

# Effects of PCSK9 Inhibitor on Favorable Limb Outcomes in Patients with Chronic Limb-Threatening Ischemia

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**Aim:** The aim of this study was to examine the effects of evolocumab on favorable limb events in patients with chronic limb-threatening ischemia (CLTI).

**Methods:** A single-center, prospective observational study was performed on 30 patients with CLTI. The subjects were divided into 2 groups based on evolocumab administration: evolocumab-treated (E) group ( $n=14$ ) and evolocumab non-treated (non-E) group ( $n=16$ ). The primary outcome was 12-month freedom from major amputation. The secondary outcomes were 12-month amputation-free survival (AFS), overall survival (OS), and wound-free limb salvage. The mean follow-up period was  $18 \pm 11$  months.

**Results:** No significant difference was detected between the two groups for the 12-month freedom from major amputation (log-rank  $p=0.15$ ), while the 12-month AFS rate was significantly higher in the E group than that in the non-E group (log-rank  $p=0.02$ ). The 12-month OS rate in the E group was shown a tendency for improvement, as compared with that in the non-E group (log-rank  $p=0.056$ ). Evolocumab administration was not associated with a significant change in freedom from major amputation (HR, 0.23, 95% CI, 0.03-2.07,  $p=0.19$ ). However, evolocumab administration was related to a tendency for improvement of AFS and OS (HR, 0.13, 95% CI, 0.02-1.06,  $p=0.056$ ; HR, 0.16, 95% CI, 0.02-1.37,  $p=0.09$ , respectively). Moreover, The E group had a higher proportion of wound-free limb salvage at 12 months (92% vs. 42%,  $p=0.03$ ).

**Conclusion:** Evolocumab administration was associated with a better AFS outcome in patients with CLTI. Long-term administration of evolocumab over 12 months contributed to improving proportion of wound-free limb salvage.

**Key words:** Amputation, Chronic limb-threatening ischemia, Evolocumab, Peripheral artery disease, Proprotein convertase subtilisin/kexin type 9 inhibitor

## Introduction

Peripheral artery disease (PAD) is a major cardiovascular disease; the number of patients with PAD is estimated to exceed 200 million worldwide<sup>1</sup>. PAD is a known risk factor associated with increased mortality from other types of atherosclerotic cardiovascular disease<sup>2</sup>. Hyperlipidemia also has been reported to be a cardiovascular risk factor, and thus several guidelines for management of PAD strongly recommend lipid-

lowering treatment with statins<sup>3, 4</sup>.

Evolocumab, a proprotein convertase subtilisin/kexin type 9 inhibitor (PCSK9-I), has been shown to reduce low-density lipoprotein (LDL) cholesterol and the frequency of cardiovascular events in patients with atherosclerotic cardiovascular disease, including PAD<sup>5</sup>. Furthermore, evolocumab has been shown (in the FOURIER clinical trial) to prevent the development of major adverse limb events in patients with PAD<sup>6</sup>.

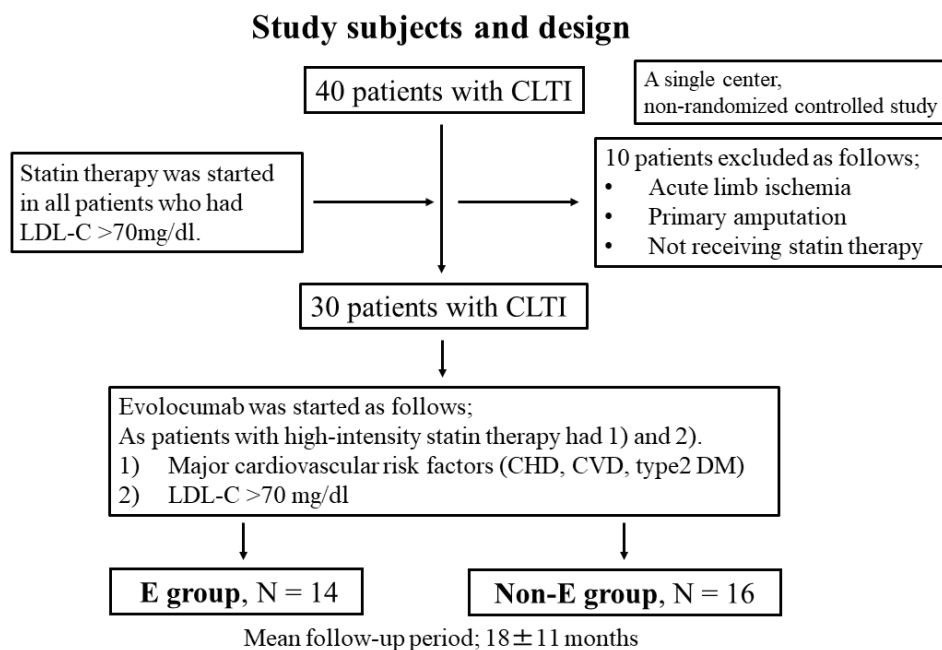
All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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**Fig. 1.** Study flowchart

CHD, coronary heart disease; CLTI, chronic limb-threatening ischemia; CVD, cerebrovascular disease; DM, diabetes mellitus; E group, evolocumab-treated group; LDL-C, low-density lipoprotein cholesterol; non-E group, evolocumab non-treated group

Chronic limb-threatening ischemia (CLTI) is the most serious version of PAD and is characterized by ischemic rest pain and tissue loss. It has long been recognized that treatment for patients with CLTI is challenging, because of comorbidities and extremely poor prognosis for life and limb<sup>7, 8</sup>. Previous studies have shown that lipid-lowering therapy with statins is associated with better limb outcomes in patients with PAD and CLTI<sup>9, 10</sup>. However, there have been (to our knowledge) no studies investigating the effects of aggressive lipid-lowering therapy with PCSK9-I on limb outcomes in patients with CLTI. Therefore, the goal of this study was to evaluate the impacts of evolocumab on favorable limb outcomes and lipid profiles in patients with CLTI.

## Methods

### Study Subjects and Design

We performed a prospective, non-randomized controlled study at a single center. Patients were recruited from the Department of Cardiovascular Medicine of the University of Fukui Hospital. This study included 40 consecutive patients admitted with CLTI between November 2016 to May 2019. The mean  $\pm$  SD follow-up period was  $18 \pm 11$  months. CLTI was defined by the presence of one of the following criteria: (1) ischemic foot rest pain with ankle-brachial index (ABI)  $< 0.4$ , ankle pressure  $< 50$  mm

Hg, toe pressure  $< 30$  mm Hg, or skin perfusion pressure (SPP)  $< 30$  mm Hg; or (2) foot ulcer/gangrene with significant PAD, such as ABI  $< 0.8$ , ankle pressure  $< 100$  mm Hg, toe pressure  $< 60$  mm Hg, or SPP  $< 60$  mm Hg<sup>4</sup>. Lipid-lowering therapy with statins was considered in all patients who had an LDL cholesterol level of 70 mg/dL or higher. A total of 10 patients were excluded due to the presence of one of the following exclusion criteria: acute limb ischemia, receiving primary amputation, or not receiving statin therapy; thus, a total of 30 patients was enrolled. Patients were considered eligible for treatment with the PCSK9-I, evolocumab if the individual receiving high-intensity statin therapy had one or more major cardiovascular risk factors and an LDL cholesterol level of 70 mg/dL or higher. High-intensity statin therapy was defined as receiving atorvastatin at a dose of 20 mg daily or its equivalent<sup>5</sup>. Major cardiovascular risk factors included coronary artery disease, non-cardiogenic cerebral infarction, chronic kidney disease, and type 2 diabetes. Accordingly, 14 patients were treated with evolocumab, administered as either 140 mg every 2 weeks or 420 mg every month. The patients who were included in this study were divided into two groups based on evolocumab administration: evolocumab-treated (E) group ( $69.4 \pm 11.7$  years,  $n=14$ ) and evolocumab non-treated (non-E) group ( $74.2 \pm 8.0$  years,  $n=16$ ) (Fig. 1). Revascularization, namely surgical and endovascular treatment, was con-

sidered in all patients. The treatment strategy for patients with CLTI was determined by a team of vascular specialists working at University of Fukui hospital, a team that included vascular surgeons, interventional cardiologists, and plastic surgeons.

After enrollment, patient characteristics and lesion characteristics were assessed. The geriatric nutritional risk index (GNRI) at admission was calculated using the following formula:  $GNRI = 14.89 \times \text{serum albumin (g/dL)} + 41.7 \times (\text{present body weight/ideal body weight})$ . In cases where the present body weight exceeded the ideal body weight, the ratio of present body weight to ideal body weight was assigned as a value of 1<sup>11</sup>. The severity of lesions was determined using the Trans-Atlantic Inter-Society Consensus (TASC) II classification system for aortoiliac and femoropopliteal lesions<sup>12</sup> and TASC classification system for infrapopliteal lesions<sup>13</sup>. The severity of CLTI was assessed using the Rutherford classification system and the Wound, Ischemia, and foot infection (WIFI) classification system<sup>14</sup>. Lipid profiles, including LDL cholesterol, triglyceride (TG), high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, malondialdehyde-modified LDL (MDA-LDL), and lipoprotein a (Lp(a)), were evaluated at admission and at 1, 3, 6, and 12 months. ABI was also evaluated after procedure and at 12 months. In addition, changes in ABI levels from post-procedure to 12 months ( $\Delta$ ABI) were measured. Regarding the safety outcome, adverse events, including those that were muscle-related, cataracts, injection site, allergic, and neurocognitive, were assessed in the follow-up period<sup>5</sup>.

This investigation conformed to the principles outlined in the 1975 Declaration of Helsinki and later amendments. Ethics committee approval and informed consent from all patients were obtained. This study was registered in the Universal Hospital Medical Information Network Clinical Trials Registry as UMIN 000032844.

### Outcome Measures

The primary outcome was 12-month freedom from major amputation. The definition of major amputation was any above-ankle amputation<sup>10</sup>. The secondary outcomes were assessed as 12-month amputation-free survival (AFS), 12-month overall survival (OS), and the wound-free limb salvage rate. AFS was defined as freedom from the composite of major amputation and all-cause mortality; OS was defined as freedom from all-cause mortality; the wound-free limb salvage rate was defined as freedom from the composite of major amputation and unhealed wounds<sup>15</sup>. The proportion of wound-free limb salvage was analyzed in patients with Rutherford classifications of 5 and 6

(E group,  $n=12$ , vs. non-E group,  $n=12$ ). Specifically, the proportion of wound-free limb salvage was compared between the two groups at admission and at 1, 3, 6, and 12 months.

### Measurements of Blood Samples

Following overnight fasting, samples of whole blood were obtained from the forearm or femoral vein and held on ice. Plasma collected with EDTA-2Na as anti-coagulant and serum samples were separated by centrifugation within 30 min, frozen, and stored at  $-80^{\circ}\text{C}$  until use. Samples used for analysis had not been thawed previously. On the day of measurement, the samples were thawed at room temperature and analyzed immediately. MDA-LDL levels were determined using a commercially available sandwich enzyme linked immunosorbent assay (Sekisui Medical Co, Tokyo, Japan). The remaining blood parameters were determined by standard methods. Levels of serum creatinine were assessed in patients without hemodialysis.

### Statistical Analysis

Data are presented as frequencies and percentages for categorical variables, medians for non-normally distributed parameters, and mean  $\pm$  SD for continuous variables. Comparisons of continuous measurements between two or more groups were performed via a two-tailed unpaired Student's *t*-test or the Mann-Whitney test. Categorical variables were compared by the chi-squared test or Fisher's exact test. Inter-group differences in lipid parameters were assessed via a repeated-measures linear mixed-effects model using all measurements from baseline through the end of the study. Event-free survival curves were constructed using the Kaplan–Meier method and were compared using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were determined by the Cox proportional hazards model. A Cox proportional hazards regression model was used to examine the effect of other variables on event-free survival. Variables included in the model were age, sex, use of PCSK9-I, and WIFI classification. A multivariate logistic regression analysis was performed to identify the predictors of wound-free limb salvage at 12 months. A value of  $p < 0.05$  was considered significant. All statistical analyses were conducted using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, EZR is a modified version of R commander programmed to incorporate statistical functions used frequently in biostatistics<sup>16</sup>.

## Results

### Baseline Characteristics

**Table 1** shows baseline characteristics of the patients enrolled in this study. The patient's characteristics matched between the E group and the non-E group; notably, there were no significant differences in non-ambulatory status and GNRI between the two groups. Additionally, medications and laboratory data on admission corresponded between the E group and the non-E group. **Table 1** also presents lower limb characteristics of the enrolled patients. No significant differences were observed in the history of revascularization, the disease severity (as assessed by Rutherford classification system), or the severity of CLTI (as assessed by WIfI classification system) between patients in the E and non-E groups. There were no significant differences in post-procedure ABI and  $\Delta$  ABI at 12 months between the two groups. No patients discontinued evolocumab as a result of adverse effects.

Lesion characteristics are listed in **Table 2**. There were no significant differences in the severity of aortoiliac lesions and femoropopliteal lesions between the two groups. The proportion of type C and D lesions in infrapopliteal lesions did not differ between the patients in the two groups. Twenty-eight of 30 patients received endovascular treatment and one patient received surgical reconstruction. Procedural success was obtained in all patients. One patient in the non-E group received conservative management and interventional non-revascularization treatments including pharmacotherapy, wound care, hyperbaric oxygen therapy and spinal cord stimulation.

### Differences in Lipid Profiles

**Fig. 2** presents differences in LDL cholesterol, non-HDL cholesterol, and MDA-LDL between the two groups during the follow-up period. Over time, the patients in the E group exhibited attenuation in the levels of LDL cholesterol and non-HDL cholesterol compared to those in the non-E group. The reduction in the MDA-LDL level was maintained through 6 months. However, there was no significant difference between the E and non-E groups in the MDA-LDL levels at 12 months ( $p=0.26$ ). In addition, the changes in other lipid parameters, such as TG, HDL cholesterol, and Lp(a), did not differ between the patients in the two groups at any of the tested time points.

### Outcome Measures

**Fig. 3** shows the Kaplan–Meier curves of 12-month freedom from major amputation and for

12-month AFS and OS in the two groups. No significant difference was detected between the two groups for the 12-month freedom from major amputation (log-rank  $p=0.15$ ). On the other hand, the 12-month AFS rate was significantly higher in the E group than that in the non-E group (log-rank  $p=0.02$ ). Additionally, the 12-month OS rate in the E group was shown a tendency for improvement, as compared with that in the non-E group (log-rank  $p=0.056$ ). Evolocumab administration was not associated with a significant change in freedom from major amputation (HR, 0.23; 95% CI, 0.03-2.07;  $p=0.19$ ). In addition, evolocumab administration was not related to a significant change in the 12-month AFS and OS. However, there was a tendency for improvement of AFS and OS (AFS: HR, 0.13, 95% CI, 0.02-1.06,  $p=0.056$ ; OS: HR, 0.16, 95% CI, 0.02-1.37,  $p=0.09$ ).

For time points up to 12 months, there was no significant difference between the two groups in the wound-free limb salvage rate. However, significant differences were observed in the wound-free limb salvage rate between the two groups at 12 months (E group [92%] vs. non-E group [42%];  $p=0.03$ ) (**Fig. 4**). In multivariate analysis, PCSK9-I and WIfI classification were significantly related to the 12-month freedom from major amputation, the 12-month AFS, and the 12-month OS. PCSK9-I was an independent factor associated with the wound-free limb salvage at 12 months (odds ratio, 56.7; 95% CI, 1.71-1880;  $p=0.024$ ) (**Supplemental Table 1 and 2**).

## Discussion

This study investigated the effects of evolocumab on the lipid profile and favorable limb outcomes in patients with CLTI. The main findings were as follows: first, evolocumab significantly reduced the levels of lipid parameters (e.g., LDL cholesterol, non-HDL cholesterol, and MDA-LDL) in patients with CLTI over time; second, the patients administered evolocumab had significantly higher 12-month AFS and wound-free limb salvage rates than patients not treated with this PCSK9-I; and third, these differences in outcome exhibited significance at 12 months.

### Efficacy of Evolocumab on Lipid Profiles in Patients with CLTI

The FOURIER trial reported that evolocumab significantly reduced levels of LDL cholesterol, non-HDL cholesterol, TG, and Lp(a) in patients with atherosclerotic cardiovascular disease<sup>5</sup>. Moreover, treatment with evolocumab attenuated LDL cholesterol values in symptomatic PAD patients to very low levels, and thus reduced the risk of major adverse limb

**Table 1.** Patient characteristics

	E group	Non-E group	<i>p</i> -value		E group	Non-E group	<i>p</i> -value
<i>n</i>	14	16		<b>Lower limb characteristics</b>			
Age, years	69.4 ± 11.7	74.2 ± 8.0	0.19	History of revascularization			
Male, <i>n</i> (%)	11 (78.8)	11 (68.8)	0.69	Endovascular treatment, <i>n</i> (%)	7 (50)	4 (25)	0.3
Body weight, kg	52.8 ± 7.8	19.5 ± 8.8	0.29	Surgery, <i>n</i> (%)	2 (14.3)	1 (6.3)	0.59
BMI, kg/m <sup>2</sup>	20.1 ± 2.5	19.6 ± 2.5	0.55	Rutherford classification, <i>n</i> (%)			
ADL at admission				R4	3 (21.4)	4 (25)	1
Ambulatory, <i>n</i> (%)	10 (71.4)	10 (62.5)	0.71	R5	8 (57.2)	8 (50)	
In wheelchair, <i>n</i> (%)	4 (28.6)	6 (37.5)		R6	3 (21.4)	4 (25)	
Geriatric nutritional risk index	85.8 ± 9.3	83.7 ± 14.5	0.64	Wifl classification, <i>n</i> (%)			
<b>Risk factors</b>				1	3 (21.4)	0 (0)	0.06
Hypertension, <i>n</i> (%)	13 (92.9)	13 (81.3)	1	2	2 (14.3)	6 (37.5)	
Hyperlipidemia, <i>n</i> (%)	14 (100)	16 (100)	1	3	3 (21.4)	7 (43.8)	
Diabetes, <i>n</i> (%)	10 (71.4)	10 (62.5)	0.71	4	6 (42.9)	3 (18.7)	
Hemodialysis, <i>n</i> (%)	6 (42.9)	5 (31.3)	0.78	Wound grades, <i>n</i> (%)			0.56
Smoking				0	3 (21.4)	4 (25)	
Previous, <i>n</i> (%)	7 (50)	8 (50)	1	1	4 (28.6)	5 (31.3)	
Current, <i>n</i> (%)	3 (21.4)	3 (18.8)	1	2	3 (21.4)	5 (31.3)	
Coronary artery disease, <i>n</i> (%)	13 (92.9)	10 (62.5)	0.09	3	4 (28.6)	1 (6.3)	
Cerebrovascular disease, <i>n</i> (%)	2 (14.3)	1 (6.3)	0.59	Ischemia grades, <i>n</i> (%)			0.32
Heart failure, <i>n</i> (%)	7 (50)	2 (12.5)	0.05	0	1 (7.1)	0 (0)	
<b>Medications</b>				1	6 (42.9)	3 (18.8)	
Antiplatelet therapy, <i>n</i> (%)	14 (100)	16 (100)	1	2	5 (35.7)	8 (50)	
Aspirin, <i>n</i> (%)	12 (85.7)	11 (68.8)	0.4	3	2 (14.3)	5 (31.2)	
Thienopyridine, <i>n</i> (%)	13 (92.9)	14 (87.5)	1	Foot Infection grades, <i>n</i> (%)			0.14
Cilostazol, <i>n</i> (%)	1 (7.1)	4 (25)	0.34	0	4 (28.6)	10 (62.5)	
Antithrombotic agents, <i>n</i> (%)	1 (7.1)	1 (6.3)	1	1	5 (35.7)	5 (31.2)	
ACE-I/ARB, <i>n</i> (%)	10 (71.4)	8 (50)	0.41	2	2 (14.3)	0 (0)	
Statin, <i>n</i> (%)	14 (100)	16 (100)	1	3	3 (21.4)	1 (6.3)	
Oral hypoglycemic agent, <i>n</i> (%)	9 (64.3)	7 (43.8)	0.45	ABI			
Insulin therapy, <i>n</i> (%)	3 (21.4)	4 (25)	1	Pre-procedure	0.55 ± 0.29	0.45 ± 0.24	0.31
<b>Laboratory data</b>				Post-procedure	0.74 ± 0.04	0.76 ± 0.04	0.88
Hemoglobin, g/dL	10.8 ± 1.4	11.3 ± 1.8	0.42	ΔABI	0.02 ± 0.24	0.08 ± 0.06	0.68
Total protein, g/dL	6.7 ± 0.9	6.4 ± 0.7	0.29	Skin perfusion pressure, mm Hg			
Albumin, g/dL	3.2 ± 0.6	3.1 ± 0.7	0.72	Dorsal surface	26.7 ± 21.8	14.8 ± 16.9	0.2
Serum creatinine, mg/dL	0.99 ± 0.5	0.9 ± 0.2	0.61	Plantar surface	19.1 ± 16.5	13.7 ± 15.3	0.48
CRP, mg/dL	2.6 ± 3.8	3.3 ± 5.6	0.83				
HbA1c, %	6.7 ± 1.2	6.6 ± 2.2	0.9				
1, 5-anhydroglucitol, µg/mL	8.0 ± 8.1	10.1 ± 9.8	0.56				
Fasting blood glucose, mg/dL	146.4 ± 72.2	147.7 ± 93.4	0.97				
Total cholesterol, mg/dL	157.1 ± 28.4	152.4 ± 52.2	0.77				
Triglyceride, mg/dL	105.7 ± 54.5	104.8 ± 58.8	0.97				
LDL-C, mg/dL	89.2 ± 19.5	82.7 ± 37.5	0.55				
HDL-C, mg/dL	45.0 ± 9.7	43.6 ± 12.2	0.72				
EPA, µg/mL	46.1 ± 27.7	66.7 ± 53.8	0.21				
DHA, µg/mL	109.1 ± 41.7	101.4 ± 48.7	0.66				
AA, µg/mL	178.0 ± 46.7	166.9 ± 47.9	0.54				
EPA/AA ratio	0.27 ± 0.16	0.51 ± 0.52	0.18				
MDA-LDL, U/L	77.0 ± 20.9	85.5 ± 36.5	0.46				
Lp(a), mg/dL	20.1 ± 11.8	23.9 ± 24.5	0.62				

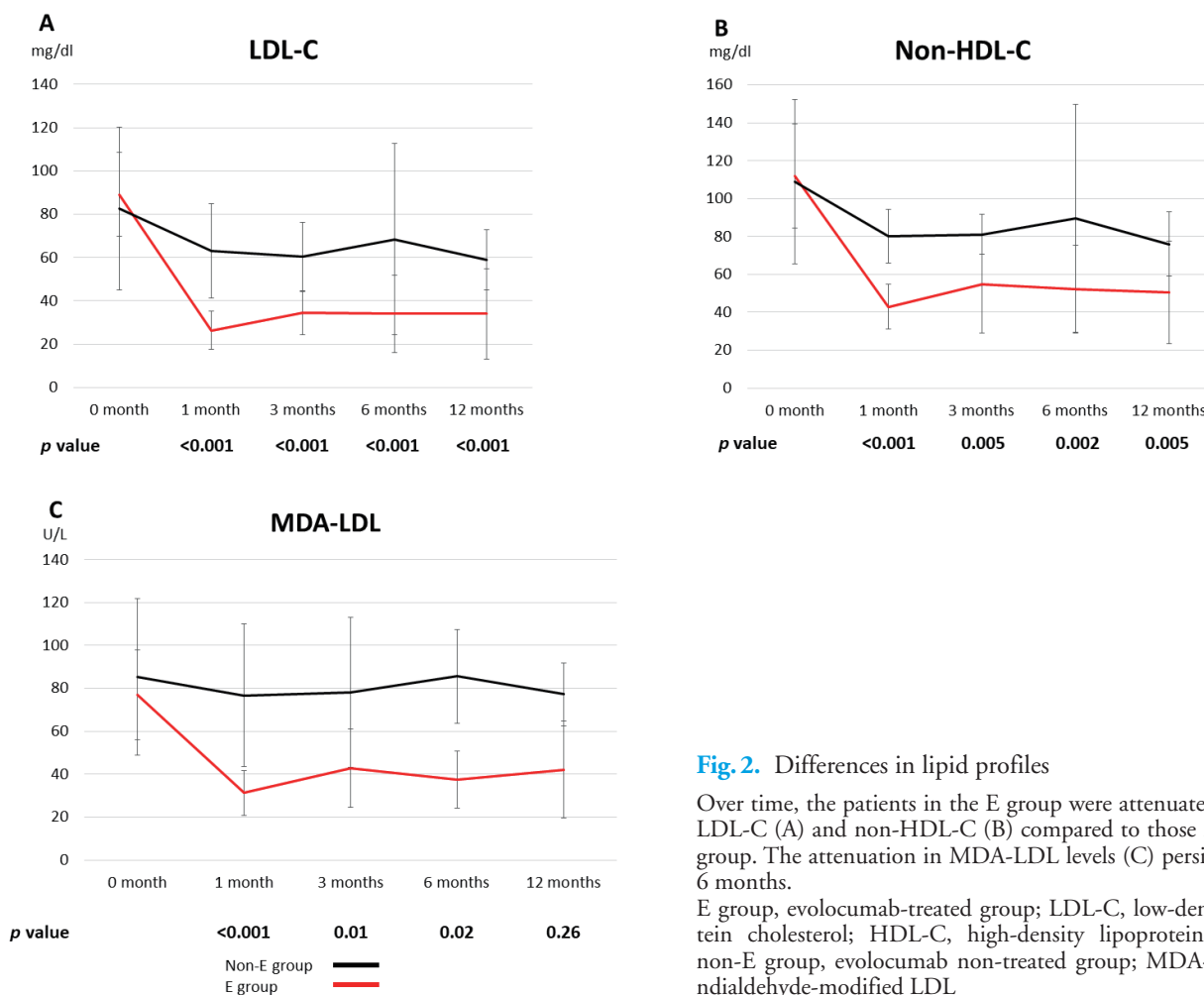
Values are presented as mean ± standard deviation or percentage and frequency.

BMI = body mass index; ADL = activities of daily living; ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CRP = C-reactive protein; HbA1c = hemoglobin A1c; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; MDA-LDL = malondialdehyde-modified LDL; Lp(a) = lipoprotein a; Wifl = the Wound, Ischemia and foot infection; ABI = ankle-brachial index; ΔABI = changes in ABI levels from post-procedure to 12 months

**Table 2.** Lesion characteristics

	E group (n = 14)	Non-E group (n = 16)	p-value
<b>Aortoiliac lesions</b>			
No lesions, n (%)	9 (64.3)	11 (68.7)	0.67
TASC II class A	0 (0)	1 (6.3)	
TASC II class B	1 (7.1)	2 (12.5)	
TASC II class C	0 (0)	0 (0)	
TASC II class D	4 (28.6)	2 (12.5)	
<b>Femoropopliteal lesions</b>			
No lesions, n (%)	2 (14.3)	5 (31.2)	0.18
TASC II class A	0 (0)	0 (0)	
TASC II class B	0 (0)	2 (12.5)	
TASC II class C	4 (28.6)	1 (6.3)	
TASC II class D	8 (57.1)	8 (50)	
<b>Infrapopliteal lesions</b>			
No lesions, n (%)	3 (21.4)	3 (18.8)	0.52
TASC class C	4 (28.6)	8 (50)	
TASC class D	7 (50)	5 (31.2)	
<b>Treatment</b>			
Revascularization			
Endovascular treatment, n (%)	13 (93)	15 (93.8)	1
Surgery, n (%)	1 (7)	0 (0)	0.47

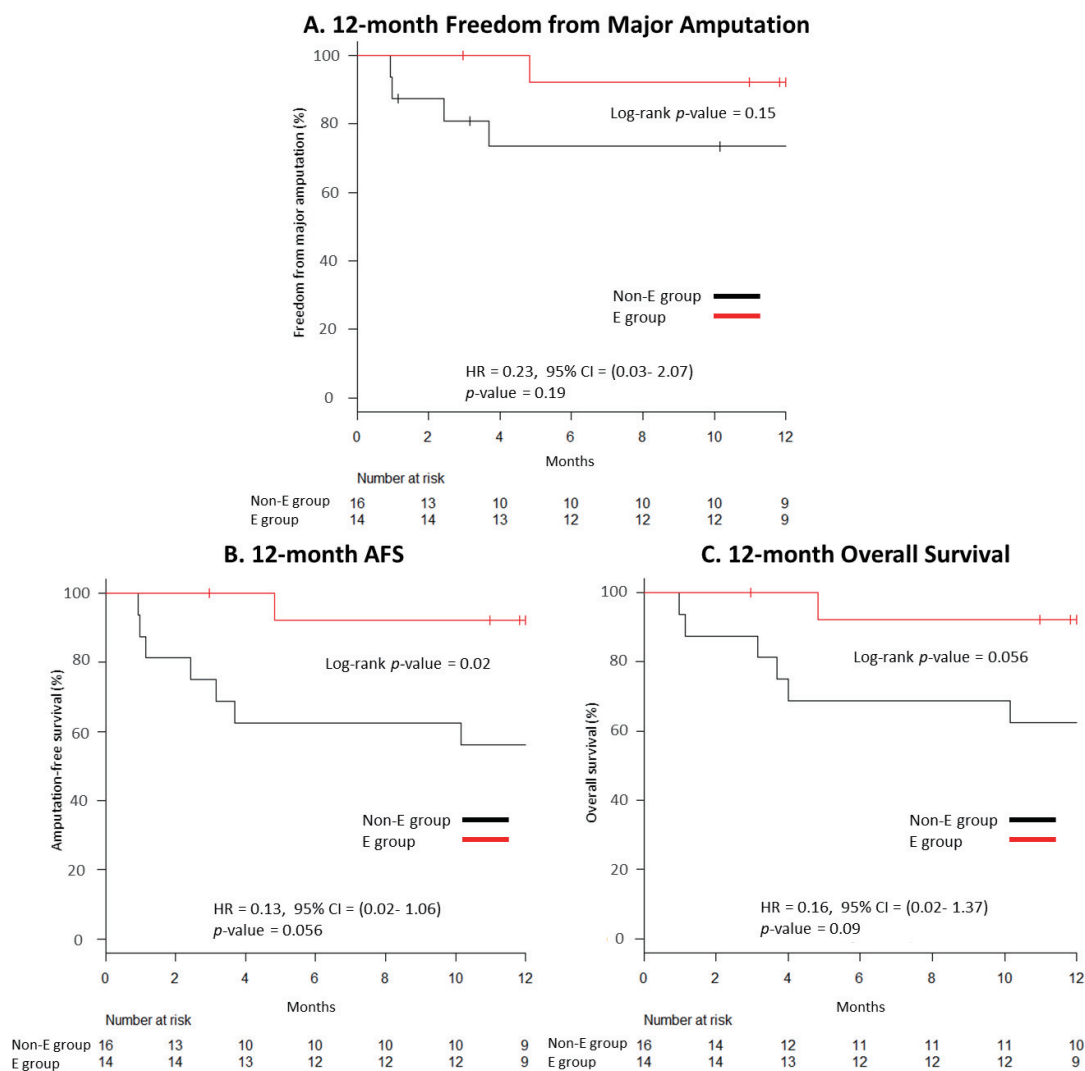
TASC = Trans-Atlantic Inter-Society Consensus



**Fig. 2.** Differences in lipid profiles

Over time, the patients in the E group were attenuated in levels of LDL-C (A) and non-HDL-C (B) compared to those in the non-E group. The attenuation in MDA-LDL levels (C) persisted through 6 months.

E group, evolocumab-treated group; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; non-E group, evolocumab non-treated group; MDA-LDL, malondialdehyde-modified LDL



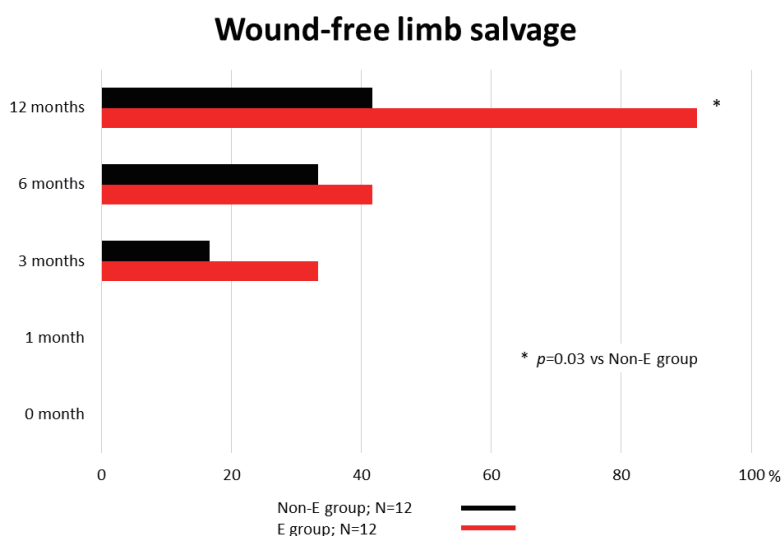
**Fig. 3.** Kaplan–Meier curves of 12-month freedom from major amputation, amputation-free survival, and overall survival for the patients with and without evolocumab

A, No significant difference was observed in the 12-month freedom from major amputation between patients with and without evolocumab (log-rank  $p=0.15$ ). Evolocumab administration was not associated with a significant change in freedom from major amputation (HR, 0.23; 95% CI, 0.03-2.07;  $p=0.19$ ). B, The 12-month AFS rate was significantly higher in the E group than that in the non-E group (log-rank  $p=0.02$ ). Evolocumab administration was not related to a significant change in AFS, however there was a tendency to improve AFS (HR, 0.13; 95% CI, 0.02-1.06;  $p=0.056$ ). C, The 12-month OS rate in the E group was shown a tendency for improvement, as compared with that in the non-E group (log-rank  $p=0.056$ ). There was a tendency to improve OS (HR, 0.16; 95% CI, 0.02-1.37;  $p=0.09$ ).

AFS, amputation-free survival; E group, evolocumab-treated group; non-E group, evolocumab non-treated group; OS, overall survival; HR, hazard ratio; CI, confidence interval

events<sup>6</sup>). Our data demonstrated that LDL cholesterol, non-HDL cholesterol, and MDA-LDL levels were improved significantly by evolocumab administration, especially in patients with CLTI. In addition, the LDL cholesterol level ( $34 \pm 21$  mg/dL) that was achieved after 12 months of evolocumab treatment was similar to that reported in a previous study, suggesting that decreased LDL levels may be associated with better limb outcomes in patients with CLTI.

Our results clearly showed that evolocumab treatment significantly reduces MDA-LDL levels in patients with CLTI; to our knowledge, this work is the first report of this association. Previous studies have shown that the accumulation of MDA-LDL, a major type of oxidized LDL, is related to the presence and progression of cardiovascular disease<sup>17</sup>). Additionally, it has been shown that the majority of MDA-LDL corresponds to small-dense LDL cholesterol, a



**Fig. 4.** Wound-free limb salvage rate

The proportion of wound-free limb salvage was analyzed in the patients with Rutherford classifications of 5 and 6 (E group,  $n=12$ , vs. non-E group,  $n=12$ ). The wound-free limb salvage rate was significantly higher in the E group at 12 months (E group [92%] vs. non-E group [42%];  $p=0.03$ ).

E group, evolocumab-treated group; non-E group, evolocumab non-treated group

known risk factor for the progression of PAD, and is associated with poor limb outcomes in patients with PAD who receive endovascular treatment<sup>18, 19</sup>). In previous studies, evolocumab administration significantly decreased the number of small LDL particles<sup>20</sup>). Therefore, the results of the present study suggested that the reduction in the MDA-LDL level observed in evolocumab-treated patients results in improved adverse limb outcomes in patients with CLTI.

### The Reduction of Adverse Limb Outcomes in Evolocumab-Treated Patients with CLTI

Our clinical data revealed that evolocumab administration is related to favorable limb outcomes, including the 12-month AFS and the 12-month wound-free limb salvage rate, in patients with CLTI. A sub-analysis of the FOURIER trial data demonstrated that the lower LDL cholesterol level achieved in evolocumab-treated patients was associated with a decreased risk of adverse limb events<sup>6</sup>). The study described here could not distinguish whether the pleiotropic effects of PCSK9-I on limb events were mediated by the lipid-lowering mechanism.

Based on a previous study of vessel pathology in patients with CLTI, most of the femoropopliteal arteries had significant atherosclerotic lesions with pathologies including intimal thickening, fibroatheroma, and fibrocalcific lesions. In fact, the majority of CLTI events included infrapopliteal lesions consisting of luminal thrombi that were presumed to originate from aortic, femoral, and popliteal atherosclerotic plaques

in the absence of significant atherosclerosis. Moreover, vessel pathology features were examined using an intravascular imaging modality<sup>21</sup>). Therefore, the authors of that report recommended the use of anti-thrombotic therapy to prevent the progression to CLTI<sup>22</sup>). Several studies have reported that PCSK9 inhibitors influence blood coagulation resulting from depletion of blood-clotting factor VIII and tissue factor, both of which play key roles in arterial thrombosis<sup>23</sup>). Regarding antiplatelet effects, it was found that PCSK9-I enhanced platelet function to decrease platelet aggregation while increasing platelet responsiveness to acetylsalicylic acid (aspirin)<sup>24</sup>). Oxidized LDL was robustly associated with thrombus formation, serving as an activator of CD36 and LOX-1 receptors on platelets. In addition, activated platelets themselves may promote further progressive oxidation of LDL<sup>23</sup>). Our data on the depletion of MDA-LDL, a form of oxidized LDL, supports the idea that PCSK9-I may counteract platelet activation. In the current study, 24 of 30 patients had severe infrapopliteal lesions, and all of the patients received antiplatelet therapy including aspirin, thienopyridine, or cilostazol. As noted above, these results collectively appear to show that the antithrombotic effects of PCSK9-I may be a means for decreasing adverse limb events, especially in patients with CLTI.

### Long-Term Administration of Evolocumab in Patients with CLTI

In the present study, we showed that evolocumab



administration significantly improved lipid profiles and that these effects were maintained during a 12-month follow-up period, even in patients with CLTI. The OSLER-1 clinical study demonstrated consistent efficacy and safety for evolocumab during 5 years of treatment in patients with hyperlipidemia. Good tolerance of long-term administration also was found, given that the discontinuation rate of evolocumab due to adverse events was 1.4% per year, a value that remained stable for up to 5 years<sup>25</sup>).

Importantly, as compared with antithrombotic agents, the effects of PCSK9-I on cardiovascular and limb events were obtained regardless of the intensity of statin treatment, and did not result in excess adverse events such as bleeding<sup>6, 26</sup>). In our study, no patients discontinued evolocumab because of adverse effects. Additionally, the wound-free limb salvage rate in patients receiving evolocumab remained significantly improved 12 months later. And, we could not find that the changes in ABI during the follow-up period. A previous study demonstrated that PCSK9 inhibitors may lessen the inflammatory response in the arterial wall without altering the levels of plasma C-reactive protein<sup>27</sup>). We are speculating that the impact of evolocumab on inflammation may be one of several mechanisms of the delayed effect on wound-free limb salvage, even if the improvement in circulation was not obtained in the long term.

Therefore, it seems reasonable to consider evolocumab administration for 12 months in patients with CLTI.

### Clinical Implications

Several world-wide major guidelines note that PAD is associated with high cardiovascular mortality; thus, all patients with symptomatic PAD should be considered for treatment by lipid-lowering therapy with statins<sup>28</sup>). In Japan, one such guideline also recommends maintaining LDL cholesterol < 120 mg/dL in patients with PAD. In contrast, a large cohort study in Japan has shown that only 36% of patients with PAD were treated with statins. These data suggest that statins remain underutilized for treatment of Japanese patients with PAD<sup>29</sup>).

In the present study, most patients with CLTI received statin therapy and achieved LDL cholesterol levels that remained below the limits specified in a Japanese guideline (Table 1). The results in the present study indicated that administration of evolocumab in combination with high-intensity statin therapy can contribute to robust reductions in the levels of lipid parameters and the improvement of limb outcomes in patients with CLTI. Additionally, long-term administration of evolocumab over 12 months contributed to

improvement of the wound-free limb salvage rate. Therefore, our data suggested that aggressive lipid-lowering therapy with evolocumab could be a favorable treatment option in patients with CLTI who are receiving statin therapy.

### Study Limitations

There were some limitations to the current study. First, our study included a small number of patients, which may have led to selection bias. Second, we conducted a non-randomized study of a single center's experience. However, the baseline characteristics matched between the patients with and without evolocumab. Additionally, all the patients without evolocumab received statin therapy, which has been reported to improve AFS in patients with CLTI<sup>10</sup>). Furthermore, almost all of the patients in this study achieved the desired LDL cholesterol levels (< 100 mg/dL) proposed in several guidelines. Third, evolocumab administration in the patients with CLTI was not associated with a significant change in the limb-specific outcome, namely, freedom from major amputation. However, multivariate analysis suggested that evolocumab administration may be related to the limb-specific outcome. Therefore, a future randomized, multicenter, large-scale study will be required to confirm the outcomes of the present study.

### Conclusions

Collectively, our findings suggested that aggressive lipid-lowering therapy with evolocumab in patients with CLTI was associated with robust reductions in lipid parameters and increased 12-month AFS. Additionally, long-term administration of evolocumab over 12 months contributed to improving wound-free limb salvage without excess adverse effects. We conclude that evolocumab as an add-on to statin therapy provides a therapeutic option to improve limb outcomes for patients with CLTI.

### Author Contributions

Yusuke Sato collected and analyzed data and wrote the manuscript. Hiroyasu Uzui contributed to conceptualization, investigation, and critical revision. Kanae Hasegawa, Hiroyuki Ikeda, Naoto Tama, Yoshitomo Fukuoka, and Kentaro Ishida and Shinsuke Miyazaki contributed to data curation. Testuji Morishita contributed to data curation and formal analysis. Hiroshi Tada contributed to supervision.

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## Conflicts of Interest

None declared.

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**Supplemental Table 1.** Predictors for event-free survival in Cox multivariate analysis

	HR	95%CI	<i>p</i> -value
Freedom from major amputation at 12 months			
Age (years)	0.961	0.844-1.095	0.544
Sex (male)	1.691	0.229-12.46	0.606
PCSK9-I	0.036	0.002-0.718	0.03
WIfI classification	23.1	1.921-277.7	0.013
12-month AFS			
Age (years)	0.958	0.868-1.057	0.394
Sex (male)	1.589	0.309-8.261	0.576
PCSK9-I	0.029	0.002-0.365	0.006
WIfI classification	8.318	2.107-32.83	0.002
12-month OS			
Age (years)	0.961	0.843-1.095	0.554
Sex (male)	1.691	0.23-12.46	0.606
PCSK9-I	0.036	0.002-0.718	0.03
WIfI classification	9.776	1.924-49.67	0.006

HR = hazard ratio; CI = confidence interval; PCSK9-I = proprotein convertase subtilisin/kexin type 9 inhibitor; WIfI = the Wound, Ischemia and foot infection; AFS = amputation free-survival; OS = overall survival

**Supplemental Table 2.** Predictors for wound-free limb salvage at 12 months in multivariate logistic regression analysis

	OR	95%CI	<i>p</i> -value
Wound-free limb salvage at 12 months			
Age (years)	1.01	0.891-1.14	0.923
Sex (male)	2.2	0.208-23.2	0.513
PCSK9-I	56.7	1.71-1880	0.024
WIfI classification	0.127	0.014-1.12	0.063

OR = odds ratio; CI = confidence interval; PCSK9-I = proprotein convertase subtilisin/kexin type 9 inhibitor; WIfI = the Wound, Ischemia and foot infection