SUPPLEMENTAL MATERIAL

Material and Methods

Study population

Between February 2011 and November 2012, patients who were referred to coronary catheterization laboratory for evaluation of coronary artery disease and were found to have non-obstructive disease, and who had a coronary endothelial function study were screened. Among them, patients with early coronary artery disease, defined as diameter stenosis <30% throughout entire coronary arteries on diagnostic coronary angiography, and vasoconstriction in epicardial coronary artery on endothelial function test were consecutively enrolled. Patients with ejection fraction less than 45%, acute coronary syndrome, angioplasty or bypass surgery within 6 months prior to study, uncontrolled hypertension, valvular heart disease, significant endocrine, renal disorder, or pregnancy were excluded from enrollment. Long-acting nitrates or calcium channel blockers were withheld for 36-48 hours before the study to allow assessment of baseline coronary physiology. The study protocol was approved by the Mayo Clinic Institutional Review Board and informed consent was obtained from all of the patients.

Endothelial function assessment

Coronary angiography was performed according to standard techniques using femoral approach. Coronary endothelial function assessment was performed as previously described.[1,4-6] Endothelium-dependent coronary vascular function was assessed by selective infusion of increasing concentrations of intracoronary acetylcholine for 3 min at each concentration (10^{-6}, 10^{-5} and 10^{-4} mol/L) into the left anterior descending artery (LAD). Hemodynamic data (heart

rate and mean blood pressure) and coronary angiography were obtained after each infusion. The infusion was terminated when the highest molar concentration of acetylcholine (10^{-4} mol/L) was reached. Nitroglycerin (100 μg) was then injected as an intracoronary bolus. The coronary artery diameter (CAD) at the corresponding segment of OCT in the proximal and middle LAD was quantitatively measured using QCA-CMS 6.0 (Medis, Leiden, the Netherlands) by a technician who was blinded to the clinical data and OCT analysis. The maximal effect of acetylcholine was expressed as percent change in CAD, which represents the degree of epicardial endothelial function. Endothelial dysfunction was defined as a decrease in percent change of epicardial CAD below the median value in response to the maximum dose of acetylcholine.[7] Endothelium-independent epicardial coronary artery function was determined by the change in CAD in response to intracoronary nitroglycerin bolus.

IVUS image acquisition and analysis

The IVUS examination was performed as previously described.[8] In brief, after intracoronary administration of 100-200 mg nitroglycerin, a 20-MHz, 2.9F phased-array IVUS catheter (Eagle Eye Gold, Volcano Corporation, Rancho Cordova, CA) was advanced into the LAD and automatic pullback at 0.5 mm/s was performed. The IVUS image was recorded on a DVD-Rom for later offline analysis. Offline volumetric reconstructions and analyses were performed by two experienced investigators in a blinded manner using Volcano Image Analysis Software V3.1 (Volcano Corporation). At the site of minimum lumen area in each coronary segment described below, quantitative analysis was performed and vessel and lumen area, plaque area (vessel area-lumen area) and plaque burden (plaque area/vessel area×100) were determined.[9]

OCT image acquisition and analysis

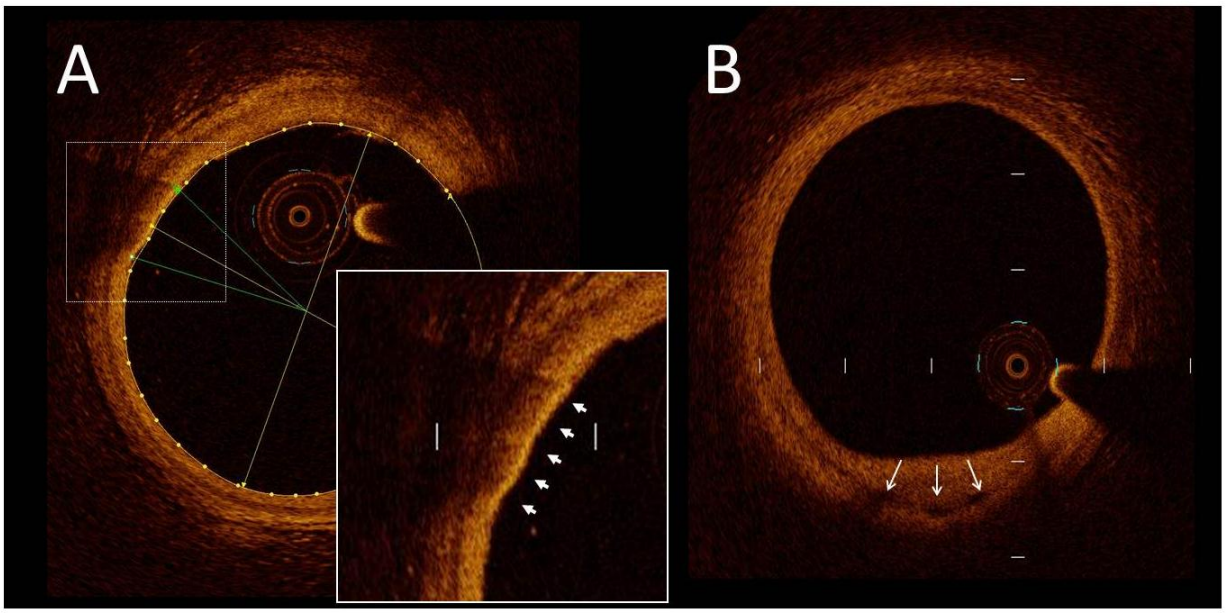
For acquisition of OCT images, C7-XR OCT Intravascular Imaging System (St Jude Medical, St Paul, MN) was used. The intracoronary OCT technique has been described previously.[10] Imaging catheter (Dragonfly, St Jude Medical, St. Paul, MN) was advanced into the mid distal segment of the LAD, and automatic pull-back at a speed of 20 mm/sec (100 frames/sec) was initiated in concordance with blood clearance by infusion of contrast media. All OCT images were digitally stored and analyzed offline using proprietary software (St Jude Medical). In order to co-register identical segments on OCT and coronary angiography, anatomical landmarks such as side branches were used. For segmental analysis, the length of each segment was defined as 20 mm and separated from the adjacent segment by at least 10 mm for matching to endothelial function data of the corresponding segment. Image review and analysis were performed by two independent examiners who were blind to clinical characteristics and the results of endothelial function assessment. Any discrepancies between two observers were resolved by consensus. Each segment was evaluated in terms of plaque type and additional 2 OCT-based characteristics including macrophage image and microchannels. Plaques were classified into 2 categories according to plaque type: lipid or fibrous.[11,12] A lipid plaque has low signal region with diffuse border. A plaque with lipid occupying two or more quadrants of any cross-sectional area within the plaque was considered as a lipid-rich plaque. A fibrous plaque was defined as a lesion with homogeneous high backscattering region. Macrophage image was defined as signal-rich distinct or confluent punctate regions that exceed the intensity of background speckle noise, which are accompanied by high behind signal attenuation.[12-14] In plaques with macrophage image, angles of macrophage arc were measured using a protractor centered on the lumen at every frame. Maximum angle and longitudinal length were recorded (**Supplemental figure A**). [15]

Microchannels were defined as intraplaque signal-voiding tubular structures with a diameter of 50-300 μm which were sharply delineated and identified on more than three consecutive cross-sectional OCT images. Maximum number and longitudinal length of microchannels were measured (**Supplemental figure B**). [16,17] Longitudinal length was measured on longitudinal view. Interobserver agreement for the presence or absence of macrophage image and microchannels were 0.84 (95%CI 0.69 to 0.99) and 0.82 (95% CI 0.66 to 0.97), respectively.

Statistics

Continuous variables are summarized as mean ± standard deviation or median [25th to 75th percentiles] as appropriate. Discrete variables are presented as frequency (percentage). Generalized estimating equations (GEE) was used to test for differences in baseline characteristics and IVUS and OCT findings between segments with and without endothelial dysfunction. GEE performed with gamma-log, binomial-logit, and an exchangeable structure in the correlation matrix for all baseline characteristics and with an independent structure for IVUS and OCT findings. GEE was necessary because of the clustered nature of ≥ 1 individual coronary segments measured from LAD, resulting in unknown correlations among measurements within these segment clusters. Similarly, generalized linear models with GEE adjustment was used to model % change in coronary artery diameter with the presence of individual OCT parameters, plus a 4-level group defined by the presence of macrophages, microchannels, or both. CAD response was grouped into quartiles and scored linearly. We tested for a trend in the prevalence of OCT characteristics across quartiles by using GEE in a generalized linear model with a logit link. All statistical tests were 2-sided and a p value < 0.05 was considered to be statistically significant. Statistical analysis was performed using SAS 9.3 software (SAS Institute, Cary, NC).

Supplemental figure



Supplemental Figure. Representative optical coherence tomography images. **A**, Macrophage image is shown as depict signal-rich distinct punctate regions with high signal attenuation (arrow heads). **B**, Microchannels are demonstrated as intraplaque signal-voiding tubular structures (arrows).

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