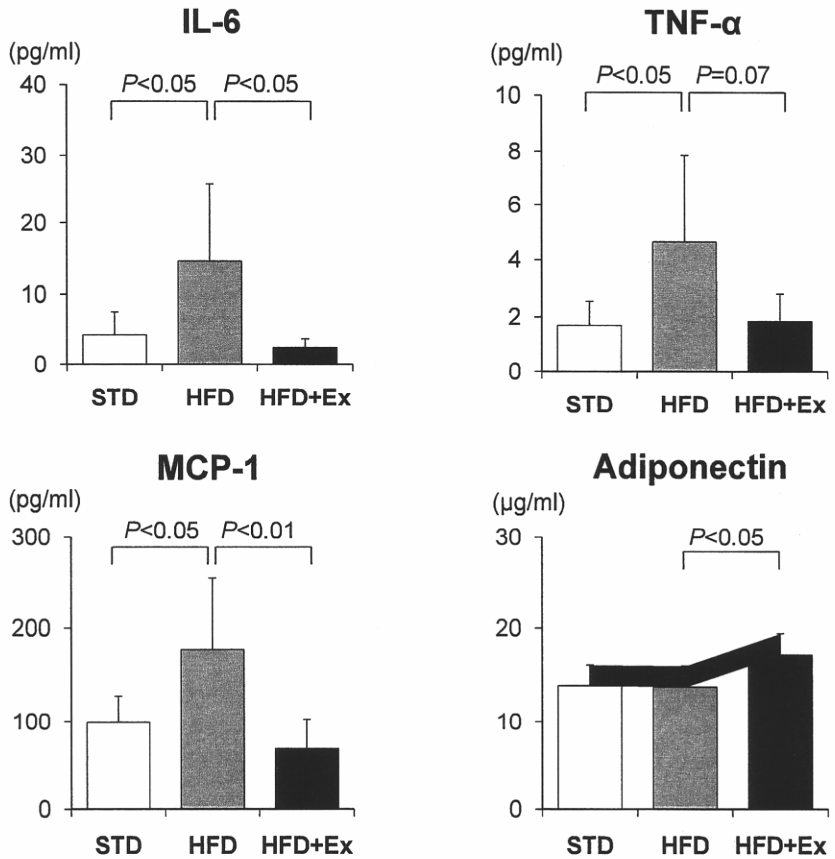

Fig. 6. Comparison of serum interleukin (IL)-6, tumor necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1, and adiponectin levels among the STD, HFD, and HFD + Ex groups.

Serum IL-6 and TNF-α levels were measured using the Cytometric Bead Array Mouse Soluble Protein Master Buffer Kit in the STD (n=8), HFD (n=6), and HFD + Ex (n=6) groups at 18 weeks of age. Serum MCP-1 and adiponectin levels were measured using commercial mouse ELISA kits in the STD (n=6), HFD (n=6), and HFD + Ex (n=6) groups at 16 weeks of age. Error bars represent SD.

physical activity benefits cardiovascular diseases in a non-stressful environment without disruption of the normal diurnal rhythm. Recent studies have reported that Ex increases the number of circulating endothelial progenitor cells in wild-type[19] and ApoE-deficient mice[20] as well as promoting anti-oxidative and anti-inflammatory effects[20, 21], and preserves endothelial function in ApoE-KO mice[21]. Our results provide new evidence that Ex reduces atherosclerotic lesion formation via an increase in adiponectin levels following a reduction in adiposity, especially white adipose tissue.

Adiponectin, which is derived only from adipose tissue and is abundantly present in circulating blood, has been identified as an "adipocytokine" with protective actions against the initiation and progression of atherosclerosis through anti-inflammatory and anti-atherogenic effects[22, 23]. The present study is the first to demonstrate that Ex may reduce atherogenesis with consequent increased circulating adiponectin levels in atherogenic ApoE-deficient mice fed HFD. Although body weight changes did not differ in the HFD + Ex group compared with those in the HFD group, the fat mass of white adipose tissues decreased significantly. A recent clinical interventional study demonstrated that the increase in adiponectin concentration in response to physical exercise was related to a reduction in fat mass and inflammatory factor levels[24]. Adiponectin mRNA levels in epididymal fat tissue increased in rats after treadmill exercise[25]. Furthermore, 3T3-L1 adipocytes treated with TNF-α showed decreased adiponectin mRNA expression[26], suggesting that TNF-α

may be responsible for regulating adiponectin expression and the production of adiponectin[23, 27]. In the present study, TNF-α, IL-6 and MCP-1 levels decreased. These cytokines and chemokines play important roles in the pathogenesis of atherosclerosis and an increase in their levels leads to endothelial dysfunction. Therefore, we speculate that Ex may ameliorate endothelial dysfunction and atherosclerotic lesion formation via anti-inflammatory effects as a consequence of increased adiponectin by a reduction in fat mass. Indeed, a significant and negative correlation between adiponectin levels and atherosclerotic areas in the thoracoabdominal aorta was observed (data not shown). In addition, we assessed aortic mRNA expression of adiponectin receptor 1 (*AdipoR1*) and adiponectin receptor 2 (*AdipoR2*) in the three groups. HFD significantly decreased aortic mRNA expression of *AdipoR1* and *AdipoR2* in ApoE-deficient mice. Expression of *AdipoR2* was higher in the HFD + Ex group than in the HFD group (data not shown). To date, the changes in the arterial expression of adiponectin, *AdipoR1*, and *AdipoR2* before and after exercise training remain unclear. Adiponectin has vascular actions that directly stimulate the production of nitric oxide (NO) in endothelial cells using phosphatidylinositol 3-kinase-dependent pathways involving the phosphorylation of eNOS at Ser1179 by AMPK[28]. Thus, the increase in adiponectin may contribute to improved vasodilatory action in the HFD + Ex group. Two studies have investigated the association between Ex and adiponectin levels in mouse models. Kimura *et al.* reported that Ex does not affect circulating adiponectin levels in Otsuka Long Evans Tokushima Fatty rats[29]. Bradley *et al.* also showed no significant changes in plasma adiponectin concentration after Ex in wild-type mice made obese by feeding a high fat and sucrose diet[30]. An explanation for the different results between the present and previously reported studies may be derived from the difference in mouse models.

Kishimoto *et al.* reported that NO and antioxidants may play an important role in the effects of exercise[13, 18]. In addition, the vascular response to acetylcholine is related to an increase in NO bioavailability as a result of the upregulation of the antioxidant defense system[31]. In the present study, markedly enhanced endothelium-dependent vasorelaxation in mice with Ex was observed compared with mice fed a standard diet. This result suggests that the NO-related system may also contribute to the preserved endothelial function.

Ajijola *et al.* recently reported that voluntary wheel-running exercise for 8 weeks reduces atherosclerotic plaque formation in 20-week-old ApoE-KO mice fed a normal chow diet, but not in those fed HFD, suggesting that Ex may reduce atherosclerosis progression in mice with early lesions but not in those with advanced complex lesions[20]. In the present study, Ex for 10 weeks reduced atherosclerotic lesion formation in 8-week-old ApoE-KO mice even on HFD. Laufts *et al.* reported that endothelium-dependent but not endothelium-independent vasorelaxation improved after voluntary wheel running exercise for 6 weeks in ApoE-deficient mice fed HFD[21]. Significant differences in endothelium-dependent vasorelaxation induced by acetylcholine between the HFD + Ex and HFD groups as well as between the HFD + Ex and STD groups were observed in the present study. These results suggest that a long period (at least 10 weeks) of voluntary running exercise may reduce the progression of atherosclerosis even in mice fed HFD that have advanced atherosclerotic lesions. In addition, endothelium-dependent vasorelaxation in the STD + Ex as well as HFD + Ex groups was significantly higher than in the STD group. These results may suggest that exercise itself could improve acetylcholine-induced relaxation regardless of the diet composition in ApoE-deficient mice. No significant differences were observed in lipid profiles between the HFD and HFD + Ex groups in the present study; however, the levels of total cholesterol and low-density lipoprotein cholesterol were significantly lower in the STD + Ex group than in the HFD and HFD + Ex groups. Furthermore, low-density lipoprotein cholesterol levels were also significantly lower in the STD + Ex group than in the STD group (data not shown); therefore, we could not reject the fact that the difference in lipid profiles between the STD + Ex and STD groups could affect endothelium-dependent vasorelaxation. We would like to clarify the precise mechanism underlying the anti-inflammatory effect of voluntary exercise in the four groups (STD/STD + Ex/HFD/HFD + Ex) in the next step.

A strong and significant correlation between the amount of exercise and the area of atherosclerotic lesion in the thoracoabdominal aorta was observed in the present study. We also confirmed the correlation between rotations (rpm/day) and atherosclerotic lesions in the aortic sinus. A moderate and negative correlation between rotations and atherosclerotic lesions in the aortic sinus was also observed, but it was not statistically significant (data not shown). There are several possible explanations, including the small number of observations in this experiment and differences in atherosclerotic process in the aortic sinus and thoracoabdominal aorta. This point will be clarified in future studies.

Conclusion

In conclusion, Ex ameliorated the progression of endothelial dysfunction and atherosclerotic lesion formation with a strong negative correlation between atherosclerotic areas and the mean running distance per day in ApoE-deficient mice, despite continued consumption of HFD. These protective roles of Ex against the progression of atherosclerosis might be derived from its anti-inflammatory effects, including an increase in adiponectin concentration followed by a decrease in the weight of white adipose tissue without a decrease in that of brown adipose tissue.

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Clinical significance of the measurements of plasma N-terminal pro-B-type natriuretic peptide levels in patients with coronary artery disease who have undergone elective drug-eluting stent implantation

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Summary
Background: N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a diagnostic biomarker for patients with congestive heart failure (CHF). However, the clinical significance of measurements of NT-proBNP levels in patients with coronary artery disease (CAD) who have undergone drug-eluting stent (DES) implantation has not been fully elucidated.
Methods and results: We recruited 280 patients with documented CAD who were scheduled for elective coronary intervention and also age- and gender-matched 140 healthy subjects. Subjects with acute coronary syndrome, ongoing CHF, and stage IV or V chronic kidney disease were excluded. We measured the plasma NT-proBNP levels and followed the CAD patients who have undergone DES implantation for up to 62 months until occurrence of major adverse cardiovascular events (MACE). Plasma NT-proBNP levels were significantly higher in CAD patients compared to control subjects ($p < 0.0001$). In the CAD group, 25 patients developed MACE and the NT-proBNP levels in the MACE group were significantly higher compared to that in the non-MACE group ($p = 0.005$). After adjusting for the confounding factors, high NT-proBNP levels were observed to be independent factors for CAD ($p < 0.0001$) and MACE ($p = 0.021$).

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Conclusions: These results demonstrated that the measurements of NT-proBNP levels may be useful in identifying high-risk subjects among CAD patients who have undergone elective DES implantation.

Introduction

A high level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a useful marker in diagnosis of acute and chronic congestive heart failure (CHF) and also an important predictor for future cardiovascular events in those patients [1–6]. NT-proBNP levels increase in patients with chronic kidney disease (CKD) and are significantly correlated with estimated glomerular filtration rate (eGFR) in patients irrespective of CHF [7,8]. Several reports also demonstrated that NT-proBNP levels were significantly higher in patients with stable coronary artery disease (CAD) than in comparison to patients without CAD [9]. Also a high NT-proBNP level predicts cardiovascular morbidity and mortality independent of traditional risk factors in patients with stable CAD [7,10].

Currently, drug-eluting stents (DES) have become the standard of care for the treatment of CAD [11]. Recent advances in DES such as sirolimus eluting-stents (SES) have substantially reduced angiographic and clinical restenosis across broad lesion and patient subsets. However, there are no data available regarding the predictive value of NT-proBNP levels for future cardiovascular events in patients with stable CAD who have undergone elective DES implantation.

The purpose of this study is to assess the clinical significance of the measurements of NT-proBNP levels in stable CAD patients who have undergone elective DES implantation. We hence compared NT-proBNP levels between the stable CAD patients with CKD stages from I to III and who were scheduled for percutaneous coronary intervention (PCI), and age- and gender-matched apparently healthy subjects. We then followed the CAD patients for up to 62 months after DES implantation until occurrence of major adverse cardiovascular events (MACE).

Methods

Subjects

We recruited 280 consecutive stable CAD patients, who were scheduled to undergo PCI from September 2004 to December 2006 at Juntendo University Hospital. The documented CAD, defined as more than 75% stenosis in at least one major coronary artery, was diagnosed by coronary angiography at nearly 2 weeks prior to PCI procedure as a safety-check for dual antiplatelet therapy. Patients with acute coronary syndrome, ongoing CHF, and CKD stage IV or V were excluded. We also recruited 352 apparently healthy subjects who had undergone a medical check-up at a medical center in the urban area during December 2004 to January 2005. After computer-based random selection, 140 age- and gender-matched healthy subjects were enrolled as the control group. None of the control subjects had a history of cardiovascular disorders or systemic inflammatory diseases. All subjects gave written informed consent and the ethical committee approved this study.

Blood sampling and biochemical analysis

Following overnight fasting, whole blood samples were drawn from the subjects. Samples from the CAD group were collected immediately prior to PCI. Following centrifugation, plasma samples were aliquoted and stored at −80 °C until further use. The plasma NT-proBNP levels were measured using a commercially available immunoassay kit (Elecsys proBNP, Roche Diagnostics, Basel, Switzerland). The lower limit of detection was observed to be 5 pg/ml. The intraassay and interassay coefficients of variation at different concentrations of NT-proBNP were as follows: 2.7% and 3.2%, respectively, at 175calculated using the following /ml; 1.8% and 2.3%, respectively, at 4962 pg/ml. The eGFR was calculated using the following equation: $eGFR = 194 \times age^{-0.287} \times Cre^{-1.094} \times 0.739$ (if female), as described previously [12]. CKD was classified into five different stages, defined with $eGFR \geq 90$, $90 > eGFR \geq 60$, $60 > eGFR \geq 30$, $30 > eGFR \geq 15$, and $eGFR > 15$ ml/min/1.73 m^2 for stages I, II, III, IV, and V, respectively [12]. Levels of total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) were measured using the standard methods. The values of low-density lipoprotein cholesterol (LDL-C) were calculated using Friedewald's formula. HbA1c (JDS) (%) was measured by the previous Japanese standard substance and measurement methods and HbA1c method (National Glycohemoglobin Standardization Program-certified).

Angiographic analyses

Selective coronary angiography was performed at baseline. The number of stenotic vessels was recorded as 1-, 2-, 3-vessel disease or stenosis of the left main artery. Lumen narrowing by >75% of the prestenotic diameter was considered to be clinically significant for stenosis, except for the left main artery where a narrowing by >50% was considered significant. Quantitative coronary angiography (QCA) assessments were carried out in all patients. The PCI was performed by implantation of DES (Cypher®, Cordis, Johnson & Johnson, Miami Lakes, FL, USA). All procedural decisions, including device selection and adjunctive pharmacotherapy were made at the discretion of the individual PCI operator. Intravenous unfractionated heparin and intracoronary nitroglycerin were administered before the PCI. After stent implantation, angiographic optimization was performed by high-pressure dilatation to achieve an acceptable angiographic result. Intravascular ultrasound (IVUS) was carried

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out according to the operator's decision. Procedural success was defined as a residual stenosis <20% without major complications. Dual antiplatelet therapy (aspirin 100 mg and ticlopidine 200 mg or clopidgrel 75 mg) was prescribed for at least 2 weeks to all the patients treated with DES. In the CAD group, selective coronary angiography was performed after PCI to assess restenosis.

Follow-up

All the patients were implanted with DES. A total of 262 patients, who had undergone a follow-up coronary angiography, were followed for up to 62 months (median 46 months). The primary endpoint of the study was MACE. MACE was defined as all cause death, nonfatal myocardial infarction (MI), unstable angina, refractory angina requiring PCI or coronary artery bypass grafting (CABG), and admission for stroke.

Statistical analyses

Continuous variables are expressed as mean ± SD and categorical variables are reported in percentages. Statistical intergroup differences were analyzed by the chi-square test, one-way ANOVA, and the Student's t test. Correlation between the two parameters was determined by simple linear regression analysis. A value of $p < 0.05$ was considered to be significant. Chi-square tests for homogeneity across strata were applied for categorical variables. We selected log NT-proBNP levels, which were normally distributed, for analysis because the plasma NT-proBNP levels were not normally distributed. Logistic regression analysis was performed to identify independent factors for the CAD including the following variables: age, gender, body mass index, eGFR, LDL-C, HDL-C, prevalence of hypertension (HT), prevalence of diabetes mellitus (DM), and log NT-proBNP levels. Cox proportional hazard analysis was performed to identify independent predictors for the MACE including the following variables; age, gender, eGFR, multivessel disease, prevalence of DM, and log NT-proBNP levels. We examined the sensitivity and specificity of various cut-off values of independent predictive factors for predicting survival and created receiver operating characteristic (ROC) curves. We divided each group into two sub-groups based on their cut-off values (determined by the ROC curve analysis), examined the results of Kaplan–Meier survival analysis, and compared the difference in survival rates using the log-rank tests.

Results

Characteristics of the subjects

The characteristics of the subjects are shown in Table 1. No significant difference for age, gender, prevalence of smoking history, distribution of CKD stage, triglyceride, or blood glucose levels between the two groups were observed. The CAD group showed higher prevalence of HT ($p < 0.0001$), DM ($p < 0.0001$), and metabolic syndrome ($p = 0.01$), and also significantly higher levels of body mass index ($p = 0.009$), waist size ($p = 0.0003$), and HbA1c (JDS) ($p = 0.011$) compared to the control group. Levels of total cholesterol ($p = 0.009$), HDL-C ($p = 0.009$), and LDL-C ($p = 0.009$) in the CAD group were significantly lower compared to the control group ($p < 0.0001$, $p < 0.0001$, $p = 0.009$, respectively).

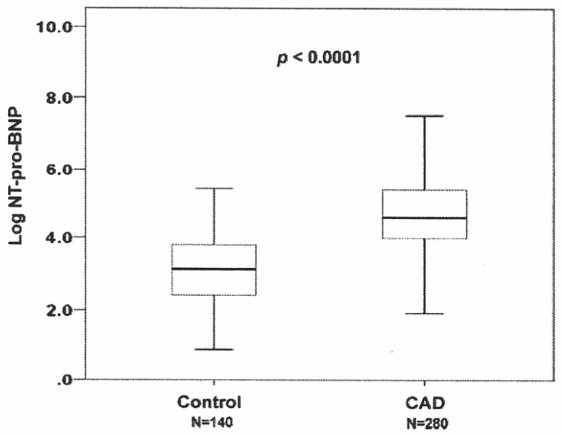

Figure 1 Comparison of logarithmically transformed N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels between the control and the coronary artery disease (CAD) groups. Lines within boxes represent median values, with top and bottom lines of boxes representing the 75th and 25th percentiles.

Plasma NT-proBNP levels in the CAD group

Plasma levels of log NT-proBNP were significantly higher in the CAD group compared to the control group ($p < 0.0001$) (Fig. 1). Negative correlation between NT-proBNP levels and left ventricular ejection fraction has been previously reported [4]. We then divided the CAD group into the Previous Event (+), including previous history of MI, PCI, and CABG ($n = 133$) and Previous Event (−) ($n = 147$) groups. Plasma levels of log NT-proBNP were significantly higher in the Previous Event (+) group compared to the Previous Event (−) group ($p = 0.006$) (Fig. 2A). However, plasma levels of log NT-proBNP were also significantly higher in the Previous Event (−) group compared to the control group ($p < 0.0001$) (Fig. 2A). It has also been reported that NT-proBNP levels were increased in patients with left ventricular diastolic dysfunction [13]. To study this effect, we excluded the patients with HT and DM from the Previous Event (+) group. Plasma levels of log NT-proBNP were observed to be significantly higher even in the Previous Event (−) HT (−) DM (−) group ($n = 23$) compared to the control group ($p = 0.0003$) (Fig. 2B).

Follow-up

MACE was observed in 25 patients (death: 3 patients; nonfatal MI: 2 patients; unstable angina requiring PCI: 8 patients; CHF admission: 4 patients; CABG: 4 patients; and stroke: 4 patients). The characteristics of the MACE (−) and MACE (+) groups are shown in Table 2. There were no significant differences between the MACE (−) and MACE (+) groups except for prevalence of DM ($p = 0.004$) and HbA1c (JDS) levels ($p = 0.004$). There were also no

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Table 1 Comparison of clinical characteristics between the control and CAD groups.

	Control	CAD	*p* value
No. of patients	140	280	
Age (years)	58 ± 7	58 ± 6	NS
Male (%)	126 (90)	252 (90)	NS
Body mass index (kg/m^2)	24.0 ± 2.6	24.9 ± 3.1	0.009
Waist (cm)	85.1 ± 7.7	88.1 ± 8.2	0.0003
Hypertension (%)	31 (22)	193 (69)	<0.0001
Diabetes mellitus (%)	16 (11)	130 (46)	<0.0001
Current smoker (%)	47 (34)	95 (34)	NS
Metabolic syndrome (%)	47 (34)	131 (46)	0.010
Chronic kidney disease			NS
Stage I (%)	10 (7)	40 (14)	
Stage II (%)	98 (70)	181 (65)	
Stage III (%)	32 (23)	59 (21)	
Estimated GFR (ml/min/1.73m^2)	70 ± 13	72 ± 16	NS
Total cholesterol (mg/dl)	213 ± 34	185 ± 36	<0.0001
Triglyceride (mg/dl)	145 ± 85	150 ± 73	NS
HDL-cholesterol (mg/dl)	63 ± 18	43 ± 11	<0.0001
LDL-cholesterol (mg/dl)	122 ± 34	113 ± 31	0.009
Blood glucose (mg/dl)	108 ± 28	112 ± 37	NS
HbA1c (%)	5.8 ± 1.0	6.1 ± 1.4	0.011
Previous myocardial infarction (%)	(−)	98 (35)	
Previous CABG (%)	(−)	27 (10)	
Previous coronary revascularization (%)	(−)	51 (18)	
Ejection fraction (%)	(N.D.)	62 ± 13	
No. of diseased vessels			
One (%)	(−)	97 (34)	
Two (%)	(−)	95 (34)	
Three (%)	(−)	84 (31)	
LMT	(−)	4 (1)	

Values are mean ± SD. CAD, coronary artery disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; CABG, coronary artery bypass graft surgery; LMT, left main trunk.

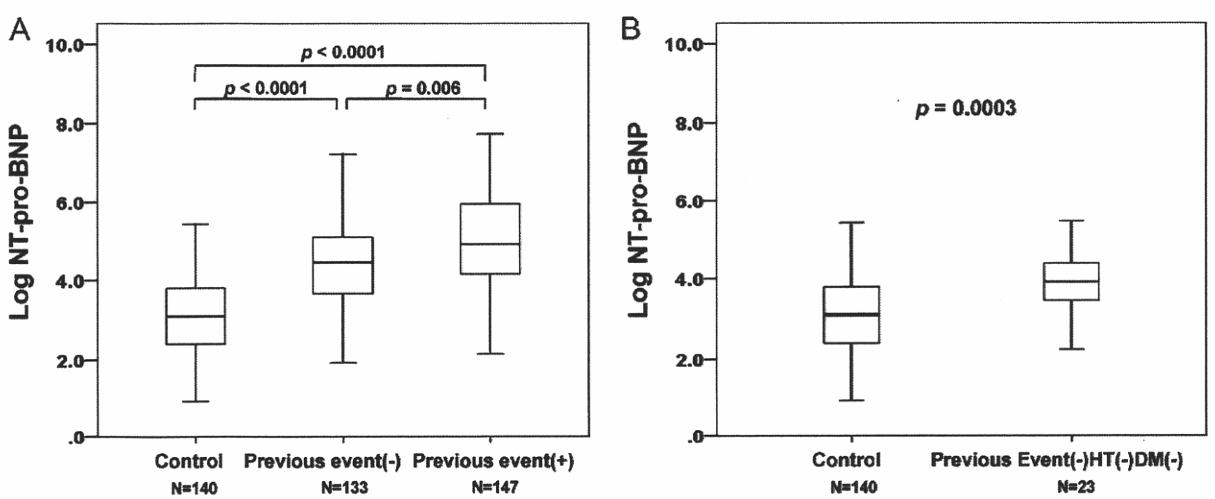

Figure 2 Comparison of logarithmically transformed N-terminal pro-B-type natriuretic peptide NT-proBNP levels (A) among the control, the Previous Event (−), and the Previous Event (+) groups and (B) between the control and the Previous Event (−) HT (−) DM (−) groups. Lines within boxes represent median values, with top and bottom lines of boxes representing the 75th and 25th percentiles HT, hypertension; DM, diabetes.

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Table 2 Comparison of clinical characteristics of MACE (−) and MACE (+).

	CAD MACE (−)	CAD MACE (+)	*p* value
No. of patients	237	25	
Age (years)	59 ± 6	59 ± 6	NS
Male (%)	215(91)	21 (85)	NS
Body mass index (kg/m^2)	24.8 ± 3.1	25.0 ± 3.5	NS
Waist (cm)	87.6 ± 7.3	90.1 ± 9.4	NS
Hypertension (%)	161 (68)	19 (76)	NS
Diabetes mellitus (%)	100 (42)	18(72)	0.004
Current smoker (%)	78 (33)	12 (48)	NS
Metabolic syndrome (%)	106 (45)	15 (60)	NS
Chronic kidney disease			NS
Stage I (%)	29 (12)	5 (20)	
Stage II (%)	157 (66)	14 (56)	
Stage III (%)	51 (22)	6 (24)	
Estimated GFR (ml/min/1.73 m^2)	72 ± 15	71 ± 17	NS
Total cholesterol (mg/dl)	186 ± 34	180 ± 40	NS
Triglyceride (mg/dl)	150 ± 72	150 ± 85	NS
HDL-cholesterol (mg/dl)	43 ± 11	44 ± 14	NS
LDL-cholesterol (mg/dl)	114 ± 31	106 ± 34	NS
Blood glucose (mg/dl)	109 ± 34	115 ± 38	NS
HbA1c (%)	6.0 ± 1.3	6.8 ± 1.8	0.004
Previous myocardial infarction (%)	82 (35)	10 (40)	NS
Previous CABG (%)	24 (10)	3 (12)	NS
Previous coronary revascularization (%)	44 (19)	7 (27)	NS
Ejection fraction (%)	63 ± 13	63 ± 14	NS
No. of diseased vessels			NS
One (%)	83 (35)	6 (24)	
Two (%)	82 (35)	7 (28)	
Three (%)	69 (29)	12 (48)	
LMT	3 (1)	0 (0)	
Lesion type			NS
A	7 (3)	0 (0)	
B1	37 (16)	5 (20)	
B2	68 (29)	5 (20)	
C	125 (53)	15 (60)	
Stent size (mm)	2.92 ± 0.39	2.86 ± 0.34	NS
Stent length (mm)	23.1 ± 5.6	23.6 ± 5.3	NS
MLD			
Pre-PCI (mm)	0.47 ± 0.32	0.42 ± 0.33	NS
Post-PCI (mm)	2.72 ± 0.46	2.66 ± 0.43	NS
Reference diameter			
Pre-PCI (mm)	2.74 ± 0.45	2.71 ± 0.46	NS

Values are ±SD. MACE, major adverse cardiac events; CAD, coronary artery disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; CABG, coronary artery bypass graft surgery; LMT, left main trunk; MLD, minimumal luminal diameter; PCI, percutaneous coronary intervention.

significant differences between the two groups regarding concomitant use of medications including antiplatelets, beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, nitrates, insulin use, and statins. Plasma levels of log NT-proBNP were significantly higher in the MACE (+) group compared to the MACE (−) group ($p = 0.0045$) (Fig. 3). The cut-off value of log NT-pro-BNP for MACE determined by ROC curve analysis was 4.93 (NT-proBNP level 139 pg/ml). Kaplan–Meier analysis demonstrated that the patients with high log NT-pro-BNP levels had a significantly higher prevalence of MACE during the entire follow-up period (log-rank test, $p = 0.0058$) (Fig. 4). The same trend was observed after dividing the CAD group into two groups using cut-off value of 125 pg/ml of NT-proBNP level (data not shown).

Multivariate analyses

Log NT-proBNP levels as well as body mass index, LDL-C, HDL-C, HT, and DM were observed to be independent for CAD by the multivariate analysis [odds ratio 3.79, 95% confidence interval (CI) 2.62–5.48, $p < 0.0001$] (Table 3A). In addition, multivariate Cox proportional hazard analysis showed that

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Table 3 Univariate and multivariate analyses.

	Univariate analysis			Multivariate analysis		
	OR	95%CI	*p*-value	OR	95%CI	*p*-value
A. Analysis for CAD						
Age	1.01	0.98–1.04	0.314			
Male	1.00	0.50–1.96	0.999			
BMI	1.10	1.02–1.18	0.001			
Estimated GFR	1.00	0.99–1.02	0.217			
LDL-C	0.99	0.98–0.99	0.010			
HDL-C	0.90	0.88–0.92	<0.0001	0.90	0.87–0.93	<0.0001
HT	7.80	4.86–12.51	<0.0001	4.45	2.23–8.88	<0.0001
DM	6.72	3.79–11.89	<0.0001	4.39	1.83–10.52	0.0009
Log NT-proBNP	3.59	2.74–4.69	<0.0001	3.79	2.62–5.48	<0.0001
	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-value	HR	95%CI	*p*-value
B. Analysis for MACE in patients with CAD						
Age	1.02	0.96–1.09	0.491			
Male	0.61	0.21–1.77	0.359			
Estimated GFR	0.99	0.97–1.02	0.824			
Multivessel disease	2.29	0.99–5.28	0.051	2.75	1.23–6.14	0.014
DM	3.52	1.42–8.76	0.007	2.64	1.09–6.43	0.032
Log NT-proBNP	1.61	1.15–2.26	0.006	1.48	1.06–2.07	0.021

CAD, coronary artery disease; MACE, major adverse cardiac events; OR, odds ratio; CI, confidence interval; HR, hazard ratio; BMI, body mass index; GFR, glomerular filtration rate; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; HT, hypertension; DM, diabetes mellitus; NT-proBNP, N-terminal pro B-type natriuretic peptide.

the levels of log NT-proBNP were a significant and independent predictor of MACE. The adjusted hazard ratios for MACE were higher by 1.48 (95%CI 1.06–2.07, $p = 0.021$) times in the high log NT-proBNP group patients compared to the low log NT-proBNP group patients (Table 3B).

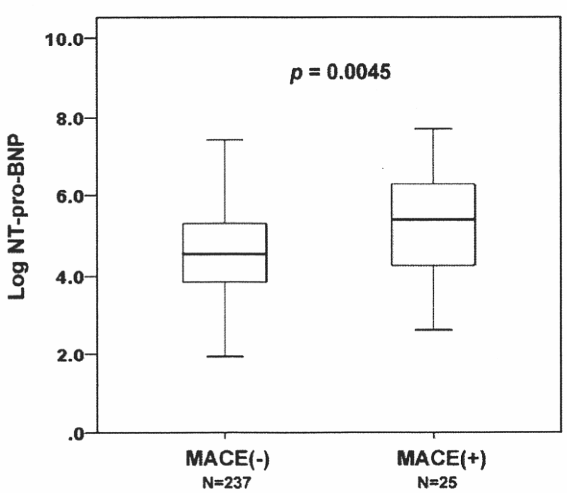

Figure 3 Comparison of logarithmically transformed N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels between the MACE (−) and the MACE (+) groups. Lines within boxes represent median values, with top and bottom lines of boxes representing the 75th and 25th percentiles. MACE, major adverse cardiovascular event.

Discussion

This study demonstrated that the plasma NT-proBNP levels were significantly higher in CAD patients even without a history of HT, DM, CHF, MI, or coronary revascularization compared to the apparently healthy subjects. In addition, high NT-proBNP level was a significant and independent predictor of MACE in CAD patients after the elective DES implantation. To the best of our knowledge, this is the first report that demonstrates the significance of the

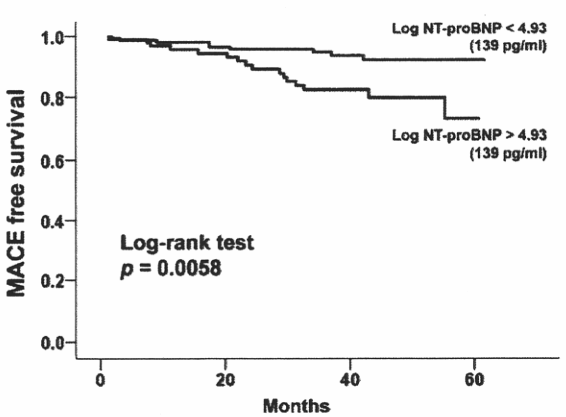

Figure 4 Kaplan–Meier curves for MACE-free survival in patients with coronary artery disease according to high and low N-terminal pro-B-type natriuretic peptide (NT-proBNP) groups. MACE, major adverse cardiovascular event.

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measurements of NT-proBNP levels in identifying the high-risk subjects in CAD patients who have undergone elective DES implantation.

Our findings are consistent with those of reported studies, which have demonstrated that the elevation of NT-proBNP level is associated with CAD [7,9,10]. BNP is synthesized as pre-proBNP$_{1-134}$ mainly in the ventricular myocardium by various stimuli such as mechanical stretch, myocardial injury, ischemic injury, endothelin-1, angiotensin II, interleukin-1β, and α-adrenergic agonists [14]. Pre-proBNP$_{1-134}$ undergoes rapid removal of a 26-amino acid signal peptide, which results in the formation of proBNP$_{1-108}$. Subsequently, proBNP$_{108}$ is enzymatically cleaved to biologically active BNP$_{1-32}$ (BNP) and biologically inactive NT-proBNP$_{1-76}$ (NT-proBNP) [14]. BNP and NT-proBNP have differential modes of clearance. BNP is cleared by receptor-mediated binding and removal by natriuretic peptide receptor-C as well as through the activity of neutral endopeptidases [15,16]. On the other hand, NT-proBNP lacks active clearance mechanisms and is cleared by organ beds with large degrees of blood flow such as kidneys [14]. Therefore, the NT-proBNP levels, rather than BNP, are thought to be associated with renal function as well as age and gender differences [17,18]. In the present study, prevalence of CKD stages from I to III was identical between the CAD patients and the age- and gender-matched controls. Moreover, higher NT-proBNP level may reflect subclinical levels of ventricular systolic or diastolic dysfunctions. However, NT-proBNP levels were significantly high even in CAD patients with no history of confounding factors for ventricular function such as HT, DM, CHF, MI, and coronary revascularization when compared to the apparently healthy subjects. Therefore, ventricular dysfunction and renal insufficiency may not sufficiently explain elevation of the NT-proBNP levels in CAD patients.

Previous studies have reported that NT-proBNP level predicts cardiovascular events, independent of traditional risk factors in patients with stable CAD [7,10]. The present study also demonstrated that the higher NT-proBNP level is an independent predictor of MACE even in CAD patients who have undergone elective DES implantation. After the adjustment of factors such as age and DM, higher NT-proBNP levels were still found to be significant for MACE. The reason why elevations of NT-proBNP levels predict future cardiovascular events may reflect subclinical levels of inducible ischemia [19]. Natriuretic peptides are secreted from the ventricle in response to ventricular stress from volume and pressure overload. Therefore, elevations of NT-proBNP level may reflect adverse hemodynamic alterations. This can probably explain the mechanism by which elevation of NT-proBNP level is associated with CAD, as discussed above. It has been reported that elevations of NT-proBNP level may also reflect vascular dysfunction, in which the natriuretic peptides produce the proliferation of vascular smooth muscle cells and change its contractility [10,20]. We did not study these vascular functions in the present study. Clinical studies to investigate the association between NT-proBNP level and vascular function (e.g. flow-mediated dilatation of brachial artery) are required in the future.

Different prognostic values have been reported between BNP and NT-proBNP [6,21]. Compared to BNP, higher NT-proBNP level is superior in predicting mortality and morbidity in patients with CHF [6] and in patients with stable CAD [21]. NT-proBNP is more stable than BNP in the blood stream due to lack of both biological activity and active clearance mechanisms. Indeed, BNP and NT-proBNP have different half-lives, which are 20 min and 120 min, respectively. In addition, NT-proBNP is stable for at least 72 h in whole blood at room temperature and requires no additives [17]. In this study, we could not elucidate the difference in clinical significances between BNP and NT-proBNP. Nevertheless, a single measurement of NT-proBNP level may prove to be a sensitive and an accurate marker to predict future cardiovascular risks in patients with stable CAD. However, further studies are needed to elucidate this probability.

The following are some limitations in our study. Firstly, it is a single center study with a small sample size. However, we prospectively enrolled consecutive CAD patients, who had undergone elective DES implantation and observed a significant association between NT-proBNP levels and MACE. Studies with larger sample size are needed to confirm this association. Secondly, we did not measure the plasma NT-proBNP levels during the follow-up period as we needed to clarify the meaning of reassessment. Thirdly, the plasma NT-proBNP level might have been affected by the treatments, including the use of antiplatelets, anti-hypertensive agents, and lipid-lowering drugs; however, the prevalence of medications at baseline was not significantly different between the patients with and without MACE. Fourthly, the detailed data of systolic and diastolic functions evaluated by echocardiography were not available for all subjects. The values of left ventricular ejection fraction were identical for the two groups. In addition, a higher NT-proBNP level was a still significant factor for MACE after the adjustment of risk factors, which may link to ventricular function such as age, HT, and DM.

Conclusions

These results demonstrated that the measurement of plasma NT-proBNP level may be useful in identifying high-risk subjects among CAD patients who have undergone elective DES implantation.

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Original Article

Plaque Regression Determined by Intravascular Ultrasound Predicts Long-Term Outcomes of Patients with Acute Coronary Syndrome

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Aim: The usefulness of drugs to treat plaque regression is assessed by intravascular ultrasound (IVUS); however, the impact of plaque regression on clinical outcomes in patients with acute coronary syndrome (ACS) has not been established; therefore, we investigated the relationship between coronary plaque regression and long-term clinical outcomes.

Methods: We analyzed data from 86 patients who underwent percutaneous coronary intervention (PCI) and who were assessed in detail at baseline and at 6 months of follow-up by measuring proximal non-culprit sites of PCI lesions using volumetric IVUS. Patients were divided according to changes in plaque volume over 6 months into one group with plaque regression ($n=55$; 64.0%) and another with progression ($n=31$; 36.0%). They were followed up observationally for a mean of 1,736 days.

Results: Baseline characteristics at the time of ACS were similar between the groups. The probability of event-free survival was significantly higher in the regression group than in the progression group as estimated by the Kaplan-Meier method (Log-rank test, $p=0.032$). Furthermore, the Cox hazards model revealed the relative contribution of plaque regression as a predictor of cardiovascular events (hazard ratio: 0.26; 95% CI, 0.07 to 0.83; $p=0.023$).

Conclusions: Plaque regression determined by volumetric IVUS over a period of 6 months was associated with a lower rate of cardiovascular events among patients with ACS. This study also demonstrated that plaque regression could be a surrogate marker of future cardiovascular events.

J Atheroscler Thromb, 2011; 18:231-239.

Key words; Plaque regression, Prognosis, Acute coronary syndrome, Atherosclerosis

Introduction

Data derived from intravascular ultrasound (IVUS) have provided important insights into the progression of atherosclerosis and remodeling in the arterial wall[1, 2]. In addition, volumetric findings of plaque change using IVUS have served as a clinical endpoint of pharmacological interventions in clinical trials[3-8]. Furthermore, IVUS meta-analysis has demonstrated that statins promote significant coronary plaque regression as assessed by IVUS[9]. The validity of plaque regression as an endpoint in clinical trials would be enhanced if a close association between regression and future clinical events could be illustrated; however, the relationship between plaque burden determined by IVUS and future cardiovascular events has not been defined.

The ESTABLISH trial demonstrated a beneficial effect of atorvastatin on plaque regression over a period of 6 months in patients with acute coronary syndrome (ACS)[10]. We extended the study of the recruited patients and applied the same protocol as the ESTABLISH trial to verify the effect of atorvastatin or changes in coronary plaque on long-term clinical outcomes of patients with ACS. That study was referred to as the Extended-ESTABLISH trial[11]. Overall, little evidence has indicated how changes in plaque volume, such as progression or regression, influence clinical

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outcomes after ACS. Thus, the present study investigated whether short-term plaque regression can become a surrogate endpoint and long-term predictor of clinical outcomes in patients with ACS.

Methods

Study Protocol

This observational cohort study included patients with ACS who were treated with PCI and underwent a complete sequential IVUS evaluation over a period of 6 months (the Extended-ESTABLISH: IVUS study). The objective was to assess the long-term clinical impact of changes in coronary plaque. The Extended-ESTABLISH trial, which was an extended version of the ESTABLISH trial, has been described in detail[10-11]. The Extended-ESTABLISH follow-up ($n=180$) demonstrated that starting atorvastatin therapy within 48 h of ACS onset followed by intensive lipid-lowering therapy for 6 months is associated with a reduced incidence of long-term cardiovascular events in patients with ACS after PCI.

The inclusion criteria for the present study was a change in the proximal site from an ACS culprit lesion that could be precisely measured by IVUS. We considered that plaque on the proximal side of a coronary artery leads to more adverse cardiac events than that on the distal side in clinical practice[12]. We also considered that plaque evaluation on the proximal side is more useful than that on the distal side for PCI under IVUS guidance, because taking the IVUS catheter to the distal side is more complicated and invasive during acute-phase treatment. Thus, this study estimated the impact of plaque volume, particularly in the proximal coronary artery segment, on long-term clinical outcomes.

We defined regression as a decrease or no change in plaque volume from baseline, and progression as an increase in the amount of plaque. Patients were assigned to either a regression or progression group and their long-term clinical outcomes from the viewpoint of follow-up IVUS were compared. Structured lipid management of all patients was discontinued after an IVUS examination at 6 months; thereafter, the attending physicians administered all patients with statins to achieve and maintain LDL-C values as close to <100 mg/dL as possible[13]. All baseline data were used at the time of ACS onset in each patient. Our institutional review board approved this study and each patient provided written informed consent to participate in the follow-up.

End Points

The primary endpoint was the first occurrence of major adverse cardiac and cerebrovascular events (MACCE), namely, all-cause death, recurrent ACS and stroke. We defined ACS as high-risk unstable angina, non-ST-elevated myocardial infarction (MI) or ST-elevated MI. An increase ($\geq$2-fold) in serum creatine phosphokinase and troponin T positivity indicated a diagnosis of MI. Moreover, recurrent ACS was defined as AMI and unstable angina requiring emergency hospitalization for either PCI or coronary artery bypass grafting. Stroke was diagnosed based on the presence of a neurologic deficit that was confirmed by computed tomography or magnetic resonance imaging.

Outcome data were collected by serial contact with the patients or their families until March 2008. The medical records of patients who died or who were treated at our hospital were analyzed. Other institutions that admitted patients provided details and causes of MACCE.

IVUS Examination and Analysis

All IVUS images were acquired as described using a 40-MHz, 2.9 F system (Boston Scientific) at baseline and follow-up[10]. After the intracoronary administration of nitroglycerin (0.2 mg), an ultrasound catheter was positioned $\geq$10 mm distal to the PCI site. The catheter was automatically retracted at 0.5 mm/s and IVUS measurements were recorded on super VHS videotape and quantified offline. Plaque volume was assessed by volumetric analysis using a Netra 3D IVUS system (ScImage, CA, USA). Baseline and follow-up IVUS images were reviewed side-by-side on a display and target segments were selected. One target segment was determined at a non-PCI site that was >5 mm proximal to the PCI site with a reproducible index side branch. Plaque was measured to determine those that were as close to 10 mm in length as possible. Segments with obvious calcification or tortuosity were avoided. An independent experienced IVUS investigator who was blinded to the patient groups and angiographic results quantified data that included vessel, lumen and plaque volumes. Standard measurements included lesion length, vessel and lumen volumes. Plaque volume was calculated as vessel volume minus lumen volume. The %change in plaque volume was defined as a change in plaque volume (follow-up minus baseline plaque volume) divided by baseline plaque volume.

Quantitative Coronary Analysis

All coronary angiographic images were analyzed

Table 1. Baseline characteristics of patients and additional therapy during follow-up

	All patients (n=86)	Regression group (n=55)	Progression group (n=31)	p value
Age (y)	62.9±9.0	63.0±10.0	62.8±8.6	0.907
Men, n (%)	71 (82.5)	45 (81.8)	26 (83.8)	0.801
Body mass index, kg/m^2	24.2±3.2	24.5±2.7	24.1±3.5	0.808
Hypertension, n (%)	50 (58.1)	35 (63.6)	15 (48.4)	0.169
Systolic blood pressure (mmHg)	139.1±23.7	139.2±24.8	139.0±22.3	0.996
Diastolic blood pressure (mmHg)	77.2±15.0	78.3±15.6	74.9±13.8	0.315
Diabetes, n (%)	34 (39.5)	20 (36.4)	14 (45.1)	0.424
Smoker, n (%)	54 (62.8)	35 (63.6)	19 (61.3)	0.829
Prior CAD, n (%)	15 (17.4)	6 (10.9)	9 (29.0)	0.037
Family history of CAD, n (%)	27 (31.4)	15 (27.3)	12 (38.7)	0.276
Classification of ACS, n (%)				0.658
AMI, n (%)	56 (65.1)	34 (61.8)	22 (70.9)	
Unstable angina, n (%)	35 (34.9)	21 (38.2)	9 (29.0)	
Culprit lesion of ACS, n (%)				0.577
Left anterior descending artery	42 (48.9)	26 (47.3)	16 (51.6)	
Left circumflex artery	16 (18.6)	12 (21.8)	4 (12.9)	
Right coronary artery	28 (32.6)	17 (30.9)	11 (35.5)	
Maximum CPK, IU/L	1638.7±1908.5	1692.2±1988.6	1551.5±1795.4	0.635
Atorvastatin therapy, n (%)	40 (46.5)	34 (61.8)	6 (19.4)	<0.0001
ACE inhibitor, n (%)	41 (48.8)	26 (49.1)	15 (48.4)	0.952
AT1 antagonist, n (%)	31 (36.9)	18 (34.0)	13 (41.9)	0.483
β-Blockers, n (%)	45 (53.6)	29 (54.7)	16 (51.6)	0.324

CAD, coronary artery disease; ACS, acute coronary syndrome; AMI, acute myocardial infarction; CPK, creatine phosphokinase; AT1, angiotensin receptor type 1. Values are the means±SD where appropriate. Categorical data analyzed by χ^2 test; continuous data analyzed by unpaired Student's t test or Mann-Whitney rank-sum test. $p<0.05$ was considered significant.

at our institution using a Cardiovascular Angiography Analysis System II (CAAS II; Pie Medical Imaging, Maastricht, The Netherlands). Experienced technicians supervised by an expert physician performed quantitative coronary analysis (QCA) of matched projections from baseline and follow-up coronary angiographic images. Reference sites were measured in every patient.

Statistical Analysis

Continuous variables are expressed as the means ±SD. Data from two independent groups were compared using a t-test or the Wilcoxon rank-sum test and intra-group data were analyzed using a paired t-test or the Wilcoxon signed-rank test. Categorical data were tabulated as frequencies and percentages and compared using the χ^2 test or Fisher's exact test. Event-free survival probabilities for MACCE were estimated using the Kaplan-Meier method and group differences were assessed using a log-rank test. Hazard ratios for each variable were calculated using a Cox proportional hazards model that included plaque regression, age, gender, BMI, hypertension, diabetes and type of ACS as variables. A two-sided p-value of <0.05 was considered significant. All data were analyzed using JMP version 7.0 for Windows (SAS Institute, Cary, NC).

Results

Characteristics of Study Subjects

Of the 114 patients who completed IVUS twice during the Extended-ESTABLISH trial, 86 (75.4%) were included in the present study. We excluded 28 patients (24.6%) if they had no adequate plaque lesions to evaluate (insufficient plaque, too short at the proximal site or poor IVUS imaging quality). Accordingly, we assessed changes in plaques using IVUS in 86 patients (mean age, 62.9±9.0 years; male, n=71; 82.5%) with ACS who underwent percutaneous coronary intervention. Plaques regressed and progressed in 55 (64.0%) and 31 (36.0%) of these patients, respectively. Baseline features at the time of ACS occurrence were similar between the two groups. Factors related to ACS, such as classification and culprit vessels, did not significantly differ between the groups.

Table 2. Baseline and follow-up intravascular ultrasound and angiographic findings

	Regression group ($n=55$)			Progression group ($n=31$)		
	Baseline	Follow-up	p value	Baseline	Follow-up	p value
IVUS measurement						
Total length, mm	89.2±24.2	89.0±22.6	0.322	84.5±24.2	85.2±22.9	0.324
Vessel volume, mm^3	180.4±64.7	170.5±64.2	<0.001	156.6±51.2	160.0±47.4	0.416
Change in vessel volume, mm^3		−9.9±15.8*			3.3±17.7	
Percent change in vessel volume		−7.3±9.5*			2.1±12.8	
Lumen volume, mm^3	95.4±41.2	95.0±43.8	0.923	80.2±27.7	76.1±26.8	0.103
Change in lumen volume, mm^3		0.4±14.9			−4.1±16.2	
Change in lumen volume (%)		−4.1±15.9			−7.6±21.1	
Plaque volume, mm^3	85.1±38.0	75.5±35.9	<0.001	76.5±36.9	83.8±37.7	<0.001
Percent plaque volume, %	46.9±11.8	44.6±13.2	<0.001	47.2±13.4	51.0±14.1	<0.001
Change in plaque volume, mm^3		−9.6±7.2*			7.4±6.5	
Percent change in plaque volume		−14.6±12.5*			10.2±8.9	
Quantitative Coronary Angiography						
Reference, mm	3.14±0.29	2.90±0.44	<0.001	3.12±0.50	2.88±0.53	<0.001
Percent change QCA, %		−7.8±9.4			−9.0±10.7	

IVUS, intravascular ultrasound; QCA, quantitative coronary angiography. Values are the means±SD where appropriate. Unpaired Student's t test or Mann-Whitney rank-sum test was used to analyze continuous data. $p<0.05$ was considered significant. Regression vs. progression group: *$p<0.01$.

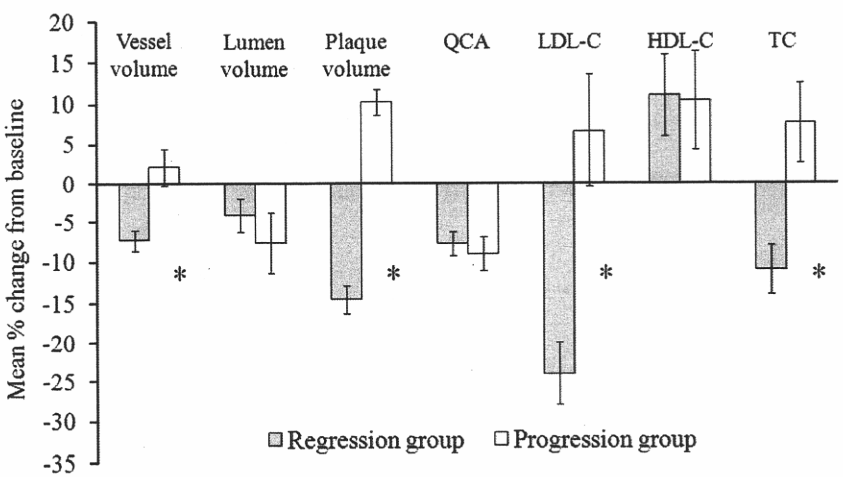

Fig. 1. Mean % change in IVUS results and lipid profile.

Plaque and vessel volumes significantly decreased in regression group, whereas the number of vessels increased with increasing plaque volume in progression group. Percent changes in LDL-C from baseline in regression and progression groups were −24.0% and 6.6%, respectively. However, % change in HDL-C did not significantly differ between groups. Regression vs. progression, *$p<0.05$.

More patients were assigned to receive atorvastatin (20 mg/day) during the first 6 months in the Extended-ESTABLISH trial in the regression group than in the progression group ($n=34$, 61.8% vs. $n=6$, 19.4%, $p<0.001$) (**Table 1**). All patients in both groups were treated after 6 months with statins, and the dose and type (including atorvastatin, pitavastatin, pravastatin, simvastatin and fluvastatin) were appropriately changed for optimal lipid management. At 6 months after the IVUS study (that is, 1 year after ACS onset), statins were administered to both groups at the same frequency (86.7% vs. 84.3%, $p=0.762$). At that time, the mean LDL-C values were 87.8±23.4 and 97.4±20.4 mg/dL, respectively ($p=0.031$).

Table 3. Blood parameters of patients at baseline and follow-up

Laboratory parameters	Baseline			Follow-up		
	Regression group (n=55)	Progression group (n=31)	p value	Regression group (n=55)	Progression group (n=31)	p value
Total cholesterol, mg/dL	190.1±37.7	181.9±35.1	0.439	165.8±41.4†	189.9±32.9	0.007
Change in TC, %				−11.0±24.4	7.6±27.7	0.002
HDL-C, mg/dL	45.8±12.2	43.1±15.9	0.112	49.3±12.5*	49.8±23.2*	0.283
Triglyceride, mg/dL	101.9±53.6	123.1±55.6	0.051	125.9±70.6*	132.1±54.2	0.269
LDL-C, mg/dL	123.9±32.5	114.3±31.0	0.263	91.4±37.9†	113.6±31.4	0.012
Change in LDL-C, %				−24.0±31.2	6.6±42.9	0.001
Change in HDL-C, %				11.0±19.8	10.3±18.3	0.371
LDL-C/HDL-C ratio	2.87±1.01	2.88±1.05	0.804	1.96±0.97†	2.63±1.11	0.009
Apolipoprotein A1, mg/dL	113.7±16.9	110.2±28.4	0.096	129.5±20.5†	125.2±25.1†	0.158
Apolipoprotein B, mg/dL	88.3±20.0	89.6±18.2	0.784	77.4±25.3†	95.0±21.2	0.003
Apo B/Apo A1 ratio	0.80±0.20	0.84±0.25	0.343	0.61±0.23†	0.78±0.24*	0.002
Apolipoprotein E, mg/dL	3.83±0.68	3.97±0.92	0.935	3.86±1.30	5.27±5.32	0.019
HbA1C, %	5.65±1.03	6.33±1.71	0.123	5.59±0.92	5.61±0.73†	0.494
hsCRP, mg/dL	0.96±1.72	1.05±2.58	0.111	0.12±0.17*	0.18±0.31*	0.373

TC, total cholesterol; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; Apo B, apolipoprotein B; Apo A1, apoprotein A1; and hsCRP, high-sensitivity C-reactive protein. Values are the means±SD when appropriate. The χ^2 test was used for categorical data and unpaired Student's t test or Mann-Whitney rank-sum test for continuous data. $p<0.05$ was considered significant. Baseline vs. follow-up: *$p<0.05$; †$p<0.01$.

Changes in IVUS Findings, QCA and Lipid Profiles

Plaque and vessel volumes significantly decreased from 85.1±38.0 to 75.5±35.9 mm^3 ($p<0.001$ vs. baseline) and from 180.4±64.7 to 170.5±64.2 mm^3 ($p<0.001$) respectively, in the regression group. In contrast, total vessel volume within the external elastic membrane increased from 156.6±51.2 to 160.0±47.4 mm^3 ($p=0.416$) with increasing plaque volume from 76.5±36.9 to 83.8±37.7 mm^3 ($p<0.001$) in the progression group. Lumen volume tended to decrease in both groups, but the difference did not reach significance (regression, from 95.4±41.2 to 95.0±43.8 mm^3, $p=0.923$; progression, from 80.2±27.7 to 76.1±26.8 mm^3, $p=0.103$). However, the reference diameter was significantly decreased in the QCA analysis of both groups (regression, from 3.14±0.29 to 2.90±0.44 mm, $p<0.001$; progression, from 3.12±0.50 to 2.88±0.53 mm, $p<0.001$) (**Table 2** and **Fig. 1**).

The level of LDL-C fell to a mean of 91.4±37.9 mg/dL (median, 85.4 mg/dL) in the regression group at follow-up IVUS ($p<0.001$), but reached a mean of 113.6±31.4 mg/dL (median, 119.6 mg/dL) in the progression group ($p=0.930$). Furthermore, the %changes in LDL-C from baseline in the regression and progression groups were −24.0±31.2% and 6.6±42.9%, respectively ($p=0.001$). The ratios of LDL-C/HDL-C and of ApoB/ApoA1 were significantly lower in the regression group than in the progression group (1.96±0.97 vs. 2.63±1.11, $p=0.009$ and 0.61±0.23 vs. 0.78±0.24, $p=0.002$, respectively; **Table 3**).

Clinical Events and Multivariate Analysis

Prognostic data were fully documented during the entire follow-up period (mean duration, 1736±589 days), during which MACCE developed in five (death, $n=2$; ACS, $n=3$) patients in the regression group and in eight (death, $n=4$; ACS, $n=3$; stroke, $n=1$) in the progression group (**Fig. 2**). Six patients developed ACS during the follow-up period, although it was not associated with the position of the measured plaque. None of the six patients with recurrent ACS had obvious stent thrombosis and three developed an obvious clinical presentation and events associated with the index stent procedure (2 of 3 in the regression group and 1 of 3 in the progression group). The remaining three patients developed new-onset ACS in a different vessel from that in which plaque was measured. The plaque volume changed significantly less in patients who developed MACCE than in those who did not (94.8±31.9 to 95.7±29.2 mm^3, $p=0.763$; 79.7±38.3 to 75.4±37.0 mm^3, $p=0.001$). Cumulative event-free survival was significantly higher in the regression group than in the progression group ($p=0.032$; log-rank test; **Fig. 3**).

The results of analysis using the unadjusted Cox proportional hazards model showed that plaque

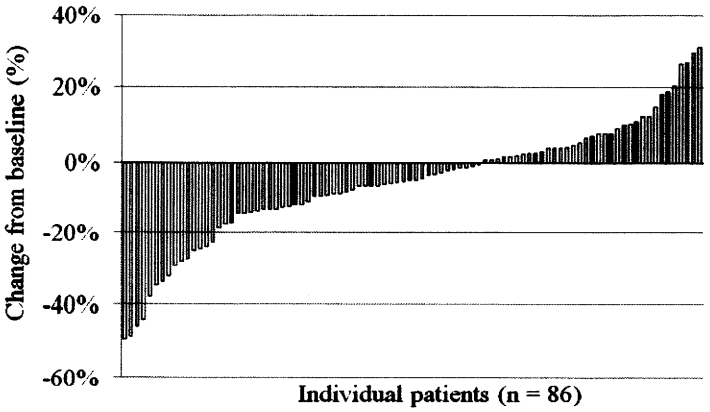

Fig. 2. Change in plaque volume from baseline using IVUS and incidence of MACCE.

Plaques regressed in 64% of patients after ACS. More patients died or developed cardiovascular events (Black bars) in progression group (25.8%) than in regression group (9.1%) during follow up.

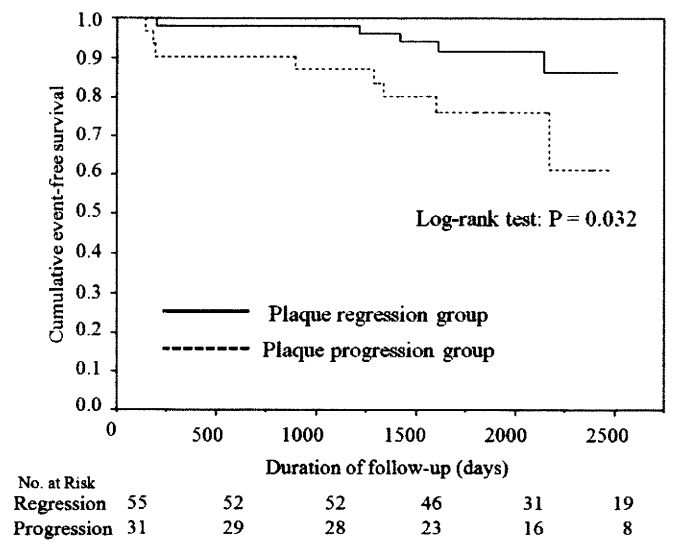

Fig. 3. Kaplan-Meier estimates of incidence of MACCE.

Cumulative event-free survival was significantly higher in regression group than in progression group (Log-rank test, $p=0.032$).

regression was significantly associated with MACCE (HR, 0.31; 95% CI, 0.09 to 0.94; $p=0.038$). The Cox proportional hazards model adjusted for plaque regression, age, gender, BMI, hypertension, diabetes and type of ACS revealed that plaque regression was significantly predictive for MACCE (HR, 0.26; 95% CI, 0.07 to 0.83; $p=0.023$; **Table 4**). In addition, when a change in plaque volume was entered in the multivariate Cox proportional hazards model as a continuous variable, a reduction in plaque volume tended to be good predictor of MACCE (10% reduction in coronary plaque volume: HR 0.69, 95%CI 0.46-1.01, $p=0.057$).

Discussion

The present study showed that short-term plaque regression determined by IVUS is associated with a

Table 4. Results of Cox proportional hazard regression analysis

Variables	Proportional hazard model					
	Unadjusted model			Adjusted model*		
	HR	95%CI	*p* value	HR	95%CI	*p* value
Plaque regression - yes	0.31	0.09-0.94	0.038	0.26	0.07-0.83	0.023
Age (1-year increase)	1.03	0.97-1.09	0.394	1.03	0.96-1.11	0.412
Female-yes	1.10	0.24-3.61	0.887	1.11	0.22-4.61	0.884
BMI (increase of 1)	1.12	0.94-1.33	0.225	1.11	0.91-1.36	0.304
Hypertension-present	1.64	0.53-6.07	0.394	1.85	0.49-8.12	0.371
Diabetes-present	1.22	0.39-3.69	0.713	0.88	0.25-2.88	0.826
Type of ACS-AMI	1.20	0.39-4.43	0.756	1.26	0.39-4.86	0.710

BMI, body mass index; ACS, acute coronary syndrome; AMI, acute myocardial infarction. $p<0.05$ was considered significant.
*Multivariate Cox model included plaque regression, age, gender, BMI, hypertension, diabetes and type of ACS as variables.

lower rate of future cardiovascular events in patients with ACS. Furthermore, this association remained significant even after adjustment for clinically important covariates. To our knowledge, this is the first study to evaluate the impact of plaque regression on the long-term prognosis of patients with ACS.

Our IVUS findings showed that both plaque and vessel volumes significantly decreased in the regression group, whereas the quantity of vessels increased with increasing plaque volume in the progression group. Atherosclerosis primarily affects the arterial wall, with atherosclerotic plaque initially growing within an outwardly expanding vessel wall (positive remodeling)[14]. Our findings indicated that the remodeling processes in progressive and regressive patients were positive and negative, respectively. Owing to this process, disease progression is not angiographically detectable during the early stages of plaque accumulation when the increasing total number of vessels occupies an increasing proportion of the plaque mass. Although angiographically determined disease progression reflects clinical prognosis, the present study found that changes in quantitative reference angiographic findings were similar between the two groups, whereas IVUS identified plaque regression or progression[15, 16]. Therefore, evaluating coronary vessel remodeling by angiography can be difficult after ACS, whereas IVUS, which can reveal arteriosclerotic changes, is more versatile. Changes in plaque measured by IVUS should lead to early risk stratification of patients after ACS over the short term.

We also believe that the targets of plaque regression are similar to those of vulnerable plaque stabilization. Vulnerable plaque results in recurrent coronary vascular events more often among patients with ACS than with stable angina[17]. We speculate that intensive lipid management of vulnerable plaque composed of various lipid constituents would result in regression compared with stable plaque. Plaques that are prone to rupture are also most likely to regress[18]. A CT angiographic study showed that patients with positively remodeled coronary lesions accompanied by low attenuation plaque are at higher risk for ACS[19]. In addition, 39.5% of patients with ACS had other coronary plaque lesions derived from a culprit lesion related to the event[20]. Vulnerable plaque in non-target vessels is reportedly an important predictor of future critical cardiac events in IVUS studies[21]; therefore, plaque regression in another coronary lesion could lead to the suppression of other events. We also considered that clinical outcome would be a more desirable and valid endpoint than simple plaque burden. Nicholls *et al.* recently reported results from six large serial IVUS imaging trials involving 4137 patients with established stable coronary artery disease who were followed up for an average of 21 months[22]. They identified a direct relationship between the burden of coronary atherosclerosis, its progression, and adverse cardiovascular events. We considered that the results of that pooled analysis revealed the prognostic significance of changes in stable plaque, whereas our findings reflect the significance of changes in unstable plaque. We thus consider that the findings of both studies demonstrate the importance of plaque progression or regression in serial IVUS studies to the clinical outcomes of patients with coronary artery disease. Consequently, the notion that coronary plaque regression could lead to an improved prognosis supports previous results.

Surrogate IVUS endpoints have recently been applied in several trials of treatment regimens based on statins for patients with coronary artery disease[4, 5].

These studies identified a close linear relationship between the degree of LDL-C reduction and changes in plaque volume. Furthermore, pleiotropic statins evidently improve endothelial dysfunction, inhibit inflammatory responses, stabilize atherosclerotic plaques and diminish smooth muscle cell accumulation and collagen deposition[18, 23]. These effects could explain the regression of atherosclerosis. Plaque regressed in about 80% of the patients enrolled in the present study who had been treated with atorvastatin. As a matter of course, we consider that the administration of statins to lower LDL-C levels is the most effective strategy for treating atherosclerotic changes such as plaque regression. We similarly postulate that blood pressure plays an important role in the promotion of coronary artery disease. Chhatriwalla *et al.* found the most attenuation of coronary plaque progression in patients with very low LDL-C (≤70 mg/dL) together with normal systolic blood pressure (≤120 mmHg)[24]. The results of another large pooled analysis also found that despite achieving intensive control of LDL-C, plaque continued to progress in 20% of patients with coronary artery disease[25]. That report also showed that these patients were more likely to progress if they had diabetes, greater increases in systolic blood pressure and smaller increases in HDL-C. Thus, we believe that these results not only provide important information about the effects of LDL-C and blood pressure on cardiovascular disease but also support the significance of multifactorial treatment for global risk in patients with coronary artery diseases including ACS.

Several limitations are associated with this study. Firstly, only 86 patients with ACS participated. Although our data are still useful because we evaluated prognosis from a novel viewpoint, further large-scale studies are warranted. Secondly, we used grayscale IVUS, so plaque composition was not evaluated; however, we believe that a reduction in the quantity of plaque approximates plaque stabilization. Future studies should evaluate the value of information generated from novel imaging techniques such as integrated backscatter and virtual histology, and whether these methods can detect changes in plaque composition as well as in plaque volume. Thirdly, we could not precisely evaluate the association among control of blood pressure, plaque volume change and clinical prognosis in the present study. Hence, the effects of multifactorial treatment, including cholesterol and blood pressure management, require further investigation to assess the prognostic significance of plaque regression.

In conclusion, plaque regression determined with volumetric IVUS over a period of 6 months predicted good long-term outcomes for patients with ACS. Furthermore, plaque regression could be a surrogate marker of prognosis from the early stage of ACS, while treatment aimed at plaque regression might improve long-term prognosis.

Acknowledgements

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