Cholesterol uptake capacity: A new measure of high-density lipoprotein functionality as a predictor of subsequent revascularization in patients undergoing percutaneous coronary…

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Methods

257 retrospective patients

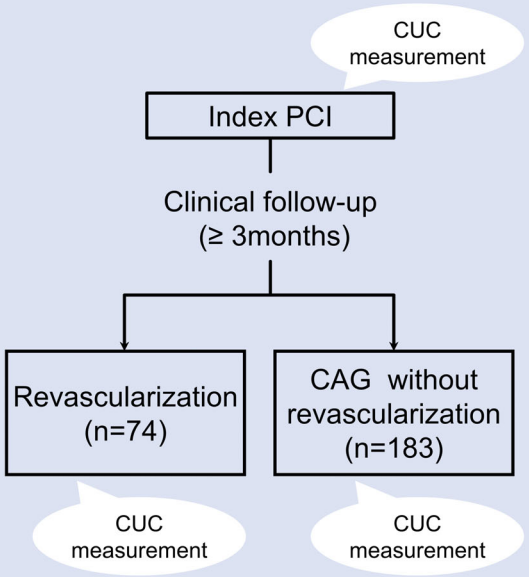
Undergoing PCI

+

Subsequent CAG
with or without revascularization

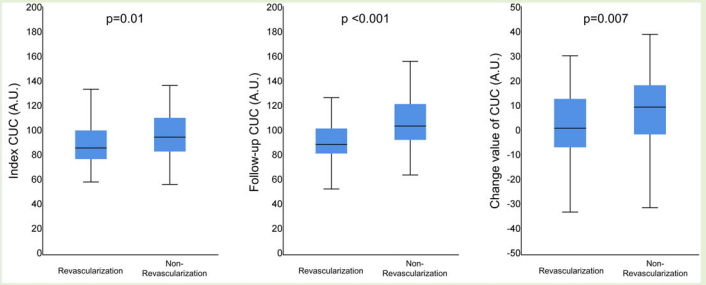
⬇

Assessment of HDL functionality
by our novel assay,
cholesterol uptake capacity (CUC)

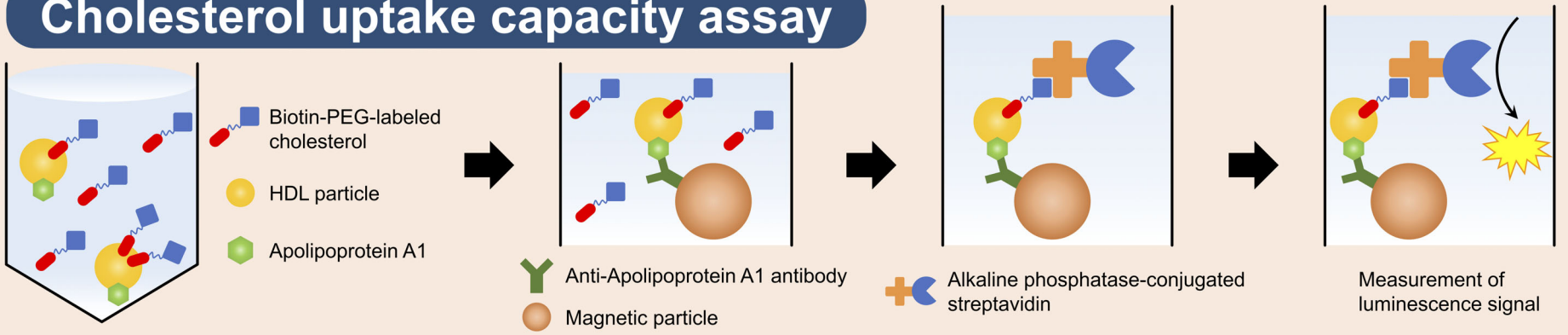


Summary

- ✓ Decreased CUC at index PCI were independently associated with subsequent revascularization
- ✓ CUC at index PCI and follow-up, change value of CUC were significantly lower in revascularization group in matched-cohort



Cholesterol uptake capacity assay



- ✓ No cells
- ✓ No radioisotopes
- ✓ Short procedure
- ✓ Automated

Cholesterol uptake capacity: A new measure of high-density lipoprotein functionality as a predictor of subsequent revascularization in patients undergoing percutaneous coronary intervention

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Number of figures and or tables: 5

Abstract

Background and aims:

High-density lipoprotein (HDL) functionality is an important determinant of coronary artery disease (CAD) development. We recently developed cholesterol-uptake capacity (CUC), a rapid cell-free assay system that directly evaluates the capacity of HDL to accept additional cholesterol. We aimed to evaluate the association between CUC and revascularization in patients who have undergone percutaneous coronary intervention (PCI).

Methods:

We retrospectively reviewed patients who underwent PCI with subsequent revascularization or coronary angiography (CAG) without revascularization. The patients who had frozen blood samples for which CUC were measurable at the index PCI and follow-up were enrolled.

Results:

We finally enrolled 74 patients who underwent subsequent revascularization and 183 patients who underwent follow-up CAG without revascularization. The serum CUC level at the index PCI was significantly lower in the revascularization group than that in the non-revascularization group (84.3 [75.2-98.9] vs. 92.0 [81.6-103.3 A.U.]; p=0.004). Multivariate logistic regression analysis revealed that decreased serum CUC level at the index PCI was independently associated with subsequent revascularization (odds ratio, 0.98; 95% confidence interval, 0.969–1.000). After adjusting for 16 cardiovascular risk factors, the serum CUC level at the index PCI and follow-up and the absolute

change in serum CUC level from the index PCI to follow-up were significantly lower in the revascularization group than those in the non-revascularization group.

Conclusion:

The serum CUC level at index PCI was independently associated with subsequent revascularization after PCI. Continuous assessment of HDL functionality by CUC might help predict subsequent revascularization after PCI.

1. Introduction

Recent studies have demonstrated an inverse relationship between high-density lipoprotein (HDL) cholesterol concentration and coronary artery disease (CAD) [1,2]. However, pharmacologic increase in HDL cholesterol concentration is not necessarily beneficial [3,4], and the genetic variants related to high HDL cholesterol concentrations are not associated with improved cardiovascular outcomes [5]. These findings suggest that quantitative assessment of serum HDL cholesterol concentration is not sufficient for risk stratification of future cardiovascular events in patients with CAD.

A key function of HDL to protect against cardiovascular events is the efflux of cholesterol from macrophages, which can be measured as cholesterol efflux capacity [6,7]. Previous studies demonstrated inverse correlations between cholesterol efflux capacity of HDL and CAD, independent of HDL cholesterol concentration [8,9]. Recently, we developed a rapid cell-free assay system to evaluate the uptake capacity of HDL. It uses biotin-polyethylene glycol (PEG)-labeled cholesterol and a specific antibody against apolipoprotein A1 (apoA1), which is the most abundant protein component of HDL. This new assay, named cholesterol-uptake capacity (CUC), enables a simpler and more rapid measurement of HDL functionality compared with the measurement of conventional cholesterol efflux capacity. In our previous report, we demonstrated that decreased serum CUC levels measured eight months after PCI or coronary artery bypass grafting were independently associated with the revascularization in patients with optimal control of LDL cholesterol [10]. Furthermore, in a recent

optical coherence tomography study of patients who underwent PCI, we demonstrated that decreased serum CUC level 24.5 months after stenting was significantly associated with the presence of in-stent neoatherosclerosis. This is associated with late-phase target lesion revascularization in patients treated with coronary stents [11]. These data revealed the clinical significance of CUC measurement in the chronic phase after PCI or coronary artery bypass grafting in patients with CAD. However, the prognostic value of CUC measurement at the time of PCI and serial assessment of CUC remain uncertain. Thus, in the present study, we aimed to clarify the clinical impact of serum CUC level at the index PCI and the absolute change in serum CUC level during follow-up on subsequent revascularization in patients with CAD.

2. Materials and Methods

2.1. Study subjects

The present study was a single-center retrospective study that used frozen blood samples from patients who underwent PCI. In our institute, blood samples were taken from all patients who underwent CAG or PCI in the catheter laboratory just before the procedure and cryopreserved in a prospective registry to evaluate the relationship between several biomarkers and clinical outcomes in patients with CAD (UMIN ID: 000030297). The inclusion criteria of this study were patients who underwent PCI with $\geq 2^{nd}$ generation drug-eluting stents or drug-coated balloons between December 2014 and March 2019, and who underwent follow-up CAG with and without revascularization during

the same period. The exclusion criteria were patients who did not have frozen blood samples at the index PCI or at follow-up (follow-up CAG or revascularization) and those undergoing hemodialysis. Indications for both index PCI and revascularization were decided based on the severity of stenosis on angiography and ischemia estimation with stress electrocardiogram, myocardial scintigraphy, invasive or non-invasive fractional flow reserve, and resting physiological indices.

We divided the enrolled patients according to the presence (revascularization group) or absence (non-revascularization group) of subsequent revascularization after the index PCI and compared the baseline and follow-up serum CUC levels between the two groups. Revascularization was defined as any subsequent PCI, including treatment for restenosis of the index PCI lesion and new or progressive lesions of the target vessel and other vessels. In patients who were diagnosed with multivessel disease and underwent multiple PCI, the first PCI procedure was defined as the index PCI. The other PCI procedures were not regarded as revascularization if such procedures were prescheduled.

In our retrospective observational study, potential biases were considered. Thus, we used propensity score matching analysis to identify the more secure relationship between serum CUC level at index PCI and follow-up, change in serum CUC level, and subsequent revascularization after PCI by matching traditional cardiovascular risk factors. This study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of Kobe University Hospital. Informed consent was obtained in the form of opt-out on the website of the Kobe University Graduate School

of Medicine, Department of Cardiology because the data were collected retrospectively.

2.2. Cholesterol up-take capacity assay

2.2.1. Preparation of the apoB-depleted serum

Serum samples were thawed on ice and incubated with the same volume of 22% PEG 4000 to remove apolipoprotein B (apoB)-containing lipoproteins. Briefly, each serum sample was mixed with a PEG solution and kept at room temperature for 20 min. The samples were then centrifuged at 860 g for 15 min to precipitate all apoB-containing lipoproteins, and the supernatant was collected as the apoB-depleted serum.

2.2.2. Generation of mouse monoclonal antibody 8E10

Hybridoma cell lines were generated by immunizing C57BL/6 mice with recombinant human apoA1 protein (Sigma Aldrich, St Louis, MO, USA). Mouse immunization and generation of hybridoma cell lines were outsourced from the Cell Engineering Corporation (Osaka, Japan). Hybridoma culture supernatants containing antibodies with the desired binding specificity for equal recognition of non-oxidized and oxidized HDL were screened by ELISA. Briefly, 1 μg/mL of recombinant human apoA1 protein or apoB-depleted serum with an apoA1 concentration of 1 μg/mL diluted in phosphate-buffered saline (PBS), were immobilized on 96-well plates at 37 °C for 1 h. After washing the wells with PBS, PBS with or without hydrogen peroxide (H_2O_2), sodium nitrite, and

diethylenetriaminepentaacetic acid (DTPA) solution (final concentrations 1, 200, and 100 μmol/L, respectively) were added to the wells and incubated at 37 °C for 1 h. The wells were washed with PBS and blocked with 2% bovine serum albumin (BSA) in PBS at 25 °C for 1 h. The plates were then incubated with hybridoma culture supernatant at 25 °C for 1 h, followed by the addition of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Dako Glostrup Denmark) at 25 °C for 30 min. The wells were washed with PBS five times, SuperSignal ELISA pico chemiluminescent substrate (Thermo Scientific, MA, USA) was added to the wells, and the chemiluminescence signal was measured using an Infinite F200 Pro microplate reader (Tecan, Mannedorf, Switzerland). mAb 8E10 was selected by screening for equal recognition of lipid-free (recombinant protein) and lipidated (apoB-depleted serum) apoA1 under native conditions, as well as after oxidation by exposure to H_2O_2/NO_2^-. To obtain sufficient antibodies for this study, mAb 8E10 was purified from the ascites fluid of ICR nude mice by Protein A-Sepharose chromatography. Preparation of mouse ascites fluid and purification of mAb 8E10 were outsourced from Kitayama Labes (Nagano, Japan).

2.2.3. Synthesis of Biotin-PEG7-cholesterol

Fifteen mg of 3β-Hydroxy-Δ^5-cholenic acid (Wako) were dissolved in 500 μl of N, N-dimethylformamide. Then 7.7 mg of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, hydrochloride (Dojindo), 4.6 mg of N-Hydroxysuccinimide (Sigma), 23.8 mg of Biotin-PEG7-amine (BroadPharm), and 8.4 μl of Triethylamine (Wako) were added to the solution and the resulting solution was stirred

at room temperature for 2 h. Silica gel column chromatography (10% methanol in chloroform) yielded Biotin-PEG7-cholesterol as a clear solid (4% yield). LC-MS (m/z): 951.4 $[M+H]^+$.

2.2.4. Automated CUC assay

The development of the CUC assay has been discussed previously [10,12]. In this study, the assay principle was applied to the HI-1000™ system (Sysmex), which is a fully automated immunoassay system for research applications. In brief, 5 µL of serum sample was diluted in PBS containing 0.2% R1 reagent of HDL-C Reagent KL "kokusai" (Sysmex) by 200 times. Next, 10 µL of the diluted apoB-depleted serum was incubated with 90 µL of 1 µM biotin-PEG-labeled cholesterol in reaction buffer (PBS containing 11% glycerol, 1.1% Pluronic F-68 (Thermo Scientific), 0.11 mM methyl-β-cyclodextrin (Sigma Aldrich), 0.055% liposome (Nippon fine chemical), 0.0047% nonion-K230 (NOF), 0.37% SF08 (NOF), and 0.009% oleamide (Kao)) at 37 °C for 1 min. Serum HDL was captured using anti-apoA1 mouse monoclonal antibody (8E10) coated on magnetic particles at 37 °C for 6 min. After washing the particles with wash buffer (HISCL ™ line washing solution containing 0.1% Pluronic F-68 and 138 mM sodium chloride), 100 µL of alkaline phosphatase-conjugated streptavidin (Vector Laboratories) in dilution buffer (0.1 M TEA (pH 7.5) containing 10 mg/mL BSA, 5 mg/mL Casein Na, 1 mM $MgCl_2$ and 0.1 mM $ZnCl_2$) was added and incubated at 37 °C for 10 min. After washing the particles with wash buffer, CDP-Star chemiluminescent substrate was added and incubated at 37 °C for 5 min, and chemiluminescence was measured as a count. The CUC assay was

standardized using pooled serum samples.

2.3. Statistical analysis

Statistical analyses were performed using SPSS for Windows version 26 (IBM SPSS Inc., Chicago, IL, USA). Baseline categorical variables are reported as percentages, and continuous variables are reported as median and interquartile. For discrete variables, comparisons were performed using chi-squared analysis or Fisher's exact test. For continuous variables, comparisons were performed using a 2-tailed, unpaired t-test, Welch test, or Wilcoxon test, according to the data of normal or non-normal distribution and equal variance, respectively. Logistic regression analysis was performed to identify the independent predictors of revascularization.

A propensity score matching analysis was performed to adjust the baseline values of age, male sex, body mass index, history of CAD, family history of CAD, current smoking, statin use, hypertension, dyslipidemia, diabetes mellitus, estimated glomerular filtration rate, HDL cholesterol level, LDL cholesterol level, triglyceride level, hemoglobin A1c (HbA1c), and index PCI for left main tract or multiple vessels. After the adjustment, baseline variables, serum CUC level at index PCI and follow-up, and absolute change in the value of serum CUC from index PCI to follow-up in the revascularization group were compared with those in the non-revascularization group using the chi-squared analysis or Fisher's exact test for discrete variables, and 2-tailed, unpaired t-test, Welch test, or Wilcoxon test for continuous variables. Receiver operating characteristic (ROC) curves were

constructed to evaluate the contribution of serum CUC level and absolute change in the value of serum CUC to the discriminatory power of revascularization. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were used to assess the contribution of serum CUC levels. R software version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria) with the PredictABEL package [13] was used to calculate NRI and IDI.

3. Results

3.1. Patients' characteristics

Among 703 consecutive patients, we enrolled 74 patients who underwent revascularization (revascularization group) and 183 patients who underwent follow-up CAG without revascularization (non-revascularization group) (see Supplementary Appendix). The median duration between index PCI and second coronary angiography was 303.0 [265.0-398.0] days. There was no significant difference in this period between the revascularization and the non-revascularization groups (337.5 [258.75-487.75] vs 299.0 [266.0-374.0 days]; p=0.22). Among 257 patients, 46 patients underwent follow-up CAG due to chest symptoms, 4 patients underwent follow-up CAG due to positive non-invasive imaging tests, and the other 207 patients underwent follow-up CAG without significant symptoms as routine clinical practice. Subsequently, 74 patients (79 lesions), including 7 acute coronary syndrome patients, underwent revascularization (revascularization group), while the remaining 183 patients did not undergo revascularization (non-revascularization group). Among

patients in the revascularization group, 31 lesions were treated for target lesions of index PCI (29 in-stent restenosis), 22 lesions for non-target lesions in target vessels, and 26 lesions for lesions in non-target vessels. The baseline characteristics and laboratory data of the study participants are presented in Table 1. Patients in the revascularization group had a significantly higher incidence of diabetes mellitus (63.5% vs. 41.0%; p=0.001), higher serum HbA1c levels (6.45 [5.90-7.10] vs. 6.00 [5.70-6.70%]; p=0.04), and lower serum HDL cholesterol level (43.0 [35.0-49.0] vs. 45.5 [39.0-54.0 mg/dL]; p=0.01) than those in patients in the non-revascularization group. In addition, patients in the revascularization group showed significantly lower serum CUC levels at the index PCI than those in patients in the non-revascularization group (84.3 [75.2-98.9] vs. 92.0 [81.6-103.3 A.U.]; p=0.004). LDL cholesterol level, triglyceride level, and the prevalence of other traditional cardiovascular risk factors were not different between the two groups. There was no significant difference in serum apoA1 levels between the revascularization and non-revascularization groups. No significant difference was noted in the usage rates of lipid-lowering therapies between the two groups (Table 1).

The serum CUC level at follow-up in the revascularization group was significantly lower than that in the non-revascularization group (87.8 [80.5-100.3] vs. 94.3 [82.6-108.5 A.U.]; p=0.03), while there was no significant difference in absolute change in value between the two groups during the follow-up period.

Multivariate logistic regression analysis of the overall population revealed that impaired HDL functionality as assessed by decreased serum CUC level at the index PCI (odds ratio [OR], 0.98; 95%

confidence interval [CI], 0.969–1.000) and the presence of diabetes mellitus (OR: 2.19, 95% CI: 1.24-3.89) were independently associated with subsequent revascularization after PCI (Table 2).

3.2. Propensity score matching analysis

There were sixty-four patients in each group. Baseline characteristics were not significantly different between the two groups in the propensity score-matched cohort (see Supplementary Appendix). Laboratory data at the index PCI and follow-up and the absolute changes of each variable from the index PCI to follow-up are shown in Table 3. The average duration between the index PCI and follow-up CUC measurement was not statistically different between the revascularization group and non-revascularization group (318.5 [255.0-497.25] vs. 315.0 [271.75-383.75 days]; p=0.94). The serum CUC level at the index PCI and at follow-up was significantly lower in the revascularization group than that in the non-revascularization group. Furthermore, the absolute changes in the serum CUC level from the index PCI to follow-up were significantly lower in the revascularization group than those in the non-revascularization group (Figure 1).

Receiver operating characteristic (ROC) curves showed that the optimal cut-off value of index serum CUC level for predicting revascularization (model 1) was 90.7 A.U. with a sensitivity of 67.2% and a specificity of 60.9% (area under the curve [AUC]; 0.63, 95% CI: 0.54-0.73), and that of follow-up serum CUC level was 92.5 A.U. with a sensitivity of 62.5% and a specificity of 71.9% (AUC; 0.72, 95% CI: 0.63-0.81) (Figure 2 (A), (B)). Patients with index CUC and follow-up CUC levels of <90.7

and <92.5, respectively, had a 6.06-fold higher risk of revascularization compared with patients who did not fulfill both criteria (Figure 2 (C)).

When absolute change in the value of serum CUC level during the follow-up was added to the index serum CUC level (model 2), AUC was numerically increased from 0.63 to 0.72 (95% CI: 0.63-0.81). Although this did not reach statistical significance (see Supplementary Appendix), model 2 showed a further increase in incremental reclassification ability as evaluated by net reclassification improvement (NRI) and integrated discrimination improvement (IDI) index compared with that in model 1 (NRI=0.47, 95% CI: 0.13-0.81, p=0.006; IDI=0.12, 95% CI: 0.06-0.17, p <0.001).

4. Discussion

The main findings of the present study can be summarized as follows: First, we demonstrated that lower serum CUC levels at the index PCI were independently associated with subsequent revascularization in non-hemodialysis patients who underwent PCI with ≥ 2nd generation drug-eluting stents or drug-coated balloons in the overall cohort. Second, in the propensity score-matched cohort, the serum CUC level at the index PCI as well as at follow-up was significantly lower in the revascularization group than that in the non-revascularization group. In addition, the absolute changes in the serum CUC level from the index PCI to follow-up were significantly lower in the revascularization group than those in the non-revascularization group. Finally, ROC curve analyses showed that the optimal cut-off value of index serum CUC level for predicting revascularization was

90.7 A.U., with a sensitivity of 67.2% and specificity of 60.9%, and that of follow-up serum CUC level was 92.5 A.U., with a sensitivity of 62.5% and specificity of 71.9%. Patients who met both criteria had a 6.06-fold higher risk for revascularization than that in patients who did not fulfill both criteria.

The traditional measurement of HDL functionality by cholesterol efflux capacity can reflect all HDL particles' capacity, including both ATP-binding cassette transporter A1 (ABCA1)-mediated efflux and aqueous diffusion of cholesterol. On the other hand, because CUC measurement is a cell-free assay using anti-apoA1 antibodies, CUC cannot necessarily reflect the capacity of HDL without apoA1 on its surface. Thus, CUC could only reflect cholesterol efflux capacity of HDL through the process of aqueous diffusion of cholesterol; however, it cannot reflect ABCA1-mediated efflux process. These features of the CUC assay implies that CUC mainly reflects the reverse cholesterol transport of phospholipid-rich measured HDL particles. Despite such theoretical difference, we previously reported that CUC is highly correlated with cholesterol efflux capacity [10]. In addition, considering that CUC enables simpler and more rapid measurement of HDL functionality, it is more suitable for daily clinical use compared with conventional cholesterol efflux capacity. Thus, we currently consider that the CUC assay could be a promising alternative in evaluating HDL functionality in daily clinical practice. Additionally, serum apoA1 level, which was previously reported to be correlated with HDL particle number [14], was not different between the revascularization and non-revascularization groups, in contrast with serum CUC level as determined in this study. Our data suggest that the quality of HDL has more important clinical implications than quantitative evaluation of HDL.

In the present study, we revealed a significant inverse relationship between serum CUC level at the index PCI and subsequent revascularization after PCI in both the overall and propensity-matched cohorts. Previous studies have demonstrated the importance of HDL functionality in halting the progression of atherosclerosis. Impaired efflux capacity of HDL was shown to be related to the incidence of de novo CAD [8,9], all-cause mortality of patients with CAD [15], and late in-stent loss [16] and neoatherosclerosis [11] after coronary artery stenting. Regarding CUC, we previously demonstrated that patients who required revascularization had a significantly lower serum CUC level at follow-up compared with that in patients who did not require revascularization [10]. However, there are no clinical studies that have evaluated the relationship between serum CUC levels at the index PCI and subsequent revascularization following PCI. In the present study, we demonstrated, for the first time, that decreased serum CUC levels measured at PCI were an independent risk factor for revascularization. This result suggests that the serum CUC level measured at PCI is useful for risk stratification for secondary prevention in patients with CAD.

Although the detailed mechanisms between serum CUC level and subsequent revascularization remain unclear, the function of HDL to directly remove cholesterol from macrophages in the vessel wall might play a central role in its relationship with future revascularization. We recently investigated the cross-sectional relationship between CUC and the morphological features of angiographic stenosis by optical coherence tomography and revealed that CUC was inversely related to lipid-rich plaque burden and the extent of macrophage accumulation [17]. In a previous

experimental study using a genetically manipulated mouse model, macrophage-specific cholesterol efflux was shown to be inversely correlated with an atherosclerotic area in the aortic root, suggesting that impaired ability of HDL to remove cholesterol from macrophages would accelerate atherosclerotic progression [18]. Additionally, attenuation of other atheroprotective properties might occur simultaneously in HDL with impaired CUC. Previous reports showed that prevention of cholesterol efflux of HDL by blocking antibody against scavenger receptor-B1 led to inhibition of endothelial nitric oxide synthase [19], and cholesterol efflux in megakaryocyte progenitors was shown to suppress platelet production and thrombocytosis [20]. We have also demonstrated that both cholesterol efflux capacity and anti-inflammatory properties were impaired in HDL isolated from patients with imbalance of myeloperoxidase and paraoxonase 1 in the bloodstream [21]. On the other hand, the removal of cholesterol from the plasma membrane by HDL might directly modulate the pro-inflammatory properties of macrophages, because previous studies have demonstrated that disorganization of lipid rafts on macrophages can lead to attenuation of its inflammatory activation [22,23]. Further studies are required to elucidate the precise mechanisms underlying the inverse relationship between CUC and the risk of repeat revascularization.

Another key finding of the present study is the importance of the continuous assessment of HDL functionality after PCI. In the overall cohort, both serum CUC levels at index PCI and follow-up were significantly lower in the revascularization group than in the non-revascularization group. However, in the multivariate analysis of this cohort, we found significant differences between the two

groups neither in the serum CUC at follow-up nor in the absolute change value during the follow-up period. On the other hand, in the propensity-matched cohort, we found that the patients with revascularization showed significantly lower serum CUC levels at follow-up and absolute change value of CUC during the follow-up compared with those without revascularization. In this matched cohort, we evaluated a more secure relationship between serum CUC level and revascularization by adjusting for 16 baseline traditional cardiovascular risk factors. As a result, we found that not only serum CUC levels at index PCI, but also those at follow-up and the absolute change in serum CUC levels were significantly associated with subsequent revascularization after PCI. Furthermore, we achieved a more accurate prediction for revascularization using a combination of serum CUC level at index PCI and change in serum CUC level during the follow-up period. Although the reasons for the lack of significance in the overall cohort remain uncertain, several confounding factors, such as diabetes, might have affected the results.

While there have been several cross-sectional studies demonstrating the clinical importance of HDL functionality, serial assessment of the relationship between HDL functionality and its clinical significance, and studies assessing the factors related to changes in HDL functionality are scarce. Imaizumi et. al. reported that the baseline cholesterol efflux capacity of HDL was independently associated with late in-stent loss; however, variation in cholesterol efflux capacity of HDL for 6-8 months was not associated with late in-stent loss [16]. In contrast, the change in CUC was shown to be inversely related to subsequent revascularization in the matched cohort in the present study.

Although still speculative, several important differences exist in the studied population between the previous study and ours, which might explain the conflicting results. A previous study enrolled 48 patients, and 30 out of 48 patients (63%) were treated with bare-metal stents. This study evaluated the relationship between changes in HDL efflux capacity and in-stent late loss. In contrast, our study enrolled a larger number of patients (257 patients) who were predominantly treated with 2nd generation drug-eluting stents (88.3%). We evaluated the impact of changes in serum CUC level during the follow-up and all revascularization, including PCI for not only target lesion restenosis, but also non-target lesions. Thus, measuring HDL functionality should be evaluated not only with target vessels but also with atherosclerotic progression in all vessels. Finally, baseline serum LDL cholesterol, triglyceride, and HbA1c levels were clearly better controlled in the present study. These differences might underestimate the importance of the change in the value of CUC on future revascularization in patients with CAD. Our results suggest that HDL functionality measured by CUC continuously after PCI is useful for assessing the risk of cardiovascular events in patients with CAD.

Our findings suggest that the HDL functionality measured by CUC could be a new therapeutic target. In an examination of a small number of patients, the cholesterol efflux capacity of HDL was increased after therapy with pioglitazone. In contrast, therapy with statins did not provide such an increase [8]. However, some studies have shown favorable effects of statins to improve the cholesterol efflux capacity of HDL [24,25]. In the present study, most of the patients with diabetes mellitus and dyslipidemia were already medically treated and, unfortunately, we could not identify the clinical

intervention associated with the improvement of serum CUC level. Future studies are required to clarify effective treatment strategies that improve HDL functionality.

Our study had several limitations. First, this was a single-center, retrospective study with a limited sample size. Although we tried to match known risk factors by adjusting for 16 baseline variables, several unknown risk factors might have affected the results because of the retrospective nature of the study design. Second, we enrolled patients who had frozen blood samples at both index PCI and follow-up; thus, patients undergoing PCI without follow-up CAG were not included. In the present study, we focused on the relationship between the change in serum CUC level and revascularization, in addition to that of serum CUC level at index PCI. In this study design, we rigidly compared the patients who required subsequent revascularization and those who did not by confirming the indication of revascularization with follow-up CAG followed by physiological assessment. In addition, not all patients undergoing PCI and/or follow-up CAG have frozen blood samples. Especially in some acute coronary syndrome cases, we could not obtain frozen blood samples due to time limitations; therefore, selection bias exists. A larger-scale prospective study might be necessary to confirm our results. Third, the number of female patients was small in this study. There was no previous study of CUC to verify sex-based differences and association with female-specific factors, such as menopausal status and ongoing hormone replacement therapy. Further studies are needed to clarify the clinical significance of CUC in females.

In conclusion, serum CUC level at the index PCI was independently associated with

subsequent revascularization after PCI, and an inverse relationship was shown between the absolute change in serum CUC level and revascularization. Assessment of HDL functionality by CUC at the time of PCI and during clinical follow-up might help predict subsequent revascularization after PCI.

Conflict of interest

This research received no specific financial support from any funding agency in the public, commercial, or not-for-profit sectors.

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Author contributions

D. Fujimoto: conceptualization, methodology, data curation, interpretation, writing-original drafts H. Otake, R. Toh: Conceptualization, methodology, writing-reviewing, and editing. H. Kawaori, T. Toba, M. Nagao, S. Nakano, K. Tanimura, Y. Takahashi, Y. Fukuyama, S. Kakizaki, K. Nakamura: Conceptualization, methodology. A. Harada, K. Murakami, and T. Iino: Investigation, resource, writing-reviewing, and editing. K. Hirata: Supervision. All authors have read and approved the final version of the manuscript.

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Keywords:

High-density lipoprotein, Functionality of high-density lipoprotein, cholesterol efflux capacity, cholesterol uptake capacity, coronary artery disease, secondary prevention

Abbreviations

ABCA1, ATP-binding cassette transporter A1

ApoA1, apolipoprotein A1

CAD, coronary artery disease

CAG, coronary angiography

CUC, cholesterol up-take capacity

HbA1c, Hemoglobin A1c

HDL, high-density lipoprotein

IDI, integrated discrimination improvement

LDL, low-density lipoprotein

NRI, net reclassification improvement

OR, odds ratio

PCI, percutaneous coronary intervention

ROC, receiver operating characteristic

Table 1.

Baseline patient characteristics, serum CUC level at follow-up, and lipid-lowering agent at both period

	Revascularization (n=74)	Non-revascularization (n=183)	p-value
Age (yr)	70.1 ± 10.9	70.7 ± 9.4	0.63
Male, n (%)	63 (85.1)	144 (78.7)	0.24
Body mass index (kg/m^2)	24.8 ± 4.1	24.4 ± 3.9	0.45
Current smoking, n (%)	13 (17.6)	29 (15.8)	0.74
Family history of CAD, n (%)	12 (16.2)	19 (10.4)	0.19
Hypertension, n (%)	53 (71.6)	142 (77.6)	0.31
Dyslipidemia, n (%)	63 (85.1)	157 (85.8)	0.89
Diabetes mellitus, n (%)	47 (63.5)	75 (41.0)	0.001
Prior CAD, n (%)	38 (51.4)	70 (38.3)	0.05
Acute coronary syndrome, n (%)	10 (13.5)	30 (16.4)	0.56
Multi-vessel disease, n (%)	31 (41.9)	59 (32.2)	0.14

Lipid-lowering agent at baseline			
Statin use, n (%)	59 (79.7)	144 (78.7)	0.85
Ezetimibe use, n (%)	5 (6.8)	6 (3.3)	0.18
PCSK9 inhibitor use, n (%)	0 (0)	1 (0.5)	0.71
EPA use, n (%)	5 (6.4)	19 (10.4)	0.37
Fibrate use, n (%)	1 (1.4)	5 (2.7)	0.45
Lipid-lowering agent at follow-up			
Statin use, n (%)	67 (90.5)	163 (89.1)	0.73
Ezetimibe use, n (%)	11 (14.9)	19 (10.4)	0.31
PCSK9 inhibitor use, n (%)	1 (1.4)	5 (2.7)	0.45
EPA use, n (%)	12 (16.2)	23 (12.6)	0.44
Fibrate, n (%)	1 (1.4)	3 (1.6)	0.67
Laboratory data at baseline			
Triglyceride (mg/dL)	135.5 (96.0–171.0)	119.0 (88.0–166.0)	0.10
Total cholesterol (mg/dL)	162.0 (140.0–178.0)	161.0 (140.0–190.5)	0.53
HDL cholesterol (mg/dL)	43.0 (35.0–49.0)	45.5 (39.0–54.0)	0.01
LDL cholesterol (mg/dL)	95.5 (79.0–112.0)	93.5 (79.0–119.0)	0.68
HbA1c (NGSP) (%)	6.45 (5.90–7.10)	6.00 (5.70–6.70)	0.04

eGFR (ml/min/1.73m2)	65.8 (53.7–73.1)	64.9 (53.4–73.8)	0.83
High-sensitivity CRP (mg/dL)	0.10 (0.04–0.23)	0.07 (0.03–0.14)	0.13
BNP (pg/dL)	30.5 (16.3–80.4)	43.4 (20.8–88.3)	0.13
ApoA1 (mg/dL) [a]	117.0 (103.0–128.0)	118.0 (107.0–132.0)	0.26
CUC level at baseline and follow-up			
CUC at index PCI (A.U.)	84.3 (75.2–98.9)	92.0 (81.6–103.3)	0.004
CUC at follow-up (A.U.)	87.8 (80.5–100.3)	94.3 (82.6–108.5)	0.03
Change value of CUC (A.U.)	1.9 (-7.13–13.1)	1.5 (-8.0–12.1)	0.72

Abbreviations: BNP, brain natriuretic peptide; CAD, coronary artery disease; CRP, C-reactive protein; CUC, cholesterol up-take capacity; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein cholesterol.

[a] ApoA1 are compared in 63 patients in revascularization vs 143 patients in non-revascularization group

Table 2.

Uni- and multivariate logistic regression analysis for revascularization

Variables	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Age	0.99	0.97–1.02	0.57			
Male sex	1.55	0.75–3.22	0.24			
Presence of diabetes mellitus	2.51	1.44–4.38	0.001	2.19	1.24–3.89	0.007
Prior CAD	1.70	0.99–2.94	0.06			
Multi-vessel disease	1.52	0.87–2.64	0.14			
Triglyceride level	1.00	1.00–1.00	0.13			
HDL cholesterol level	0.97	0.94–0.99	0.01			
LDL cholesterol level	1.00	0.99–1.00	0.36			
CUC level at the index PCI	0.98	0.97–1.00	0.01	0.98	0.97–1.00	0.04

Abbreviations: CAD, coronary artery disease; CUC, cholesterol up-take capacity, HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio.

Table 3.

Laboratory data at the index PCI, follow-up, and absolute changes in the propensity score-matched cohort

	Revascularization (n=64)	Non-revascularization (n=64)	p-value
Index PCI			
HbA1c (NGSP) (%)	6.40 (5.85–7.00)	6.20 (5.65–6.90)	0.28
Triglyceride (mg/dL)	133.0 (96.0–165.0)	119.5 (87.5–171.5)	0.48
Total cholesterol (mg/dL)	158.5 (137.5–177.0)	159.0 (140.5–186.5)	0.58
HDL cholesterol (mg/dL)	43.0 (36.0–49.5)	45.0 (39.0–58.5)	0.06
LDL cholesterol (mg/dL)	93.0 (79.0–112.0)	88.5 (76.0–109.5)	0.58
CUC (A.U.)	85.0 (76.0–99.9)	93.8 (82.1–109.5)	0.01
Follow-up			

HbA1c (NGSP) (%)	6.25 (5.85–6.90)	6.10 (5.80–6.75)	0.67
Triglyceride (mg/dL)	116.0 (84.0–156.0)	99.0 (73.0–145.5)	0.36
Total cholesterol (mg/dL)	134.5 (117.5–159.0)	142.0 (127.0–165.0)	0.25
HDL cholesterol (mg/dL)	43.5 (36.0–50.0)	47.0 (40.0–58.5)	0.02
LDL cholesterol (mg/dL)	75.5 (62.5–96.5)	74.0 (59.5–98.5)	0.80
CUC (A.U.)	87.8 (80.5–100.8)	103.5 (92.0–120.7)	<0.001
Absolute change value			
HbA1c (NGSP) (%)	0.00 (-0.2–0.2)	0.0 (-0.2–0.25)	0.80
Triglyceride (mg/dL)	-14.0 (-35.5–6.50)	-17.0 (-45.0–13.0)	0.88
Total cholesterol (mg/dL)	-14.5 (-38.0–0.50)	-14.0 (-32.0–8.0)	0.78

HDL cholesterol (mg/dL)	1.0 (-4.5–6.0)	2.0 (-1.0–4.5)	0.20
LDL cholesterol (mg/dL)	-12.0 (-29.0–1.0)	-11.0 (-35.5–5.5)	0.92
CUC (A.U.)	0.5 (-7.25–12.5)	9.0 (-2.0–18.0)	0.007
Abbreviations: CUC, Cholesterol up-take capacity; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein cholesterol.			

1

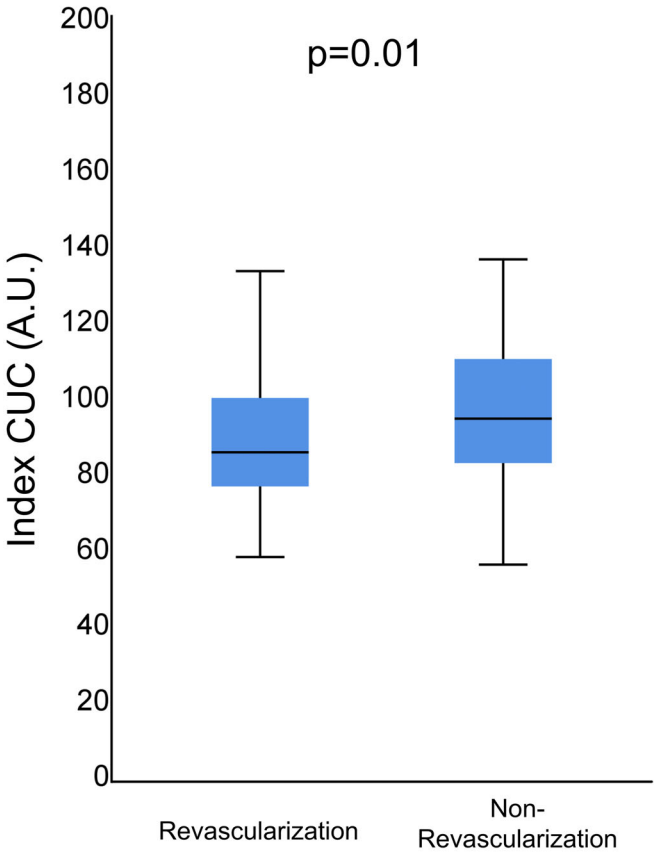
Figure legends

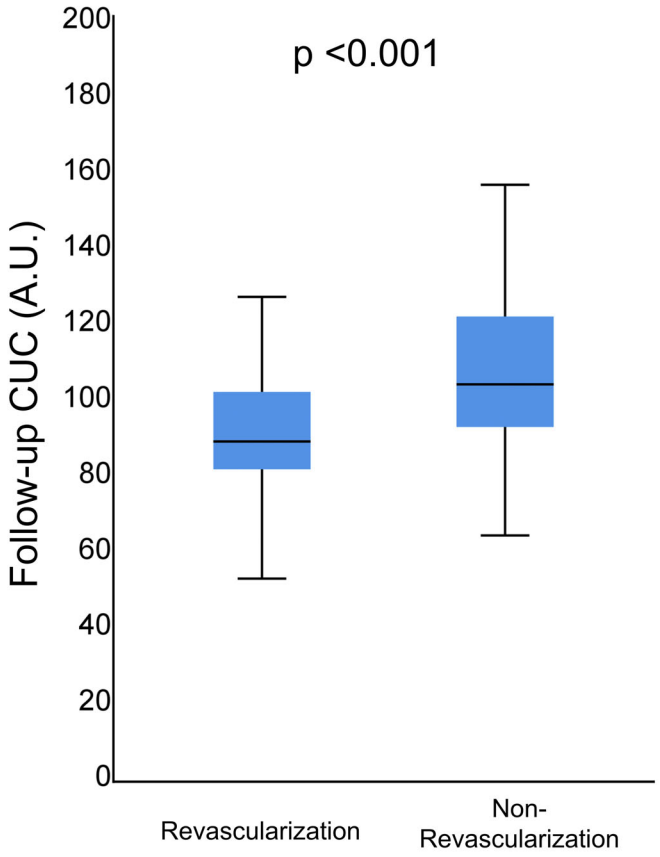
Figure 1. Serum CUC level at the time of index PCI and follow-up, and the absolute change in the value of serum CUC between the time of index PCI and follow-up.

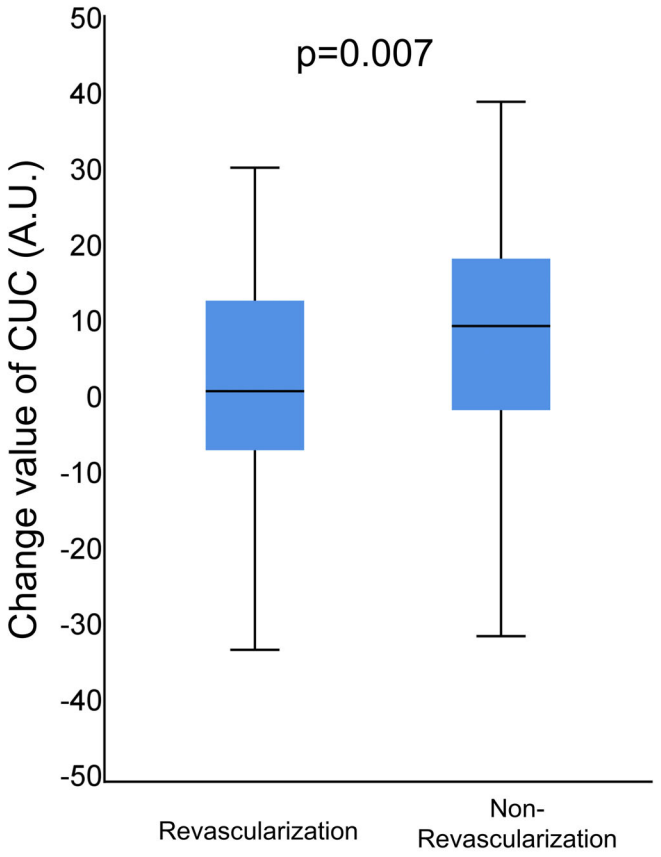
CUC, cholesterol uptake capacity; PCI, percutaneous coronary intervention

Figure 2. Diagnostic performance of index PCI and follow-up serum CUC level for revascularization. (A), (B) ROC curves for the sensitivity and specificity of the association between the revascularization and index serum CUC level (A) and follow-up serum CUC level (B). (C) The incidence rate of revascularization stratified by the cut-off value of the index and follow-up serum CUC level. (-/-): patients with index CUC >90.7 A.U. and follow-up CUC >92.5 A.U.; (+/-): patients with index CUC <90.7 A.U. and follow-up CUC >92.5 A.U.; (-/+): patients with index CUC >90.7 A.U. and follow-up CUC <92.5 A.U.; (+/+): patients with index CUC <90.7 A.U. and follow-up CUC <92.5 A.U.

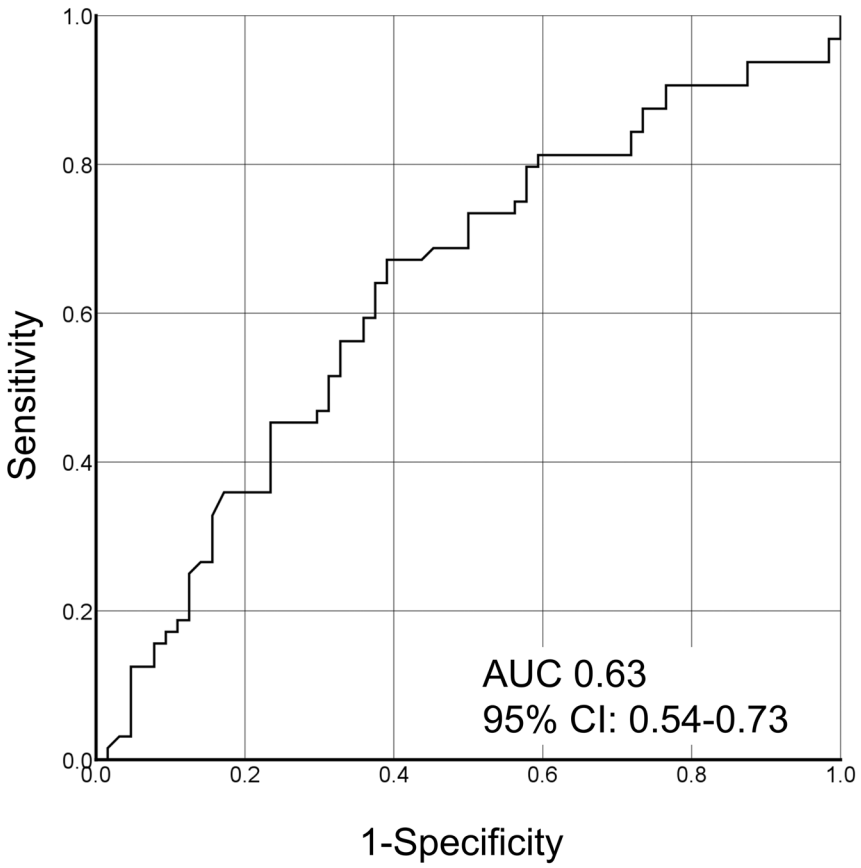
ROC, receiver operating characteristic; CUC, cholesterol uptake capacity


p=0.01
Index CUC (A.U.)
200
180
160
140
120
100
80
60
40
20
0
Revascularization
Non-
Revascularization

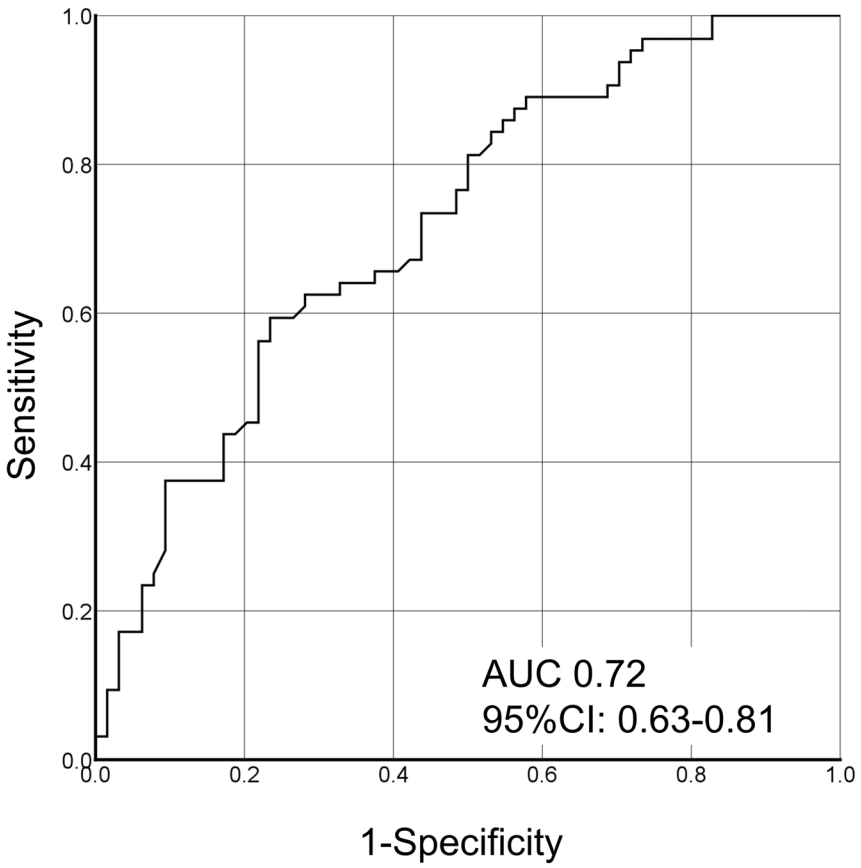

p <0.001
Follow-up CUC (A.U.)
200
180
160
140
120
100
80
60
40
20
0
Revascularization
Non-
Revascularization


p=0.007
Change value of CUC (A.U.)
50
40
30
20
10
0
-10
-20
-30
-40
-50
Revascularization
Non-
Revascularization

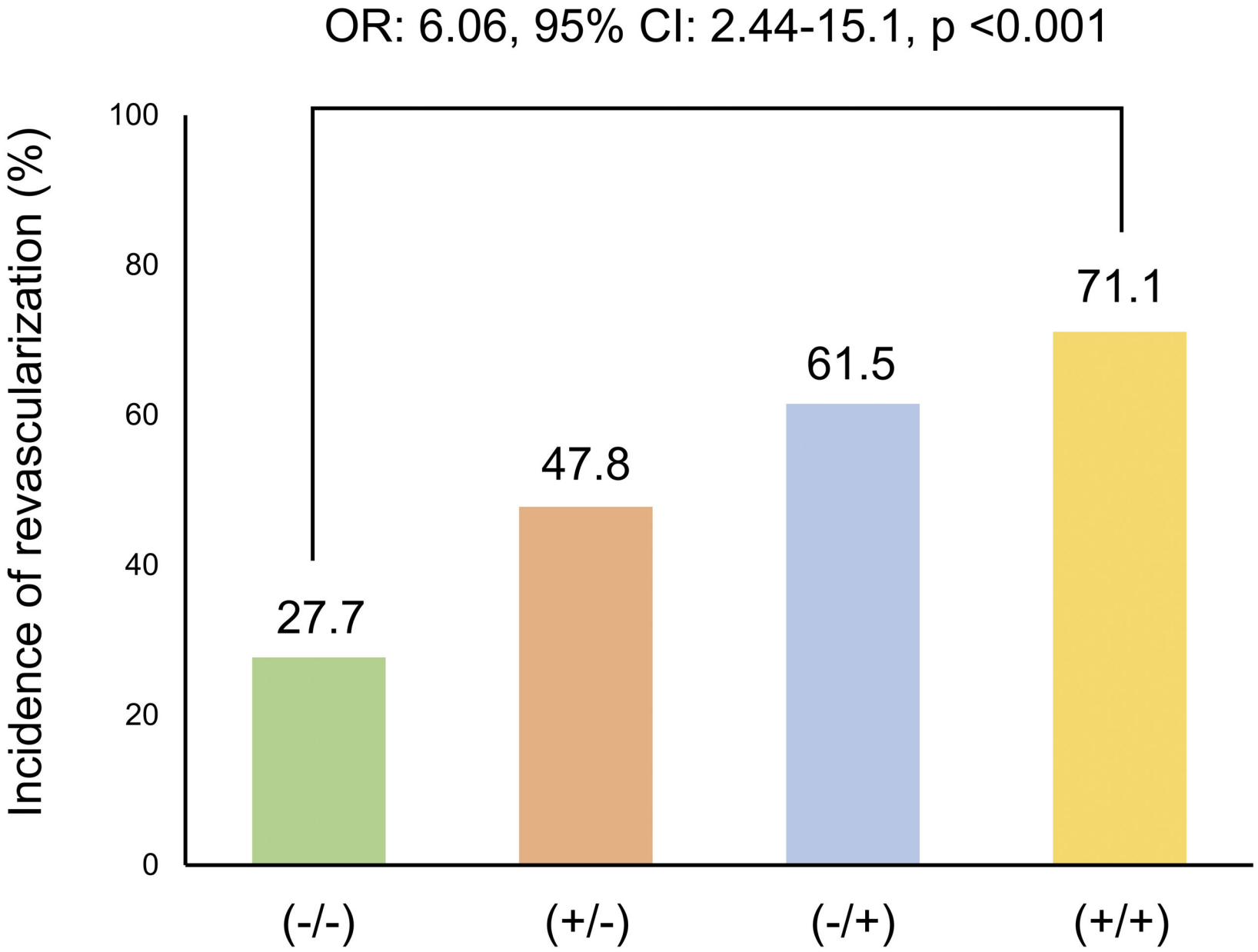
(A)


Index CUC
Sensitivity
1-Specificity
AUC 0.63
95% CI: 0.54-0.73
0.0
0.2
0.4
0.6
0.8
1.0

(B)


Follow-up CUC
Sensitivity
1-Specificity
AUC 0.72
95%CI: 0.63-0.81
0.0
0.2
0.4
0.6
0.8
1.0

(C)


OR: 6.06, 95% CI: 2.44-15.1, p <0.001
Incidence of revascularization (%)
100
80
60
40
20
0
27.7
47.8
61.5
71.1
(-/-)
(+/-)
(-/+)
(+/+)