Materials and Methods

Study Population

We prospectively enrolled 126 patients with ST elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), or stable angina pectoris (SAP) with de novo culprit lesion in the native coronary artery, who underwent percutaneous coronary intervention (PCI) at Wakayama Medical University between January 2013 and January 2015. The diagnosis criteria for STEMI was based on the concurrence of all the following: 1) continuous chest pain for at least 20 min, 2) arrival at our hospital within 6 h from symptom onset, 3) persistent ST-segment elevation >0.1 mV in two or more contiguous leads on 12-lead electrocardiogram, and 4) the presence of elevated levels of troponin T (>0.1 ng/mL).

The clinical entity of non-ST-segment elevation acute coronary syndrome (NSTEACS) essentially includes various disease subsets, including unstable angina that was difficult to determine the onset, stable angina but mimicking unstable symptoms, vasospastic angina, Takotsubo myopathy. To exclude such unclear factors, we included patients with NSTEMI instead of NSTEACS in this study. The diagnosis criteria for NSTEMI was based on the concurrence of all of the following: 1) new findings of ST-segment depression >0.1 mm or transient ST-segment elevation, and/or T-wave changes in two or more contiguous leads, 2) arrival at our hospital within 6 h from the onset of the symptoms consistent with acute MI, and 3) the presence of elevated level of troponin T (>0.1 ng/mL). The definition of SAP was based on the concurrence of all of the following: 1) symptoms had been stable for more than 2 months and 2) ischemic evidence in stress test.

Patients with hemodynamic shock, renal insufficiency with baseline creatine >1.6 mg/dL (or estimated glomerular filtration rate: eGFR < 35 mL/min/1.73 m²), known systemic inflammatory conditions requiring corticosteroid therapy, evidence of active infection diseases, a left main coronary artery lesion, an extremely tortuous vessel, reference vessel diameter of >4 mm were excluded. In addition, we excluded patients with poor optical coherence tomography (OCT) images (n = 10), spontaneous coronary artery dissection (n = 1), suspected thromboembolism (n = 1), and undetermined culprit lesion (suspected vasospastic angina) or absence of thrombus in OCT (n = 3). Finally, 111 patients (STEMI: 69; NSTEMI: 25; SAP: 17) were analyzed in the present study.

This protocol was approved by the Wakayama Medical University Ethics Committee. All patients provided written informed consent before participation. This study was in compliance with the Declaration of Helsinki with regard to investigation on humans.

Study Protocol

Primary PCI was performed on all patients with acute MI. Coronary angiography was performed using 5-Fr Judkins-type catheters via the femoral or radial approach. All patients received oral aspirin, an intravenous bolus injection of 5000 U heparin, and
intracoronary isosorbide dinitrate before angiography. After completion of diagnostic angiography, the culprit lesion was identified on the basis of coronary angiography findings as well as those of electrocardiography and echocardiography. A careful manual thromboaspiration was performed in all patients using an aspiration catheter (Export®, Medtronic Japan, Tokyo). Following thromboaspiration, frequency domain OCT (ILUMIEN and ILUMIEN OPTIS, St. Jude Medical, Minnesota, USA or LUNAWAVE, Terumo, Tokyo, Japan) was used to observe the culprit lesion morphology. Because the use of GpIIb/IIIa inhibitors is not allowed in Japan, no patients were administered GpIIa/IIIb inhibitors.

**Systemic and local inflammatory activities assessments**

Peripheral blood samples were obtained at the beginning of PCI from artery sheath for the assessment of systemic inflammatory activities. Previous studies demonstrated that intra-plaque matrix metalloproteinase 9 (MMP-9) and myeloperoxidase (MPO) were released into the coronary circulation after balloon dilatation, not into the coronary sinus. For local MMP-9 and MPO assessments, we obtained coronary blood from the distal of culprit site immediately after thrombus aspiration and right after stenting using aspiration catheters (Figure 1).

All samples were immediately centrifuged at 3,000 × g for 15 min, and then stored at -80 °C. MMP-9 and MPO levels were measured at once by enzyme-linked immunosorbent assay kits (R&D systems, Minnesota, USA).

**Clinical data collection**

Clinical and laboratory data were collected prospectively during hospitalization. Patients with acute MI underwent a blood test every three hours to determine the peak levels of creatine kinase and creatine kinase MB. We noted seven coronary risk factors as follows: hypertension (blood pressure ≥140/90 mmHg and/or a history of antihypertensive medication), diabetes mellitus (HbA1c ≥6.5% or a history of antidiabetic medication), dyslipidemia (serum low density lipoprotein cholesterol >140 mg/dL, or high density lipoprotein cholesterol <40 mg/dL, and/or a history of lipid lowering medication), history of smoking, obesity (body mass index >25 kg/m²), family history of coronary artery disease, and history of MI. Composite of MI, unplanned repeat revascularization, re-hospitalization for suspected acute coronary syndrome, and cardiac death were defined major adverse cardiac events. All patients were followed up for 2 years at our clinic in Wakayama Medical University Hospital. To analyze the impact of inflammatory activities on prognosis, we divided the patients with acute MI into 2 categories according to the local MMP-9 levels. We set the upper 3rd tertile for cutoff value of high post-stent local MMP-9 levels. The 3rd tertile of post-stent local MMP-9 levels was 115 ng/mL.

**OCT Analysis**

All OCT images were analyzed using commercially available offline OCT consoles.
For OCT image analysis, consensus reading was performed by two experienced OCT investigators (A. Taruya and H. Emori) who were blinded to the angiographic and clinical data. Presence of plaque rupture (PR) and thin-cap fibroatheroma were noted. When the presence of PR was confirmed, the longitudinal morphological features of PR were classified according to the rupture site at the plaque; proximal-type, mid-type and distal-type ruptures as described in our previous report.\textsuperscript{3,4} The cross-sectional area (CSA) of ruptured cavity was measured at the site of the largest intra-plaque cavity.\textsuperscript{3} Cap broken length and longitudinal cavity length were measured according to our previous study.\textsuperscript{3} TCFA was defined as a lipid-rich plaque with an overlying fibrous cap where the minimum thickness of the fibrous cap is less than 70 µm.\textsuperscript{5} Intracoronary thrombus was identified as a mass that protruded into the lumen from the surface of the vessel wall. Red thrombus was defined as high-backscattering protrusions with signal-free shadowing, and white thrombus was defined as low-backscattering ones with signal-rich shadowing.\textsuperscript{6} Macrophages were assessed using previously reported technique.\textsuperscript{7} In short, macrophage accumulation was defined as high-intensity, signal-rich linear regions with sharp attenuation. In addition, maximum lipid arc, lesion length, proximal and distal CSA, minimum lumen CSA were measured.\textsuperscript{8}

**Angiography Analysis**

Quantitative coronary analysis (QCA) was performed by an independent PCI cardiologist (Y. Ozaki) who was unaware of OCT findings and clinical data with the CAAS system (Pie Medical Imaging, Maastricht, Netherlands). QCA was performed in initial angiogram before any intervention. Minimum lumen diameter and reference vessel diameter were measured with standard techniques. Percent diameter stenosis was calculated. The presence of residual thrombus on the final angiogram was noted.

**Statistical Analyses**

Statistical analyses were performed with R software (The R Foundation for Statistical Computing, Vienna, Austria). Results were expressed as mean ± SD for normally distributed variables and median [interquartile range] for skewed variables. Qualitative data are presented as numbers (%). Differences were tested using unpaired Student’s t-test for comparison between STEMI and NSTEMI. ANOVA was used for approximately normally distributed variables in three groups, nonparametric Mann–Whitney U test was used for skewed variables between STEMI and NSTEMI, and Kruskal–Wallis was used in three groups; and Chi-square test or Fisher’s exact test (for an expected cell value <5) was used for categorical variables. If the p value was < 0.05, the p values were adjusted with Bonferroni correction for multiple comparisons among the three groups (STEMI, NSTEMI, and SAP). Kaplan–Meier method was used for survival analysis. The log-rank test was used to compare the survival curves. Simple regression analysis was used to analyze relationships between systemic and coronary biomarkers. Multiple logistic regression analysis was performed to determine the independent determinants of STEMI. Variables that
showed p < 0.10 in the univariate analysis were used in multivariate analysis. A value of p < 0.05 was considered statistically significant.

References