesterolemia and diabetic retinopathy.

Methods: We performed a sub-analysis of the intEnsive statin therapy for hyper-cholesteroleMic Patients with diAbetic retinopaTHY (EMPATHY) study (n=5042), in which patients were assigned randomly to intensive or standard statin therapy targeting low-density lipoprotein cholesterol <70 mg/dl or 100-120 mg/dl. Using the survival analysis, the risks for CV events and the need for laser treatment were evaluated according to the lipids one year after registration.

Results: The patients were 63±11 years old. LDL-C and sdLDL-C levels were 98±25 and 32±14 mg/dl, respectively, one year after registration. The sdLDL-C level had a strong positive correlation with apolipoprotein B level (r=0.83 at registration). SdLDL-C was a sensitive marker for predicting CV events when comparing among the quartiles according to sdLDL-C levels (hazard ratios: HR for quartiles 1-4 were 1.0, 1.4, 1.6, and 2.5, respectively; p for trend <0.01). Also, sdLDL-C was a sensitive marker for predicting the need for laser treatment among lipids (log rank, p=0.009), especially in patients with elderly (≧65 yrs) and obesity (BMI ≧25 kg/m^2).

Conclusions: SdLDL-C is a sensitive target marker to predict cardiovascular events as well as the need for laser treatment in patients with hypercholesterolemia and diabetic retinopathy.

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Trial registration: UMIN000003486, www.umin.ac.jp/ctr/.

Key words: Small dense low-density lipoprotein cholesterol, Diabetic retinopathy, Statin

Introduction

Low-density lipoprotein cholesterol (LDL-C) is a useful lipid marker for predicting cardiovascular (CV) events[1, 2]. Lipid-lowering therapy targeting LDL-C < 70 mg/dl is recommended for patients with CV diseases[3, 4]. Lipid markers other than LDL-C, such as total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), triglyceride-rich lipoprotein cholesterol (TRL-C), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB), are also potential markers for predicting CV events[1, 2, 5, 6, 7].

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One study reported that ApoB/A1 is superior to LDL-C as a marker for predicting CV events[2]. LDL-C is known to be subdivided into granular fractions, among which small dense LDL-C (sdLDL-C) with a smaller particle size has recently attracted attention as a lipid marker that may be more sensitive for predicting CV events than LDL-C[8]. LDL-C with a smaller particle size is more easily oxidized and permeable to the cell wall[9], promoting atherosclerotic changes more than LDL-C with a larger particle size, so-called large buoyant LDL-C (lbLDL-C). Several studies, including the Atherosclerosis Risk in Communities (ARIC) study, have reported sdLDL-C as a more sensitive predictive marker of CV events as compared with LDL-C[8].

Diabetic retinopathy as a common microvascular complication of diabetes is a primary cause of vision loss among adults leading to 0.8 million blindness and 3.7 million visual impairment in 2010 [10]. As timely treatment for diabetic retinopathy is known to reduce the risk of vision loss by 98%[11], predictive markers for the progress in diabetic retinopathy should be identified. Considering that diabetic retinopathy is caused by microvascular damage in retina, lipids which are known to have harmful effects on the vascular structure and function should be a predictive marker for the progress in diabetic retinopathy. Whether any lipid could be a powerful marker for the progress in diabetic retinopathy, is still inconclusive. Indeed, a randomized controlled trial in diabetic patients showed that treatment with fibrate reduced the need for laser treatment for diabetic retinopathy[12]. In this study, however, the mechanism of this effect did not seem to be related to lipid profile.

Aim

Here, we confirmed the previous evidence that sdLDL-C predicts the occurrence of CV events and investigated whether sdLDL-C predicts the need for laser treatment more sensitively than other lipid markers in patients with hypercholesterolemia and diabetic retinopathy using the dataset from the intEnsive statin therapy for hyper-cholesteroleMic Patients with diAbetic retinopaTHY (EMPATHY) study[13].

Methods

The EMPATHY study (n=5042) was performed from 2010 to 2013, to examine whether intensive lipid-lowering therapy was superior to standard therapy in reducing the incidence of primary endpoints (i.e., CV events including cardiac, cerebral, renal, and vascular events, or CV-associated death) in patients with hypercholesterolemia and diabetic retinopathy, but no history of coronary artery disease. In this multicenter, prospective, randomized, open-label, blinded endpoint (PROBE) study, patients were randomly assigned to intensive statin therapy targeting LDL-C <70 mg/dl, or standard statin therapy targeting LDL-C 100-120 mg/dl[13]. The EMPATHY study showed only a tendency toward fewer CV events in the intensive therapy group as compared with the standard therapy group because the difference in LDL-C levels between the two groups one year after registration was smaller (20 mg/dl) than expected at planning the study.

Informed consent was obtained from each patient and the study was conducted under the Declaration of Helsinki and Japanese ethical guidelines for clinical studies. The protocol was reviewed and approved by the human research committee of each participating center (in total, 774 institutions).

Study Population

From the study population of the EMPATHY study (n=5042), we excluded patients whose blood samples could not be obtained one year after registration (n=453) **(Fig. 1)**. After this exclusion, first, the correlation between lipid profile and the risk for CV events in 4589 patients was evaluated to confirm the previous evidence on the predictors for CV events. Next, patients with history of treatment for retinopathy including laser treatment (n=1424) or without routine ophthalmologic check-up until 3 years after registration (n=2189) were excluded. In the remaining 976 patients with routine ophthalmologic check-up after registration, we evaluated the correlation between lipid profile and the need for laser treatment for diabetic retinopathy.

Outcomes

The primary endpoints of this study included the occurrence of CV events and the need for laser treatment for diabetic retinopathy. CV events were defined as cardiac, cerebral, renal, and vascular events, or CV-associated death[13]. Cardiac events were defined as myocardial infarction or unstable angina requiring unscheduled hospitalization, or coronary revascularization. Cerebral events were defined as cerebral infarction or cerebral revascularization. Renal events were defined as initiation of chronic dialysis or an increase in serum creatinine levels by at least 2-fold (and >1.5 mg/dl). Vascular events were defined as aortic disease, aortic dissection, mesenteric artery thrombosis, severe lower limb ischemia, revascularization, or finger/lower limb amputation

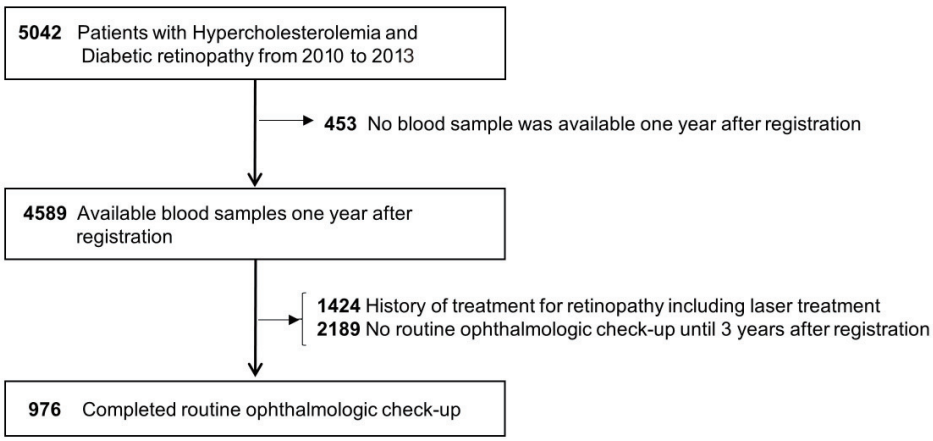

Fig. 1. Flowchart of patient enrollment

Patients whose blood samples were not available one year after registration were excluded from this study and the cardiovascular events were analyzed in remaining 4589 patients. Patients with the history of laser treatment for retinopathy and patients without routine ophthalmologic check-up until 3 years after registration were excluded from the analysis of the need for laser treatment.

caused by arteriosclerosis obliterans. Laser treatment for diabetic retinopathy was performed by ophthalmologists according to the guidelines for diabetic retinopathy[14].

Experimental Design and Laboratory Methods

In the EMPATHY study, blood samples were collected at each clinic from all patients at registration, and every year after registration, for analysis of lipid profile: TC, LDL-C, HDL-C, TG, ApoA1, ApoB, and sdLDL-C. TC levels were measured using the chemical oxygen demand method. LDL-C, HDL-C, and sdLDL-C levels were measured with direct homogenous assays using detergents (LDL-EX, HDL-EX, sdLDL-EX, Denka Seiken, Tokyo, Japan). TG levels were examined by the enzymatic assay of glycerol-3-phosphate oxidase. Apolipoprotein levels were measured using the turbidimetric immunoassay. All assays were performed at SRL (Tokyo, Japan). TRL-C was calculated as TC minus HDL-C minus LDL-C[5].

Management of Diabetic Retinopathy

In the EMPATHY study, the retinal photographs of all participants were obtained at registration, indexing the stage of diabetic retinopathy according to the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria[15]. Routine ophthalmologic check-ups were scheduled at registration and every year after registration. If the progression of diabetic retinopathy was suspected in the study period, additional ophthalmoscopy was performed according to demand. The information on the laser treatment for diabetic retinopathy was obtained from medical records in each institution. The laser treatment for macular edema was not regarded as the endpoint in the present study.

Statistical Analyses

Using SPSS ver. 22 (IBM, Chicago, IL, USA), the changes of lipids 0, 1, 2, 3, 4 and 5 years after registration were evaluated by the paired or unpaired t-test. After the Kolmogorov-Smirnov Goodness-of-fit test, Pearson's correlation coefficient (r) was calculated to assess the degree of association between two variables in the parametric tests. The Spearman rank correlation coefficient was used to examine the degree of association between two variables in the non-parametric tests.

Hazard ratios (HRs) and survival curves were analyzed to compare the risk for CV events, death, and the need for laser treatment for retinopathy. To minimize the influence of lipid changing-term after registration, we set one year after registration as the landmark starting point to compare the outcomes among the groups and participants with events occurring <1 year after registration being excluded from the analysis. Covariates at registration, including age, gender, hypertension, and current or previous smoking, were adjusted in the Cox hazard analysis. For continuous variables, HRs of the Cox proportional analyses were estimated per one standard deviation increase[16]. The Kaplan-Meier analysis and Cox hazard analysis were used to estimate survival curves of quartiles according to levels of LDL-C, TG, TRL-C, and sdLDL-C.

Since the higher prevalence of dyslipidemia including the elevated sdLDL-C level was reported in

Table 1. Baseline Characteristics at registration

	Analysis on CV events (n=4589)	Analysis on laser treatment (n=976)
Age (yrs)	63±11	63±14
Male, n (%)	2182 (48)	467 (48)
Body mass index (kg/m^2)	26±4	26±4
Systolic blood pressure (mmHg)	135±16	136±17
Diastolic blood pressure (mmHg)	75±11	75±11
Hypertension, n (%)	3259 (71)	717 (73)
Smoking, n (%)		
Current	842 (18)	165 (17)
Past	993 (22)	225 (23)
No	2754 (60)	586 (60)
Family history of CAD, n (%)	590 (13)	144 (15)
βblocker, n (%)	272 (6)	55 (6)
ACEI, n (%)	235 (5)	55 (6)
ARB, n (%)	2240 (49)	510 (52)
Intensive statin therapy, n (%)	2272 (50)	500 (51)
Hb (g/dl)	14±2	14±2
Creatinine (mg/dl)	0.8±0.3	0.7±0.2
BNP (pg/ml)	26±41	27±47
CRP (mg/dl)	1.5±5.8	1.4±4.5
HbA1c (%)	7.2±1.2	7.3±1.2
Diabetic retinopathy status, n (%)		
Simple retinopathy	3053 (67)	691 (71)
Preproliferative retinopathy	840 (18)	193 (20)
Proliferative retinopathy	664 (14)	92 (9)
Others[§]	32 (1)	0 (0)
History of treatment for retinopathy	1424 (31)	0 (0)

[§]Includes patients who had a history of laser therapy but no funduscopic findings at enrollment or had retinopathy negative after enrollment. CV: cardiovascular, CAD: coronary artery disease, ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, Hb: hemoglobin, BNP· brain natriuretic peptide, CRP: C-reactive protein

diabetic nephropathy[17], the association between sdLDL-C level and the risk of the need for laser treatment for retinopathy was calculated after adjustment for the existence of nephropathy (defined as urine albumin to creatinine ratio≧30 mg/gCr and/or estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2).

Results

Correlations among Lipids

The baseline characteristics of patients are shown in **Table 1**. The average age in the total study population was 63 years old and 48% of them were male and the level of HbA1c was 7.2±1.2%. Follow-up period was 3.2±0.9 years. The changes of all lipids, except for ApoA1, were significantly changed one year after registration, and after that, lipid profile were not significantly changed except LDL-C and TRL-C at 2 years after registration, ApoA1 at 3 years after registration, and sdLDL-C at 2 and 5 years after registration **(Table 2)**. Close correlations between sdLDL-C and other lipids, were observed at registration and one year after registration **(Table 3)**. At registration, both of LDL-C and ApoB were significantly correlated with sdLDL-C, and correlation between ApoB and sdLDL-C (r=0.83, p<0.001) was greater as compared with that between LDL-C and sdLDL-C (r=0.64, p<0.001) **(Fig. 2)**.

Survival Analysis

Next, we analyzed the risk of variables for CV events **(Fig. 3A)**. Male, higher systolic blood pressure, current or past smoker, lower level of hemoglobin, and higher levels of creatinine, brain natriuretic peptide (BNP), C-reactive protein, and HbA1c were significantly associated with the risk for CV events. The levels of all lipids at registration were not significantly related to the risk for CV events (data not shown). However, higher levels of TC, LDL-C, TG,

Table 2. The longitudinal change of Lipids

N	Registration 4589	1 year after 4589	2 year after 4155	3 year after 2432	4 year after 970	5 year after 118
TC (mg/dl)	189 ± 32	176 ± 32*	176 ± 32	176 ± 32	172 ± 33	173 ± 32
HDL-C (mg/dl)	56 ± 14	55 ± 14*	55 ± 14	55 ± 14	56 ± 14	57 ± 14
LDL-C (mg/dl)	110 ± 26	98 ± 25*	97 ± 25**	96 ± 26	96 ± 27	96 ± 26
TG (mg/dl)	140 ± 89	134 ± 88*	134 ± 88	134 ± 88	134 ± 92	129 ± 79
TRL-C (mg/dl)	24 ± 14	22 ± 13*	21 ± 14**	20 ± 13	21 ± 13	20 ± 12
ApoA1 (mg/dl)	148 ± 27	149 ± 26	149 ± 26	151 ± 27***	151 ± 26	151 ± 24
ApoB (mg/dl)	93 ± 21	84 ± 19*	83 ± 20	82 ± 20	82 ± 20	82 ± 18
ApoB/A1	0.64 ± 0.18	0.58 ± 0.17*	0.57 ± 0.17**	0.56 ± 0.17***	0.56 ± 0.16	0.56 ± 0.16
sdLD-C (mg/dl)	35 ± 15	32 ± 14*	32 ± 14**	32 ± 14	31 ± 14	28 ± 12****

The levels of lipids were compared using the *t* test. *$p<0.05$ between patients at registration and one year after registration. **$p<0.05$ between patients with 1 year and 2 years after registration. ***$p<0.05$ between patients with 2 years and 3 years after registration. ****$p<0.05$ between patients with 4 years and 5 years after registration .TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, TRL-C: triglyceride-rich lipoprotein cholesterol, Apo A1: apolipoprotein A1, Apo B: apolipoprotein B, sdLDL-C: small dense LDL-C.

Table 3. Correlation between small dense LDL cholesterol and other biomarkers

n=4589	correlation coefficient	*p*
at registration		
Age (yrs)	-0.14	<0.001
Hb (g/dl)	0.21	<0.001
Creatinine (mg/dl)	0.00	0.81
BNP (pg/ml)	-0.09	<0.001
CRP (mg/dl)	-0.02	0.23
HbA1c (%)	0.20	<0.001
Lipid at registration		
TC (mg/dl)	0.66	<0.001
HDL-C (mg/dl)	-0.16	<0.001
LDL-C (mg/dl)	0.64	<0.001
TG (mg/dl)	0.58	<0.001
TRL-C (mg/dl)	0.53	<0.001
ApoA1 (mg/dl)	0.04	<0.01
ApoB (mg/dl)	0.83	<0.001
ApoB/A1	0.63	<0.001
Lipid after one year		
TC (mg/dl)	0.67	<0.001
HDL-C (mg/dl)	-0.16	<0.001
LDL-C (mg/dl)	0.67	<0.001
TG (mg/dl)	0.58	<0.001
TRL-C (mg/dl)	0.49	<0.001
ApoA1 (mg/dl)	0.05	<0.01
ApoB (mg/dl)	0.84	<0.001
ApoB/A1	0.64	<0.001

Hb: hemoglobin, BNP: brain natriuretic peptide, CRP: C-reactive protein, TC: total cholesterol, HDL C high density lipoprotein cholesterol, LDL C low density lipoprotein cholesterol, TG: triglyceride, TRL C: triglyceride-rich lipoprotein cholesterol, Apo A1: apolipoprotein A1, Apo B: apolipoprotein B

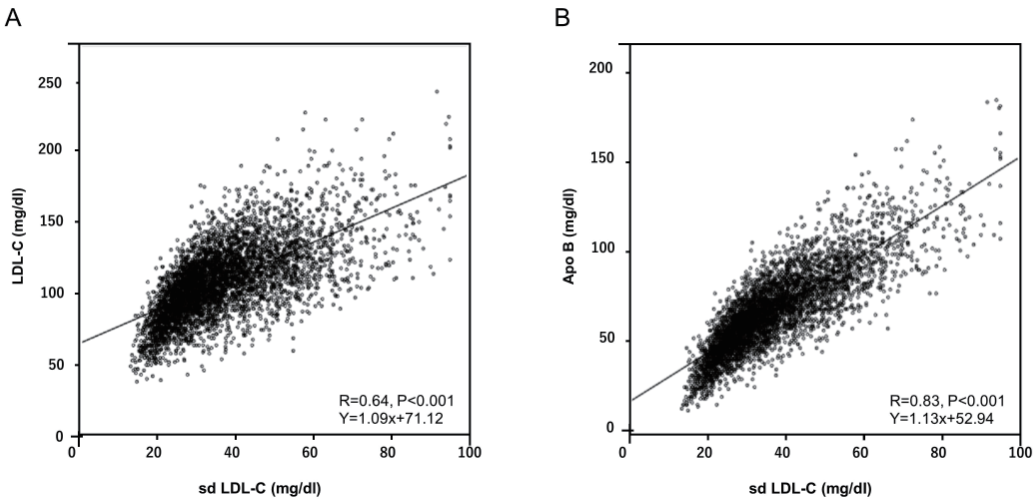

Fig. 2. Correlation between sdLDL-C and LDL-C or Apo B

A: The scatter diagrams of the correlation between LDL-C and sdLDL-C at registration. B: The scatter diagrams of the correlation between Apo B and sdLDL-C at registration. LDL-C: low-density lipoprotein cholesterol, sdLDL-C: small dense low-density lipoprotein cholesterol, Apo B: apolipoprotein B.

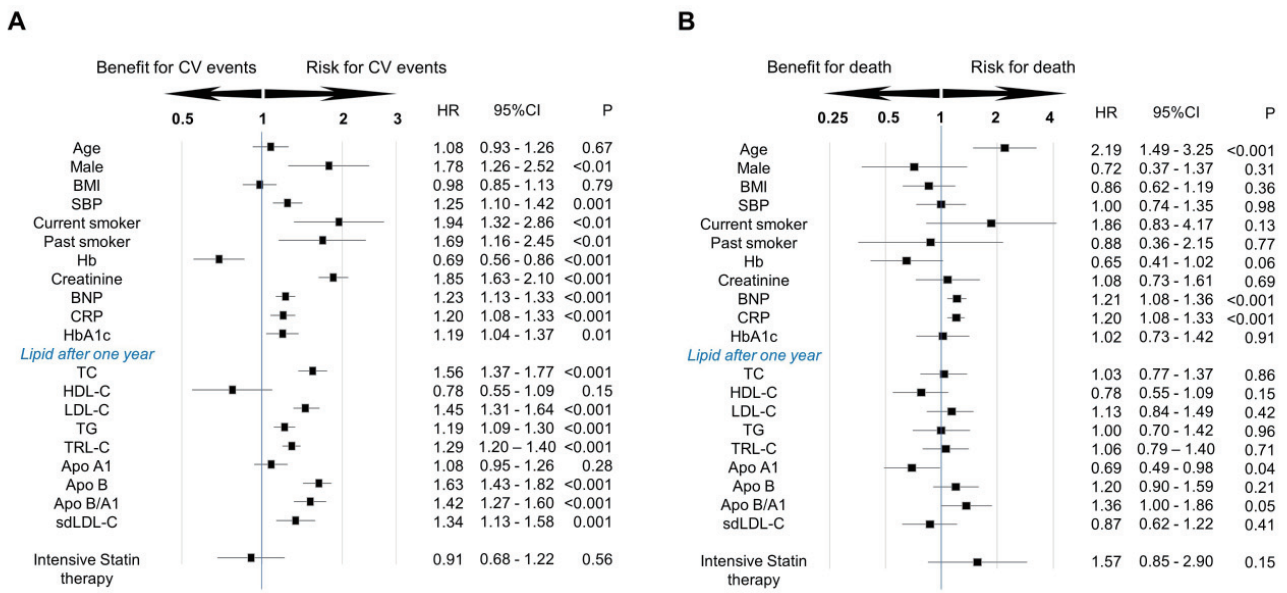

Fig. 3. Hazard ratios of each parameter for CV events and death

Hazard ratios were estimated per one standard deviation increase by a Cox proportional analysis of the risks for CV events (A) and all death (B), after adjusting for covariates including age, gender, hypertension, and current or previous smoking at registration. Hb: hemoglobin, BNP: brain natriuretic peptide, CRP: C-reactive protein, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triglyceride, TRL-C: triglyceride-rich lipoprotein cholesterol, ApoA1: apolipoprotein A1, Apo B: apolipoprotein B, sdLDL-C: small dense low-density lipoprotein cholesterol, CV: cardiovascular.

TRL-C, ApoB and sdLDL-C at one year after registration were significantly associated with the risk for CV events (HR 1.56, 95% CI 1.37-1.77; HR 1.45, 95% CI 1.31-1.64; HR 1.19, 95% CI 1.09-1.30; HR 1.29, 95% CI 1.20-1.40; HR 1.63, 95% CI 1.43-1.82; HR 1.34, 95% CI 1.13-1.58, respectively). While older age and higher levels of BNP and C-reactive protein were significantly associated with risk for all death, none of the lipid markers except for ApoA1 at one year after registration were related to the risk for death **(Fig. 3B)**.

Comparisons among the quartiles (Q) according

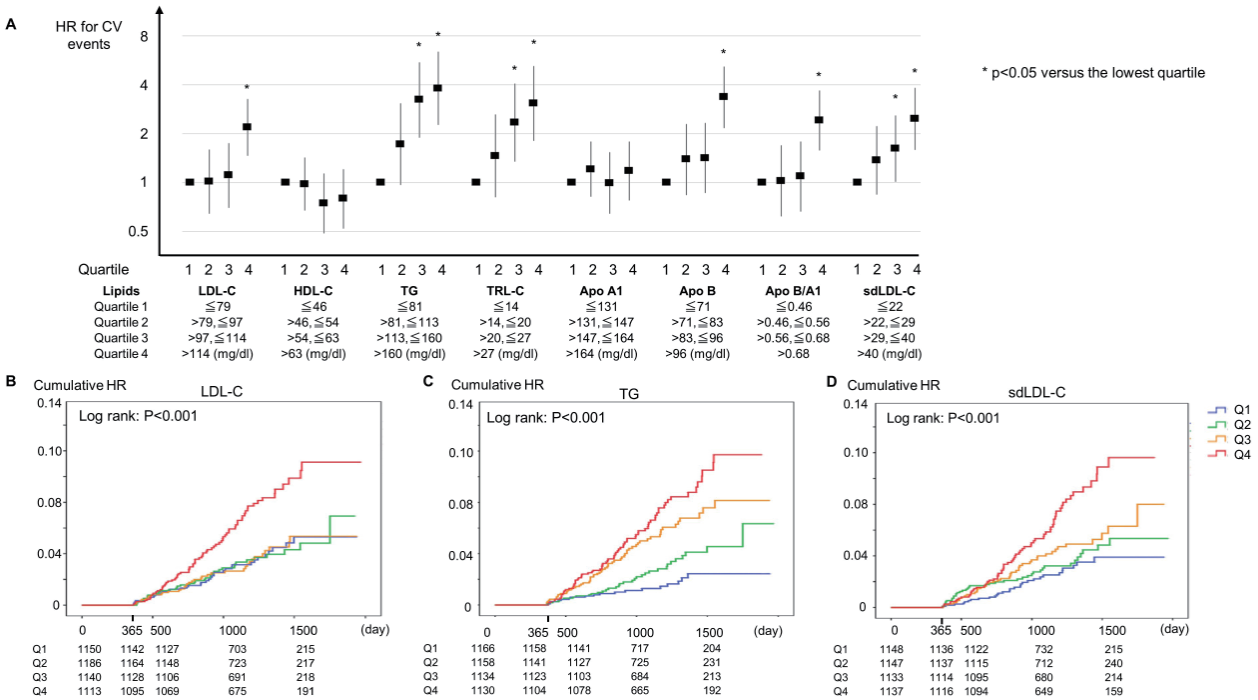

Fig. 4. Hazard ratios of lipid levels for CV events

Hazard ratios for CV events comparing among the quartiles according to levels of each lipid are shown (A). Cumulative HR of LDL-C (B), TG (C), and sdLDL-C (D) for CV events were analyzed by Kaplan-Meier analysis. HR: Hazard ratios, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride, TRL-C: triglyceride-rich lipoprotein cholesterol, ApoA1: apolipoprotein A1, Apo B: apolipoprotein B, sdLDL-C: small dense low-density lipoprotein cholesterol, CV: cardiovascular.

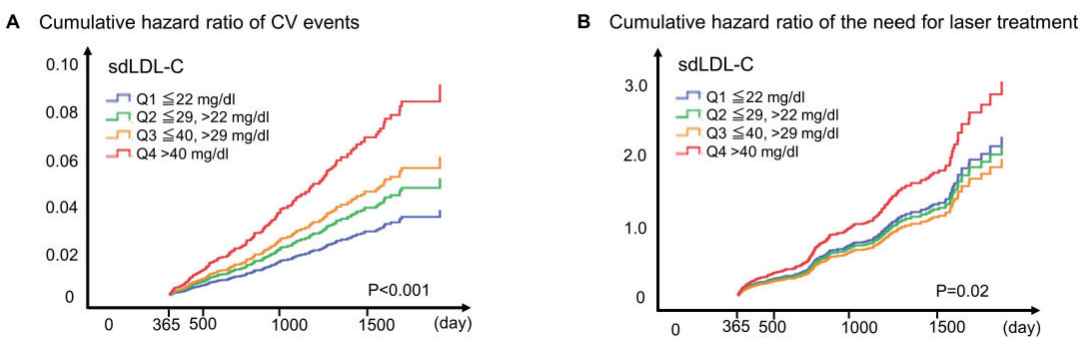

Fig. 5. Cumulative hazard ratios of lipid levels for CV events and the need for laser treatment for diabetic retinopathy

Cumulative hazard ratio of CV events and the need for laser treatment for diabetic retinopathy were analyzed by Cox hazard analysis after adjusting for covariates including age, gender, hypertension, and current or previous smoking at registration. LDL-C: low density lipoprotein cholesterol, sdLDL-C: small dense low-density lipoprotein cholesterol, CV: cardiovascular.

to levels of each lipid at one year after registration showed that TG, TRL-C, and sdLDL-C levels were positively related to the risk of CV events (TG: Q1, HR 1.0; Q2, HR 1.7; Q3, HR 3.2; Q4, HR 3.8, p for trend <0.01. TRL-C: Q1, HR 1.0; Q2, HR 1.5; Q3, HR 2.3; Q4, HR 3.1, p for trend <0.01. sdLDL-C: Q1, HR 1.0; Q2, HR 1.4; Q3, HR 1.6; Q4, HR 2.5, p for trend <0.01) **(Fig. 4A)**. The Kaplan-Meier analysis showed a significantly higher risk for CV events as the levels of LDL-C, TG, and sdLDL-C at one year after registration are higher (log rank, $p<0.001$ for all) **(Fig. 4B-D)** and Cox hazard analysis showed a significantly higher risk for CV events according to the higher levels of sdLDL-C at one year after registration ($p<0.001$) **(Fig. 5A)**.

In patients with TG levels in the lower half (≦

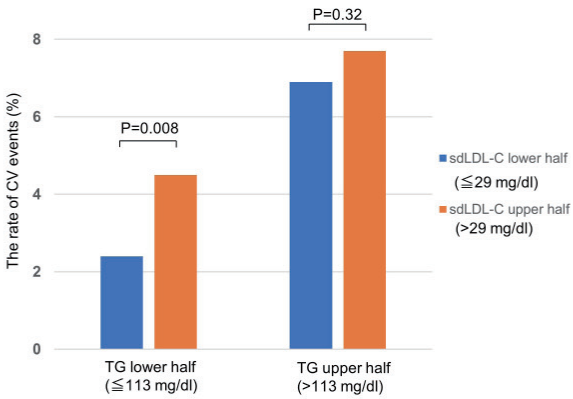

Fig. 6. The rate of CV events according to TG and sdLDL-C levels

The study population was divided into 2 subgroups, lower and upper halves of TG levels. The rates of CV events during the follow-up period were compared between the lower and upper halves of sdLDL-C levels in each subgroup by the chi squared test. CV: cardiovascular, TG: triglyceride, sdLDL-C: small dense low-density lipoprotein cholesterol.

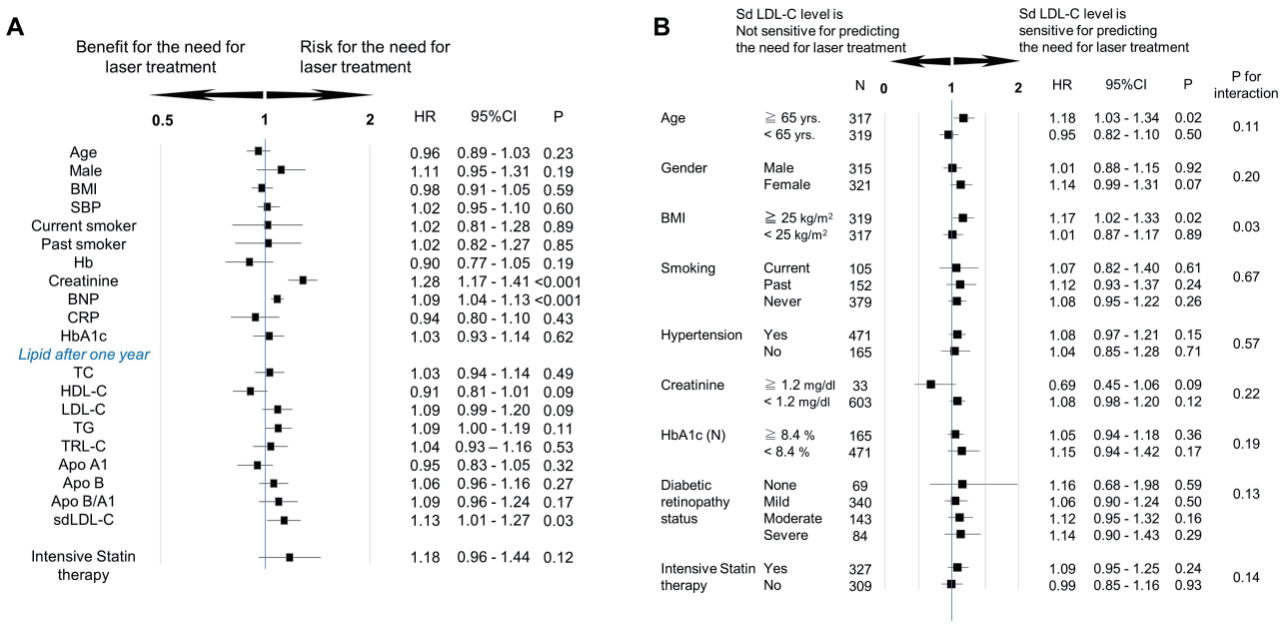

Fig. 7. Hazard ratios for the need for laser treatment

Hazard ratios were estimated per one standard deviation increase by a Cox proportional analysis on the risks for the need for laser treatment after adjusting for covariates including age, gender, hypertension, and current or previous smoking at registration (A). Subgroup analysis (B). Hb: hemoglobin, BNP: brain natriuretic peptide, CRP: C-reactive protein, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triglyceride, TRL-C: triglyceride-rich lipoprotein cholesterol, ApoA1: apolipoprotein A1, Apo B: apolipoprotein B, sdLDL-C: small dense low-density lipoprotein cholesterol.

113 mg/dl), those with sdLDL-C levels in the upper half (>29 mg/dl) had more CV events than those with sdLDL-C in the lower half (≦29 mg/dl)(CV events rate: 4.5 vs. 2.4%, respectively, $p<0.01$) **(Fig. 6)**, and the sdLDL-C (>29 mg/dl) was a significant marker for predicting CV events (HR 1.86, 95% CI 1.16-3.00, $p=0.01$). In patients with TG levels in the upper half (>113 mg/dl), sdLDL-C (>29 mg/dl) was not a significant marker for predicting CV events (HR 1.09, 95% CI 0.77-1.54, $p=0.63$).

Laser Treatment for Diabetic Retinopathy

In Cox proportional hazard analysis, higher levels of creatinine, BNP and sdLDL -C were risks of the need for laser treatment (HR 1.28, 95% CI 1.17-1.41; HR 1.09, 95% CI 1.04-1.13; HR 1.13, 95% CI 1.01-1.27, respectively) **(Fig. 7A)**. The Kaplan-Meier analysis and Cox hazard analysis showed a significantly

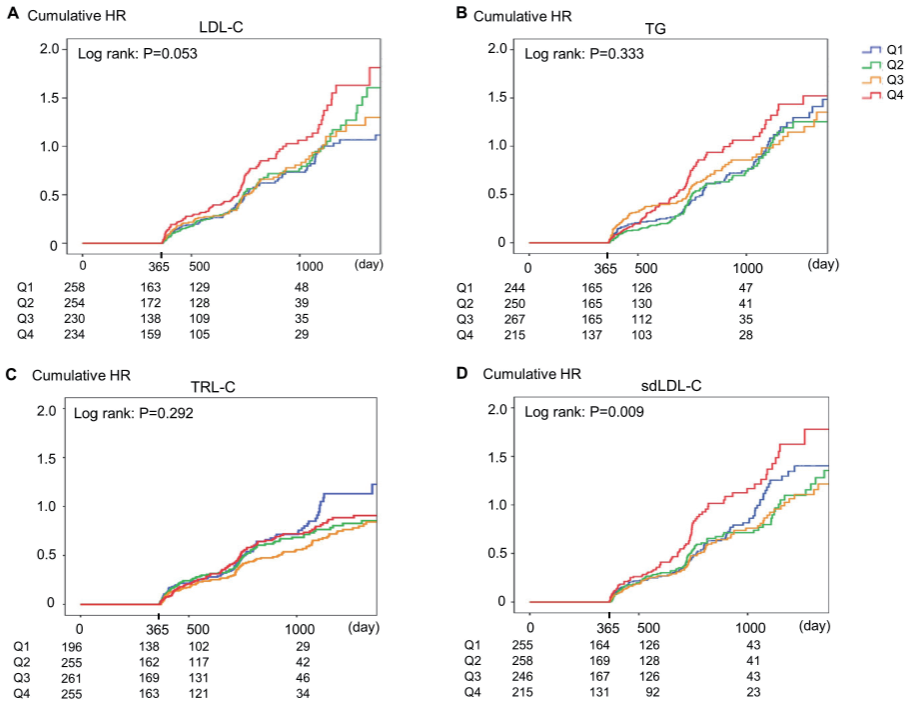

Fig. 8. Cumulative hazard ratios of lipid values for the need for laser treatment for diabetic retinopathy

Cumulative HR of LDL-C (A), TG (B), TRL-C (C) and sdLDL-C (D) for the need for laser treatment for diabetic retinopathy were analyzed by Kaplan-Meier analysis. HR: Hazard ratios, LDL-C: low density lipoprotein cholesterol, TG: triglyceride, TRL-C: triglyceride-rich lipoprotein cholesterol, sdLDL-C: small dense low-density lipoprotein cholesterol.

higher risk of laser treatment as the levels of sdLDL-C at one year after registration are higher (log rank, $p=0.009$, and $p=0.02$) **(Fig. 8D, Fig. 5B)**, and HR of the need for laser treatment in the highest quartile group (>40 mg/dl) of sdLDL-C as compared with the lowest quartile group (≦22 mg/dl) was 1.35 (95% CI 1.01-1.80) **(Fig. 5B)**. No other lipid except for sdLDL-C was significantly associated with the need for laser treatment **(Fig. 7A, Fig. 8A-C)**. Particular in the sub-populations of older age (≧65 years) and obesity (BMI ≧25 kg/m^2), sdLDL-C was significantly associated with the need for laser treatment for diabetic retinopathy (HR 1.18, 95% CI 1.03-1.34; HR 1.17, 95% CI 1.02-1.33, respectively) **(Fig. 7B)**.

There was no significant correlation between sdLDL-C and urine albumin or eGFR. After adjustment for the existence of nephropathy, the sdLDL-C level remained to be a risk of the need for laser treatment (HR 1.12, 95% CI 1.01-1.23), suggesting that nephropathy and the sdLDL-C level exert as independent risks of the need for laser treatment.

Discussion

This study clarified that TC, LDL-C, TG, TRL-C, ApoB and sdLDL-C were lipid markers to predict CV events, depending on their concentrations, and sdLDL-C was the only lipid marker to predict the need for laser treatment in patients with hypercholesterolemia and diabetic retinopathy receiving statin therapy. To our best knowledge, this is the largest study so far to examine prospectively and comprehensively which lipid marker could predict CV events and the need for laser treatment in diabetic patients receiving statin therapy.

Correlations among Lipids

SdLDL is a particle with a specific density of 1.044-1.063 g/ml and a size of 19.0-20.5 nm[9, 18]. As compared with lbLDL, sdLDL particles have lower affinity to the LDL receptor, and longer retention time in the circulation. In addition, sdLDL easily adheres to proteoglycans in the vessel wall, and easily penetrates the subendothelium of blood vessels **(Fig. 9)**[19]. Compared with other LDL particles, sdLDL particles are more susceptible to oxidative modification and more readily engulfed by macrophages leading to greater levels of oxidized-LDL particles as well as vascular damage **(Fig. 9)**. Consistently, elevated sdLDL-C values correspond to development of unstable and rupture-prone plaque

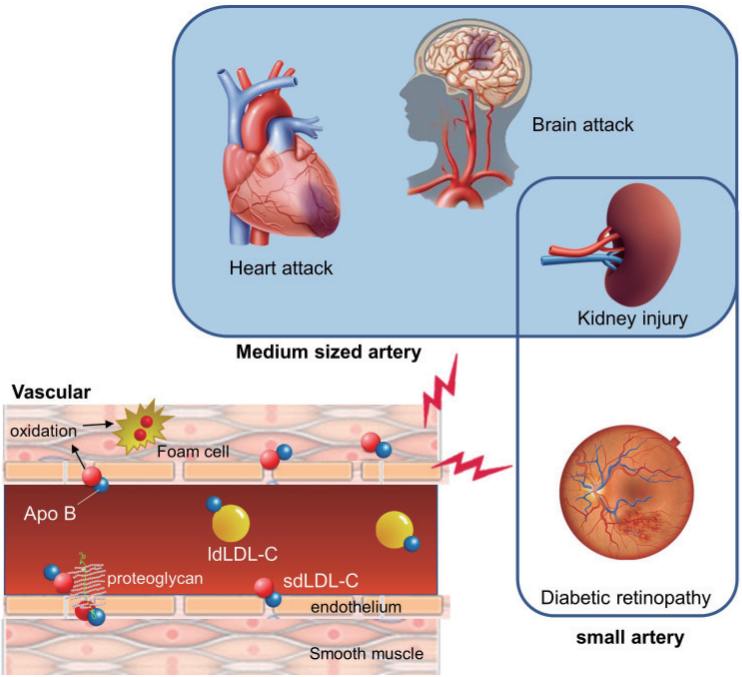

Fig. 9. The role of small dense low-density lipoprotein cholesterol

SdLDL-C easily adheres to proteoglycans in the vessel wall and easily penetrates the subendothelium of blood vessels, leading to greater levels of oxidized-LDL particles as well as vascular damage in both medium- and small-sized arteries. Apo B: apolipoprotein B, lbLDL-C: large buoyant low-density lipoprotein cholesterol, sdLDL-C: small dense low-density lipoprotein cholesterol.

phenotypes relevant to CV events.

Overproduction of Apo B-containing particles by the liver leads to the excessive biosynthesis of the heavier subfraction of LDL. ApoB reflects LDL particle number because all lipoprotein particles contain one molecule of ApoB[18], and sdLDL is the most numerous particle among LDL particles[20]. Accordingly, there should be the close correlation between sdLDL-C and ApoB levels. A high correlation between sdLDL-C and ApoB values in small study populations was reported[21, 22]. Consistent with these findings, in the present study (n=4589), sdLDL-C level was strongly correlated with ApoB level (r=0.83, $p<0.001$).

Lipid Markers for Predicting CV Events

In the population from the EMPATHY study, we explored the association between lipid values and the future onset of CV events. TC, LDL-C, TG, TRL-C, ApoB and sdLDL-C at one year after registration were significantly associated with the risk of CV events. Usefulness of measuring TG for predicting the CV events was previously reported in many studies including the EMPATHY study[23, 24]. Moreover, it was shown that non-fasting TG could be considered to be a substitute for fasting TG as a risk-stratification for future CV events[23]. The Copenhagen City Heart Study showed that the non-fasting TG level was correlated with the risks of ischemic heart diseases and stroke[25]. The European Atherosclerosis Society and the European Society of Laboratory Medicine jointly published the statement that postprandial samples may be used routinely to assess lipid profiles, and suggested their usefulness and convenience in daily clinical practice[26]. Despite these arguments, the clinical usefulness of the measurement of non-fasting TG seems still controversial[27] and we prefer the biomarker which is less susceptible to dietary influence. In this regard, sdLDL-C, which is much less susceptible to dietary influences[28], might be a better lipid marker measured in daily clinical practice as compared with TGs.

TRL-C, which contains very low-density lipoproteins, intermediate-density lipoproteins, chylomicrons, and their remnants, has been recently paid attention as the substrate causally related to development of the atherosclerotic plaque[5]. In our study, TRL-C was reduced by statin therapy and exerted as the significant marker for predicting CV events, but not for predicting the need of laser treatment.

In Framingham Heart Study, women with CAD had higher sdLDL-C values as compared with controls[29]. Sd-LDL-C values were most closely

correlated with carotid artery intima-media thickness among lipid parameters tested[30]. In the recent epidemiological studies, sdLDL-C values are associated with increased risk of CV events[8, 31] and incident myocardial infarction[32]. Consistently, in our large-scale prospective study of diabetic patients, we could clearly demonstrate that sdLDL-C is the good predictor of incident CV events among lipid parameters.

Lipid Markers for Predicting the Need for Laser Treatment

The association between lipids and risk for worsening diabetic retinopathy or the need for laser treatment is controversial[33]. In 1998, Davis *et al.* demonstrated that elevated TG, but not TC, HDL-C, or LDL-C, was significantly associated with high-risk proliferative diabetic retinopathy in diabetic patients[34]. The recent Mendelian randomization study in Copenhagen cohorts found that elevated LDL-C value was not associated with risk of retinopathy and showed that LDL-C had no causal relationship with microvascular diseases such as retinopathy[35]. The FIELD study[12] showed HDL-C, LDL-C, and TG levels were not the significant markers for laser treatment. However, fibrate[12] and statin[36] intake was shown to reduce the need for laser treatment in randomized controlled trial and meta-analysis. Taken together, there might be a weak or borderline association between LDL-C and the risk for diabetic retinopathy. LDL-C complex, which consists of lbLDL-C, sdLDL-C, and other subfractions, could not serve as a robust marker for worsening diabetic retinopathy. Theoretically, sdLDL-C could easily flow inside the retinal artery walls, augmenting the oxidative stress, followed by the advanced proliferative change in retina. As far as we know, this is the first study to demonstrate that elevated sdLDL-C level is significantly associated with increased risk of the need for laser treatment for diabetic retinopathy. Hereafter, further clinical studies are warranted to clarify whether sdLDL-C lowering therapy could have a suppressive effect on the progression of diabetic retinopathy as well as CV diseases.

Clinical Implications for the Measurement of sdLDL-C Values

Considering that higher TG values are associated with smaller LDL particle sizes, TG values should be associated with sdLDL-C values[22]. However, in the present study, a high correlation between TG and sdLDL-C was not observed ($r=0.58$). Rather, even in patients with TG levels in the lower half, sdLDL-C was a significant marker for predicting CV events. Given that, sdLDL-C was a sensitive marker to predict future CV events, independent of TG level. In addition, sdLDL-C was found to be the only lipid marker to predict the need for laser treatment. Although ApoB level was strongly correlated with sdLDL-C level ($r=0.83$, $p<0.001$), ApoB level was not associated with the need for laser treatment in our study. ApoB is a component of a variety of lipoproteins (eg. lb LDL-C[21]) whose pathogenic properties in the vascular wall might vary depending on vascular size, which could partly explain the reason why ApoB itself could not predict the prognosis of retinopathy. Taken together, sdLDL-C levels should be evaluated as a predictive maker for future CV events and the need for laser treatment in patients at risk for CV events and diabetic retinopathy.

Limitations

This study was a sub-analysis of the EMPATHY study[13]. This large-scale PROBE study design could minimize the effect of confounding factors. However, study participants were limited to patients with hypercholesterolemia and diabetic retinopathy receiving statin therapy; therefore, our findings cannot be simply generalized to the other populations. Blood sampling was performed at each clinic in the EMPATHY study[13], and we cannot deny the possibility of postprandial changes in lipid profile, especially TG. Finally, after the exclusion of patients with history of treatment for retinopathy including laser treatment or without routine ophthalmologic check-up until 3 years after registration, it was the remaining 976 patients that we used for the analysis on the need for laser treatment. Also, in this study, the need for laser treatment for diabetic retinopathy was decided by the ophthalmologists in each institution, which could have biased the ophthalmologic endpoint. Further studies with larger sample size are required to confirm the significance of sd LDL-C levels in this field.

Conclusions

SdLDL-C can serve as a sensitive target marker to predict both the occurrence of cardiovascular events and the need for laser treatment in patients with hypercholesterolemia and diabetic retinopathy. SdLDL-C levels should be evaluated in patients at risk for cardiovascular events as well as diabetic retinopathy.

Data Availability

The data that support the findings of this study are available from the EMPATHY data center, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are available from the authors upon reasonable request and with permission of the EMPATHY Investigators.

Disclosures / Conflicts of Interest

H.I. reports grants and personal fees from Shionogi & Co., Ltd during the course of the study, and grants and personal fees from Takeda Pharmaceutical Co. Ltd, Nippon Boehringer Ingelheim Co., Ltd, Daiichi Sankyo Co., Ltd, MSD K.K., Mitsubishi Tanabe Pharma Corporation, Shionogi & Co., Ltd and Taisho Toyama Pharmaceutical Co., Ltd, as well as grants from Sumitomo Dainippon Pharma Co., Ltd, Astellas Pharma Inc., Kyowa Hakko Kirin Co., Ltd, Teijin Pharma Ltd, Mochida Pharmaceutical Co., Ltd, Ono Pharmaceutical Co., Ltd, Chugai Pharmaceutical Co., Ltd, Eli Lilly Japan K.K. and personal fees from Nipro Corporation and SBI Pharmaceuticals Co., Ltd outside the submitted work. I.K. reports personal fees from Shionogi & Co., Ltd during the course of the study, grants and personal fees from Takeda Pharmaceutical Co. Ltd, Nippon Boehringer Ingelheim Co., Ltd, Astellas Pharma Inc., Daiichi Sankyo Co., Ltd, and Otsuka Pharmaceutical Co., Ltd and grants from MSD K.K., Shionogi & Co., Ltd, GlaxoSmithKline K.K., Sanofi K.K., Genzyme Japan K.K., Sumitomo Dainippon Pharma Co., Ltd, Mitsubishi Tanabe Pharma Corporation and Bristol - Myers Squibb Co. outside the submitted work.

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Authors' Contributions

AN and TS contributed to the study design. AN and TK contributed to the statistical plan. AN and TK analyzed the data and prepared the study results. HM contributed to the interpretation of the findings. NT, SK, HI, and IK supervised the study project. AN led the drafting of the manuscript, and all co-authors contributed to revising of the manuscript and approved the final version.

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