Endothelial Dysfunction by Brachial Artery Flow-mediated Dilatation as Predictor of Major Adverse Cardiovascular Event in Acute Coronary Syndrome

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Abstract

Background

Endothelial dysfunction (ED) is thought to be a risk predictor of acute coronary syndrome (ACS) outcome. Our aim is to analyse the ED by brachial artery flow-mediated dilatation (FMD) as a predictor of major adverse cardiac event (MACE) in ACS patients.

Method

This is a prospective cohort study with consecutive sampling for ACS in a hospital at Indonesia in April – May 2021. 69 patients met the inclusion were examined for brachial artery FMD with value < 5% stated as ED. The outcome was MACE during in-hospital, 1 month, and 6 months after hospitalization. Bivariate and multivariate logistic regression were used for analysis.

Result

From bivariate analysis, significant result was found for FMD < 5% (RR 7,13; 95%CI = 2,07–24,58; p = 0.001) with 1 month of follow-up MACE and diabetes mellitus (RR 2,57; 95%CI = 1,15 − 5,73; p = 0,043) and FMD < 5% (RR = 5,60; 95%CI = 2,44 − 12,86; p < 0.001) with 6 months of follow-up MACE. In multivariate analysis, FMD < 5% was found as the most significant predictor of 1 month and 6 months follow-up MACE.

Conclusions

ED by brachial artery FMD was an important independent predictor of the 1 month and 6 months MACE after ACS and might be useful for risk stratification outcome.

Introduction

Cardiovascular disease (CVD), which include acute coronary syndrome (ACS), accounts for 31% of global mortality.[1] In Indonesia, CVD is the main cause of morbidity and mortality, contributes to 1/3 of all deaths.[2] Major adverse cardiovascular events (MACE) are a significant cause of morbidity and mortality in patients with ACS. Detecting and treating MACE risk factors is critical for improving health quality and life expectancy.[3]

The vascular endothelium is a thin layer that covers all blood vessels and plays a critical anatomical and functional role in the development and progression of CVD. [4] Endothelial dysfunction (ED) is caused by a reduction in the production of nitric oxide (NO) by the endothelium, a molecule with significant anti-atherogenic properties.[5] ED is the earliest vascular abnormality that can occur in the process of

atherosclerosis formation and is also an important criterion in determining plaque vulnerability and thrombogenesis because ED is also the final result or "barometer" of the traditional risk factors combination for atherosclerosis.[6] Specifically, it has been reported that ED is correlated with coronary plaque progression, anatomic complexity, and vascular vulnerability.[4] ED enhances issues associated with vascular plaques, such as abrupt coronary thrombosis that can caused by platelet activation and aggregation.[7] In addition, ED is also correlated with increased risk of cardiovascular clinical outcomes.[8,9] Therefore, strategies based on endothelial function assessment may provide a better approach in preventing cardiovascular outcomes.[10]

Originally, endothelial function of coronary artery was measured during cardiac catheterization using invasive approaches. However, several non-invasive approaches for assessing endothelium function have been developed recently.[10] A widely applied and well-validated non-invasive method for assessing endothelial function is flow-mediated dilatation (FMD) of the brachial artery after the forearm ischemia period.[11] FMD was first explained by Celermajer et al., using the principle of the reactive hyperemia phenomenon, which is a brief rise in blood flow following an arterial occlusion interval that serves as a measure of endothelium-dependent vasodilator activity.[10,12] FMD is a sensitive method in endothelium-dependent vasomotor quantification, reflecting systemic endothelial function.[12] Previous studies have shown a close association between endothelial-dependent coronary artery vasodilation response to acetylcholine and brachial artery FMD, and also correlates with the occurrence of MACE.[13]

In light of the above mentioned, non-invasive FMD evaluation is thought to be a good predictor of coronary endothelial function and has a high predictive value in patients with coronary artery disease. Thus, in this study, we performed a prospective study to assess whether ED by brachial artery FMD examination was useful in providing a predictor role for in-hospital, 1 month, and 6 months follow-up MACE, in a cohort of patients with ACS.

Methods

Study design and population

This study protocol was approved by the Dr. Soetomo General Hospital Ethics Committee (ethical clearance number: 0175/KEPK/IV/2021). The principles of the Declaration of Helsinki are followed in this research. Before being included in the study, all subjects gave their informed consent. All information that could disclose the subjects' identities has been removed. This study was a cohort study conducted in Soetomo General Hospital Surabaya and enrolled 78 consecutive patients with ACS who were hospitalized from April 1st to May 31th, 2021. 9 patients excluded due to loss to follow-up, so there was a total of 69 patients analysed in this study. Patients were diagnosed with ACS based on clinical signs and symptoms of acute myocardial ischemia (unstable angina or new ischemia-related ECG changes) and/or detectable rise and/or fall in cardiac troponin (cTn) based on ESC guideline. [14,15] Unstable angina was defined as : angina at rest that often occurs >20 minutes; new onset angina (de novo) that can inhibit

physical activity; angina that occurs more frequently, lasts longer, or occurs with less activity than the one that triggered the previous angina (crescendo angina); and post myocardial infarction (MI).[16] All the patients underwent both coronary angiography and brachial artery FMD examination. All the patients were ethnic Indonesian and met the inclusion and exclusion criteria. The inclusion criteria were patients aged >18 years and willing to follow the study procedure by signing an informed consent. The exclusion criteria were (1) active liver disease on treatment or hepatic dysfunction with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels >1.5 times upper limit of normal; (2) renal dysfunction with decreased eGFR (<30 mL/dL/min) or a history of dialysis; (3) malignant disease; (4) inflammatory disease.

We collected baseline characteristics data of subjects including age, sex, body mass index (BMI), blood pressure (BP), creatinine serum, left ventricular ejection fraction (LVEF), coronary angiography, revascularization history, presence of comorbidities as coronary heart disease risk factors (hypertension, dyslipidemia, diabetes mellitus (DM), smoking history), and medications (aspirin, clopidogrel, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEi/ARB), beta blocker, calcium channel blocker (CCB), nitrate, and statin. Hypertension was obtained by examining systolic blood pressure ³140 mmHg and/or diastolic ³90 mmHg and/or a history of antihypertensive medication. Dyslipidemia was described as subjects who currently have or with history of low-density lipoprotein (LDL) cholesterol level ≥140 mg/dl, high-density lipoprotein (HDL) cholesterol level <40 mg/dl, triglycerides level ≥150 mg/dl, and/or taking statin medication. Diabetes mellitus (DM) was described as having fasting blood sugar level >126 mg/dl and HbA1c level ≥6.5% or taking antidiabetic medications. The clinical baseline characteristics of the patients are summarized in Table 1.

Brachial Artery FMD examination

Brachial artery FMD examination was carried out with subject preparation, protocol, and analysis as recommended.[11,17] Patients were instructed to fast for at least 6 hours and should avoid exercise, food/drinks containing caffeine or alcohol, and smoking for at least 12 hours before the examination. All patients rested in a quiet room, preferably a dark room for 20 minutes before FMD examination that performed at the same time (9-11 am). The subject was on supine position and a longitudinal image of the straight and unbranched segment of the brachial artery above the antecubital fossa brachial artery was recorded at baseline (at least 1 minute) using high-resolution ultrasound probe (GE Healthcare Vivid S60) that was well fixed by stereotactic clamp. The cuff placed distally, 1-2 cm below the antecubital fossa, then inflated to a supra-systolic pressure of 25-50 mmHg for 5 minutes. Post-deflation diameter measurement should be started >10 seconds before cuff release and taken at least 2 minutes. Arterial diameter changes were assessed using commercial digital software to detect vessel wall edges. FMD value (%FMD) is expressed as the percentage change in the vessel diameter over the baseline diameter at maximum dilatation during reactive hyperemia. ED was indicated by FMD value <5.0%. [18,19]. All measurements were performed by experienced inspecting technicians who were blinded to the patient's data.

Follow-up

The information about MACE was followed up prospectively during in-hospital, 1 month, and up to 6 months after hospitalization, or until the occurrence of MACE. The follow-up data was based on medical record data at Soetomo General Hospital Surabaya and by telephone interview to inquire about all interim hospital admissions, cardiovascular outpatient diagnoses and deaths in all patients. MACE was defined as all cause-death, recurrent angina, recurrent myocardial infarction (non-fatal), and stroke. Recurrent angina was defined as unstable angina without detectable rise and/or fall in cardiac troponin (cTn).[3,20–22]

Statistical analysis

Continuous variables were presented as mean ± standard deviation, and categorical variables as a percentage of the total. All statistical analyses were performed using IBM SPSS Statistics 23 software. Clinical baseline characteristics, comorbidities, and %FMD were set as independent variables. Each independent variable was analyzed using chi-square test, and student's t test or mann-whitney u test for bivariate analysis. The relative risk (RRs) and the 95% confidence interval (CIs) of each variable will be reported. Variables with p value <0.250 will be included in the multivariate logistic regression analysis. All of the statistical analyses were two-sided, and a p value <0.050 was considered to be significant.

Results

Clinical baseline characteristics, comorbidities, and %FMD

Based on data from 69 patients (Table 1), the mean age was 56 ± 9.4 years. Most of the patients were male (75.4%) with a mean BMI of 25.1 ± 3.7 kg/m2, systolic BP during examination was 126 ± 15.7 mmHg, and most of them having multivessel CAD (62.3%). On echocardiography examination, the mean LVEF was 52% ± 9.8. The highest prevalence of comorbid was dyslipidemia (76,8%). Endothelial dysfunction was assessed by brachial artery FMD examination with a value of <5% found in 17 patients (24.6%). The descriptive data of comorbidities and FMD prevalence can be seen in figure 1.

There was a significant correlation between several variables of clinical baseline characteristics on the results of FMD examination (Table 1), including statin medication (RR 0.31; 95%CI=0.14-0.65; p=0.029), serum creatinine (p=0.010), and LVEF (p=0.007). In patients receiving statin medication, the prevalence of FMD <5% was lower than in patients not receiving statin medication (76.5% vs 96.2%). Serum creatinine values were higher (1.4 ± 0.4 vs 0.9 ± 0.5) and lower LVEF values in patients with FMD <5% (46 ± 12 vs. 53 ± 8.2).

Predictors of in-hospital MACE

There was no significant correlation between clinical baseline characteristics and comorbidities with in-hospital MACE (N=1) (Table 2). The %FMD variable also did not has a significant correlation with the p

value=0.246 (Table 2). There was 1 patient experiencing in-hospital MACE (recurrent angina) that had DM, dyslipidemia, and FMD <5% (Table 7). There were no variables included as predictors of in-hospital MACE because at the bivariate analysis test, there were no variables that were significantly correlated.

Predictors of 1 month follow-up MACE

There was no significant correlation between clinical baseline characteristics and comorbidities with 1 month follow-up MACE (N=10) (Table 3). A significant correlation was found between the %FMD variable and 1 month follow-up MACE with p value=0.001. The presence of FMD <5% increased 1 month follow-up MACE by 7.13 times (95% CI=2.07-24.58; p=0.001). There were 10 patients experiencing 1 month follow-up MACE (Table 7), including 9 recurrent angina events (90.0%) and 1 recurrent myocardial infarction event (10.0%). Of all subjects who experienced the MACE, as many as 7 people (70.0%) had FMD <5%.

After bivariate analysis, variables with p value<0.250, namely multivessel CAD, DM, nitrate therapy, and FMD <5% (step 1) were included in the multivariate analysis to assess the main predictors using logistic regression. Based on the results of the multivariate test (Table 4), multivessel CAD and FMD <5% were obtained as predictors of 1 month follow-up MACE. FMD<5% is the strongest predictor compared to multivessel CAD.

Predictors of 6 months follow-up MACE

There was a significant correlation between beta blocker medication with p value=0,044 and LVEF with p value=0,039 on 6 months follow-up MACE (N=17) (Table 5). Beta blocker medication reduced 6 months follow-up MACE by 3.57 times (95%CI=0.13-0.59; p=0.044). The mean LVEF in subjects with the MACE was lower than in subjects without the MACE (47.7% ± 12.2 vs 53.4% ± 8.5). There was also a significant correlation between the DM (p=0.043) and %FMD variable (p<0.001) with 6 months follow-up MACE (Table 5). The presence of DM increased the incidence of 6 months follow-up MACE by 2.57 times (95%CI=1.15-5.73) and FMD <5% increased 6 months follow-up MACE by 5.60 times (95%CI=2.44-12.86). There were 17 patients experiencing 6 months follow-up MACE, including 13 recurrent angina events (76.4%), 3 recurrent myocardial infarction events (17.6%), and 1 patient experiencing death (5.8%). From the classical CVD risk factor such as hypertension, dyslipidemia, diabetes, and smoking history, only diabetes account responsible for MACE in 6 months, as can be seen in the event-free survival curve (figure 2). Meanwhile, of all patients who experienced the MACE, as many as 11 patients (64.7%) had FMD <5%, which also act as a strong predictor of MACE in 6 month (figure 3).

After bivariate analysis, variables with p value<0.250, namely gender, aspirin and beta blocker medication, serum creatinine, LVEF, DM, and FMD (step 1) were included in the multivariate analysis to assess the main predictors using logistic regression. Based on the results of the multivariate test (Table 6), beta blocker medication, DM, and FMD <5% were obtained as predictors of 6 months follow-up MACE. FMD <5% is the strongest predictor compared to beta blocker medication and DM.

Discussion

In this prospective cohort study, brachial artery FMD was performed in a total of 69 ACS hospitalized patients for analysis. The mean age of the patients was 56 ± 9.4 years and most of them were male (75.4%). There were 17 patients (24.6%) with FMD < 5% that was considered ED in this study. Then the patient was followed-up to determine the MACE that occurred during in-hospital, 1 month, and 6 months after hospitalization. Only 1 patient had in-hospital MACE, while during 1 month and 6 months follow-up there were 10 and 17 patients had MACE, respectively. The aim of this study was to determine ED by brachial artery FMD as a predictor of MACE in that time period.

The endothelium is involved in controlling vasomotor tone, blood fluidity, and the local balance of pro- and anti-inflammatory mediators, as well as procoagulant and anticoagulant activities.[23] NO is the most significant endothelium-derived factor, acting as a vasodilator, inhibiting platelet aggregation and leukocyte adhesion, reducing vascular smooth muscle cell migration and proliferation, and regulating oxidative stress and inflammation.[24,25] ED is a condition of relaxation and constriction of blood vessels imbalance resulting from reduced production and/or bioavailability of NO that is important in determining thrombogenesis and plaque vulnerability to rupture in CVD.[6,26] Several studies have shown that the process of ED in response to genetic and environmental risk factors, has occurred long before clinical cardiovascular disease emerged.[27,28] In addition, ED has an independent prognostic value of acute cardiovascular events in CAD. As many as 70% of subjects with AMI and/or sudden coronary death were due to plaque rupture in patients with no previous history of high risk factors for atherosclerotic disease. The factors that influence plaque rupture are not necessarily the same as those that lead to atherosclerotic formation.[29]

The traditional risk factors that were collected and analysed as confounding factors for the occurrence of MACE in this study included age, hypertension, DM, dyslipidemia, and smoking history. The percentage of patients who had hypertension was 53.6%, DM 30.4%, dyslipidemia 76.8%, and smoking history 53.6%. Several prospective studies suggest that all of these comorbidities are at risk for MACE.[30] However, in this study, only DM was found to have a significant correlation to 6 months follow-up MACE (RR 2.57; 95% CI = 1.15–5.73; p = 0.043). After multivariate analysis, DM was a predictor of 6 months follow-up MACE, but not the strongest predictor (Exp B 6.33; 95%CI = 1.37–29.22; p = 0.014). This is consistent with several studies.[20,31,32]

Endothelial function assessment can identify “susceptible” patients who are at high risk for recurrence of cardiovascular complications following an ACS.[33] FMD has been shown to have prognostic value in later cardiovascular disease (CVD), which may be better than traditional risk factors.[10,34,35] This prognostic utility is consistent across various population subgroups, although heterogeneity between studies and between subgroups was found.[10] A meta-analysis that summarized 14 prospective studies reported that per 1% higher FMD value, the risk of experiencing a cardiovascular event was found to be 13% lower.[36] Sawada et al. reported that low FMD value indicates overall coronary artery vulnerability and that patients are at increased risk of MACE compared with patients with high FMD value.[13]

Brachial artery FMD examination in this study was carried out with the recommended subject preparation and protocol.[11,17] It used a stereotactic clamp fitted to the ultrasound probe, a modified patient hand fixator, and commercial software for arterial edge detection, thereby increasing objectivity and accuracy in this study. Because medication therapy for CAD has direct and indirect vascular effects, if possible, subjects should not take all medications for ≥4 half-lives of the drug before FMD examination or at least 6 hours fasting to minimize drug effect. However, if this is not possible, because discontinuation of the drug can pose a risk to the patient's cardiovascular disease, documentation and analysis of confounding medications is needed.[11,37] In this study, there was a significant correlation only on statin medication to FMD < 5% (RR 0.31; 95%CI = 0.14–0.65; p = 0.029). In accordance with a study examining 1081 CAD patients, with at least 7 days of statin therapy, an independent effect was found on improving FMD value, independent of the lipid-lowering effect at a later date.[38]

In the analysis of the correlation between FMD < 5% with in-hospital, first and sixth months follow-up MACE, there was a significant correlation between first and sixth months follow-up MACE with p=0.001 and p < 0.001, respectively. The presence of FMD < 5% increased 1 month follow-up MACE by 7.13 times (95% CI = 2.07–24.58; p = 0.001) and increased 6 months follow-up MACE by 5.60 times (95% CI = 2.44–12.86; p < 0.001). For in-hospital MACE, there was no significant correlation with FMD value. In multivariate analysis with other variables having p < 0.250, FMD < 5% was the strongest predictor compared to multivessel CVD for 1 month follow-up MACE (p = 0.001). At 6 months of follow-up, FMD < 5% was still the strongest predictor compared to DM and beta blocker medication (p = < 0.001). This has confirmed several previous studies and meta-analyses, which have shown that FMD value was reported as predictors of MACE.[13,35,36,39,40]

After knowing the magnitude of the role of ED with brachial artery FMD on predicting MACE, it should be noted that the management of conditions related to improving FMD value must be carried out aggressively. Modena et al. found that increased FMD value indicated people with a better outcome for cardiovascular event after management of other risk factors, such as blood pressure, in hypertensive postmenopausal women.[41] Kitta et al. found that persistent and unfavourable FMD values following CAD risk factor treatment were independent predictors of cardiovascular events.[42] Takishima et al. recently revealed that in patients with stable chronic ischemic heart failure, persistently low FMD values after effective treatment for heart failure and atherosclerotic risk factors were independent predictors of cardiac events, even when other risk factors were successfully managed.[43] Overall, these findings indicate that patients may gain benefit from endothelial function-improving therapy.[35] CAD patients with ED by brachial artery FMD are recommended to be given optimal therapy against atherosclerosis risk factors to prevent MACE.[19]

Conclusion

This study showed a correlation in ED by brachial artery FMD with MACE during 1 month and 6 months after hospitalization and ED by brachial artery FMD is the most significant predictor of 1 month and 6

months follow-up MACE in ACS patients. In conclusion, this non-invasive ultrasound measurements of endothelial dysfunction may therefore be clinically useful for risk stratification of the outcome of patients with ACS.

Abbreviations And Acronyms

ACEi = angiotensin-converting enzyme inhibitor

ACS = acute coronary syndrome

ALT = alanine aminotransferase

ARB = angiotensin receptor blocker

AST = aspartate aminotransferase

AMI = acute myocardial infarction

BMI = body mass index

BP = blood pressure

CABG = coronary artery bypass graft

CCB = calcium channel blocker

CVD = cardiovascular disease

DM = diabetes mellitus

ED = endothelial dysfunction

FMD = flow-mediated dilatation

HDL = high-density lipoprotein

LDL = low-density lipoprotein

LVEF = left ventricular ejection fraction

MACE = major adverse cardiovascular events

MI = myocardial infarction

NO = nitric oxide

PTCA = percutaneous transluminal coronary angioplasty

Declarations

Competing interest

No competing interests were disclosed.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contribution

BN, IGR, and AS designed the study; BN and MFR accumulated the data; BN and REI drafted the manuscript. All the authors reviewed the manuscript.

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Tables

Table 1 Clinical baseline characteristics, comorbidities, and %FMD

Variables	*Mean* ± SD	%FMD		p	RR (95% CI)
	or N (%)	<5% (N=17)	³5% (N=52)		
Age (years)	56 ± 9.4	61 ± 7.4	57.5 ± 10	0.309	-
Gender				0.747	1.27 (0.52-3.09)
Female	17 (24.6%)	5 (29.4%)	12 (70.6%)		
Male	52 (75.4%)	12 (70.6%)	40 (76.9%)		
BMI (kg/m2)	25.1 ± 3.7	24.9 ± 4.3	24.7 ± 3.5	0.361	-
Systolic BP (mmHg)	126 ± 15.7	130 ± 17.8	125 ± 15.2	0.605	-
Multivessel CAD	43 (62.3%)	9 (52.9%)	34(65.4%)	0.528	0.68 (0.30-1.54)
Medications					
Aspirin	59 (85.5%)	12 (70.6%)	47 (90.4%)	0.105	0.40 (0.18-0.90)
Clopidogrel	45 (65.2%)	11 (64.7%)	34 (65.4%)	1.000	0.97 (0.41-2.31)
ACEi/ARB	55 (79.7%)	12 (70.6%)	43 (82.7%)	0.309	0.61 (0.25-1.44)
Beta blocker	65 (94.2%)	15 (88.2%)	50 (96.2%)	0.252	0.46 (0.15-1.35)
CCB	15 (21.7%)	4 (23.5%)	11 (21.2%)	1.000	1.10 (0.42-2.90)
Nitrate	50 (72.5%)	11 (64.7%)	39 (75.0%)	0.533	0.69 (0.30-1.61)
Statin	63 (91.3%)	13 (76,5%)	50 (96.2%)	0.029	0.31 (0.14-0.65)
Creatinine serum (mg/dl)	1.0 ± 0.5	1.4 ± 0.4	0.9 ±0.5	0.010	-
LVEF (%)	52 ± 9.8	46 ± 12	53 ± 8.2	0.007	-
Post PTCA	42 (60.9%)	10 (58.8%)	32 (61.5%)	1.000	0.91 (0.39-2.11)
Post CABG	4 (5.7%)	1 (5.9%)	4 (7.7%)	1.000	0.80 (0.13-4.85)
Hypertension	37 (53.6%)	11 (64.7%)	26 (50.0%)	0.438	1.58 (0.66-3.80)
DM	21 (30.4%)	7 (41.2%)	14 (26.9%)	0.421	1.90 (0.60-5.96)
Dyslipidemia	53 (76.8%)	14 (82.4%)	39 (75.0%)	0.743	1.60 (0.70-3.62)
Smoking	37 (53.6%)	7 (41.2%)	30 (57.7%)	0.365	0.60 (0.26-1.40)
%FMD		-	-	-	-
<5%	17 (24.6%)				
³5%	52 (75.4%)				

Table 2 Association of in-hospital MACE with clinical baseline characteristics, comorbidities, and %FMD

Variables	In-hospital MACE		p	RR (95% CI)
	Yes (N=1)	No (N=68)		
Age (years)	57	56.8 ± 9.5	0.763	-
Gender			1.000	-
Female	0 (0.0%)	17 (25%)		
Male	1 (100%)	51 (75%)		
BMI (kg/m2)	23.6	25.1 ± 3.7	0.689	-
Systolic BP (mmHg)	130	126.8 ± 15.8	0.687	-
Multivessel CAD	1 (100%)	42 (61.8%)	1.000	-
Medications				
Aspirin	1 (100%)	58 (85.3%)	1.000	-
Clopidogrel	0 (0.0%)	45 (66.2%)	0.348	-
ACEi/ARB	1 (100%)	54 (79.4%)	1.000	-
Beta blocker	1 (100%)	64 (94.1%)	1.000	-
CCB	0 (0.0%)	15 (22.1%)	1.000	-
Nitrate	1 (100%)	49 (72.1%)	1.000	-
Statin	1 (100%)	62 (91.2%)	1.000	-
Creatinine serum (mg/dl)	2.2	1.0 ±0.5	0.096	-
LVEF (%)	40	52.2 ± 9.7	0.152	-
Post PTCA	1 (100%)	41 (60.3%)	1.000	-
Post CABG	0 (0.0%)	4 (5.7%)	1.000	-
Hypertension	0 (0.0%)	37 (54.4%)	0.464	-
DM	1 (100%)	20 (29.4%)	0.304	-
Dyslipidemia	1 (100%)	52 (76.5%)	1.000	-
Smoking	0 (0.0%)	37 (54.4%)	0.464	-
%FMD			**0.246**	-
<5%	1 (100%)	16 (23.5%)		
³5%	0 (0.0%)	52 (76.5%)		

Table 3 Correlation of 1 month follow-up MACE with clinical baseline characteristics, comorbidities, and %FMD

Variables	1 month follow-up MACE		p	RR (95% CI)
	Yes (N=10)	No (N=59)		
Age (years)	56.9 ± 7.3	56.8 ± 9.8	0.778	-
Gender			0.431	0.34 (0.04-2.49)
Female	1 (10.0%)	16 (27.1%)		
Male	9 (90.0%)	43 (72.9%)		
BMI (kg/m2)	25.2 ± 5.2	25.1 ± 3.5	0.919	-
Systolic BP (mmHg)	125 ± 15.2	127.2 ± 15.9	0.817	-
Multivessel CAD	9 (90.0%)	34 (57.6%)	0.077	5.44 (0.73-40.5)
Medications				
Aspirin	9 (90.0%)	50 (84.7%)	1.000	1.52 (0.21-10.76)
Clopidogrel	6 (60.0%)	39 (66.1%)	0.730	0.80 (0.25-2.56)
ACEi/ARB	9 (90.0%)	46 (78.0%)	0.674	2.29 (0.31-16.60)
Beta blocker	9 (90.0%)	56 (94.9%)	0.474	0.55 (0.09-3.35)
CCB	1 (10.0%)	14 (23.7%)	0.442	0.40 (0.05-2.91)
Nitrate	5 (50.0%)	45 (76.3%)	0.124	0.38 (0.12-1.16)
Statin	9 (90.0%)	54 (91.5%)	1.000	0.85 (0.13-5.66)
Creatinine serum (mg/dl)	1.17 ± 0.4	1.04 ±0.3	0.607	-
LVEF (%)	50.2 ± 14.4	52.3 ± 8.9	0.682	-
Post PTCA	7 (70.0%)	36 (61.0%)	0.732	1.41 (0.39-4.98)
Post CABG	1 (10.0%)	3 (5.1%)	0.474	1.80 (0.29-10.95)
Hypertension	6 (60.0%)	31 (52.5%)	0.742	1.29 (0.40-4.19)
DM	6 (60.0%)	15 (25.4%)	0.057	3.42 (1.07-10.89)
Dyslipidemia	9 (90.0%)	44 (74.6%)	0.433	2.71 (0.37-19.85)
Smoking	5 (50.0%)	32 (54.2%)	1.000	0.86 (0.27-2.72)
%FMD			**0.001**	**7.13 (2.07-24.58)**
<5%	7 (70.0%)	10 (16.9%)		
³5%	3 (30.0%)	49 (83.1%)		

Table 4 Predictors of 1 month follow-up MACE

Variables	Bivariate Analysis		Multivariate Analysis	
	p	RR (95% CI)	p	Exp B (95% CI)
Multivessel CAD	0.077	5.44 (0.73-40.5)	0.022	16.60 (1.49-184.02)
DM	0.057	3.42 (1.07-10.89)	-	-
Nitrate	0.124	0.38 (0.12-1.16)	-	-
FMD <5%	0.001	7.13 (2.07-24.58)	**0.001**	22.56 (3.8-133.30)

Table 5 Correlation of 6 months follow-up MACE with clinical baseline characteristics, comorbidities, and %FMD

Variables	6 months follow-up MACE		p	RR (95% CI)
	Yes (N=17)	No (N=52)		
Age (years)	56.2 ± 8.1	57.0 ± 9.6	0.621	-
Gender			0.206	0.40 (0.10-1.60)
Female	2 (11.8%)	15 (28.8%)		
Male	15 (88.2%)	37 (71.2%)		
BMI (kg/m2)	24.7 ± 4.5	25.3 ± 3.5	0.617	-
Systolic BP (mmHg)	124.1 ± 16.6	127.8 ± 15.5	0.339	
Multivessel CAD	9 (52.9%)	34 (65.4%)	0.397	0.68 (0.30-1.54)
Medications				
Aspirin	13 (76.5%)	46 (88.5%)	0.248	0.55 (0.22-1.35)
Clopidogrel	11 (64.7%)	34 (65.4%)	1.000	0.97 (0.41-2.31)
ACEi/ARB	14 (82.4%)	41 (78.8%)	1.000	1.18 (0.39-3.57)
Beta blocker	14 (82.4%)	51 (98.1%)	0.044	0.28 (0.13-0.59)
CCB	3 (17.6%)	12 (23.1%)	0.746	0.77 (0.25-2.33)
Nitrate	11 (64.7%)	39 (75.0%)	0.533	0.69 (0.30-1.61)
Statin	16 (94.1%)	47 (90.4%)	1.000	1.52 (0.24-9.58)
Creatinine serum (mg/dl)	1.23 ± 0.4	1.00 ±0.3	0.129	-
LVEF (%)	47.7 ± 12.2	53.4 ± 8.5	0.039	-
Post PTCA	10 (58.8%)	34 (65.4%)	0.772	0.75 (0.24-2.32)
Post CABG	1 (5.9%)	5 (9.6%)	1.000	0.58 (0.06-5.41)
Hypertension	9 (52.9%)	28 (53.8%)	1.000	0.97 (0.42-2.22)
DM	9 (52.9%)	12 (23.1%)	0.043	2.57 (1.15-5.73)
Dyslipidemia	12 (70.6%)	41 (78.8%)	0.518	0.72 (0.30-1.74)
Smoking	10 (58.8%)	27 (51.9%)	0.830	1.23 (0.53-2.86)
%FMD			**<0.001**	**5.60 (2.44-12.86)**
<5%	11 (64.7%)	6 (11.5%)		
³5%	6 (35.3%)	46 (88.5%)		

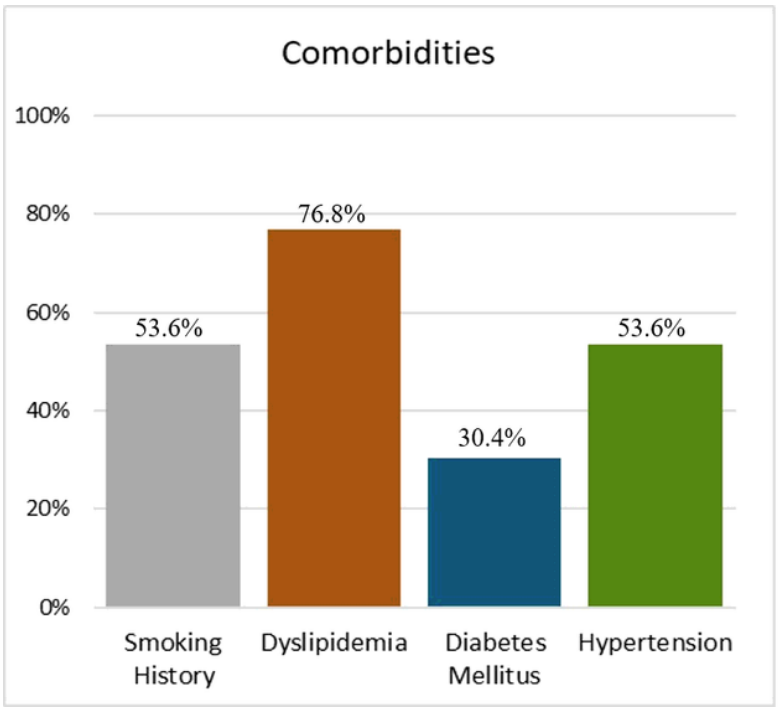
Table 6 Predictors of 6 month follow-up MACE

Variabel	Bivariate Analysis		Multivariate Analysis	
	p	RR (95% CI)	p	Exp B (95% CI)
Gender	0.206	0.40 (0.10-1.60)	-	-
Aspirin	0.248	0.55 (0.22-1.35)	-	-
Beta Blocker	0.044	0.28 (0.13-0.59)	0.024	0.03 (0.00-0.65)
Creatinine Serum	0.129	-	-	-
LVEF	0.039	-	-	-
DM	0.043	2.57 (1.15-5.73)	0.018	6.33 (1.37-29.22)
FMD <5%	<0.001	5.60 (2.44-12.86)	**<0.001**	15.11 (3.33-68.60)

Table 7 in-hospital, 1 month and 6 months follow-up MACE

Events	FMD		*p*
	<5% (N=17)	**³5% (N=52)**	
In-hospital MACE	1 (5.8%)	0 (0.0%)	**0.246**
All cause death	0 (0.0%)	0 (0.0%)	
Recurrent angina	1 (100%)	0 (0.0%)	
Recurrent myocardial infarction	0 (0.0%)	0 (0.0%)	
Stroke	0 (0.0%)	0 (0.0%)	
1 month follow-up MACE	7 (41.1%)	3 (5.7%)	**0.001**
All cause death	0 (0.0%)	0 (0.0%)	
Recurrent angina	6 (85.7%)	3 (100%)	
Recurrent myocardial infarction	1 (14.2%)	0 (0.0%)	
Stroke	0 (0.0%)	0 (0.0%)	
6 months follow-up MACE	11 (64.7%)	6 (11.5%)	**<0.001**
All cause death	0 (0.0%)	1 (16.6%)	
Recurrent angina	9 (81.8%)	4 (66.6%)	
Recurrent myocardial infarction	2 (18.1%)	1 (16.6%)	
Stroke	0 (0.0%)	0 (0.0%)	

Figures



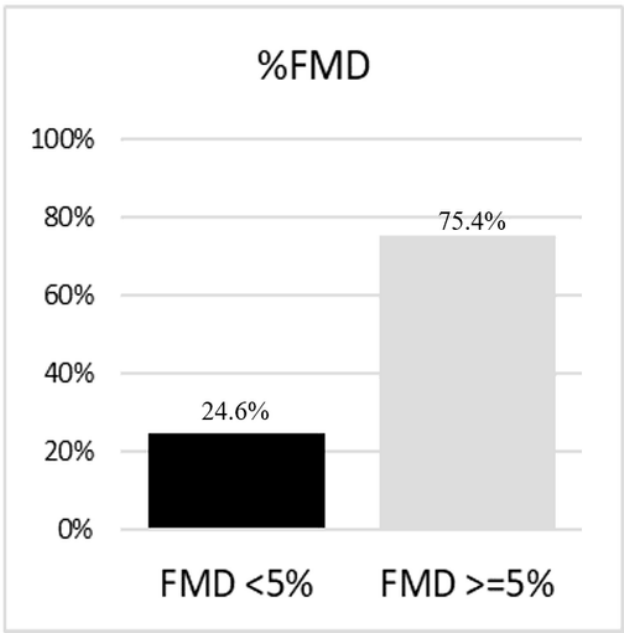


Figure 1

Comorbidities and %FMD prevalence

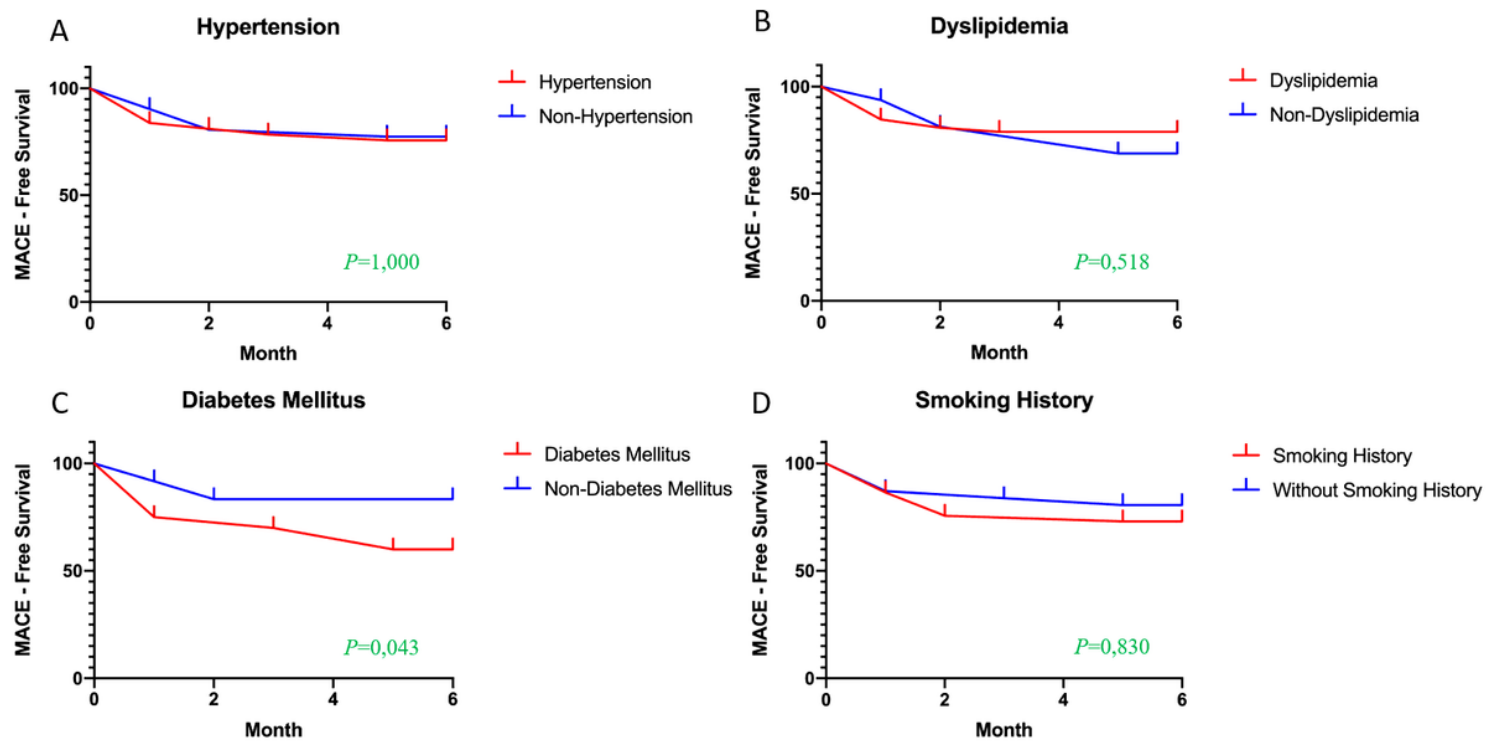


Figure 2

MACE-free survival curve based on comorbidities variable. A. Hypertension B. Dyslipidemia C. Diabetes Mellitus D. Smoking history

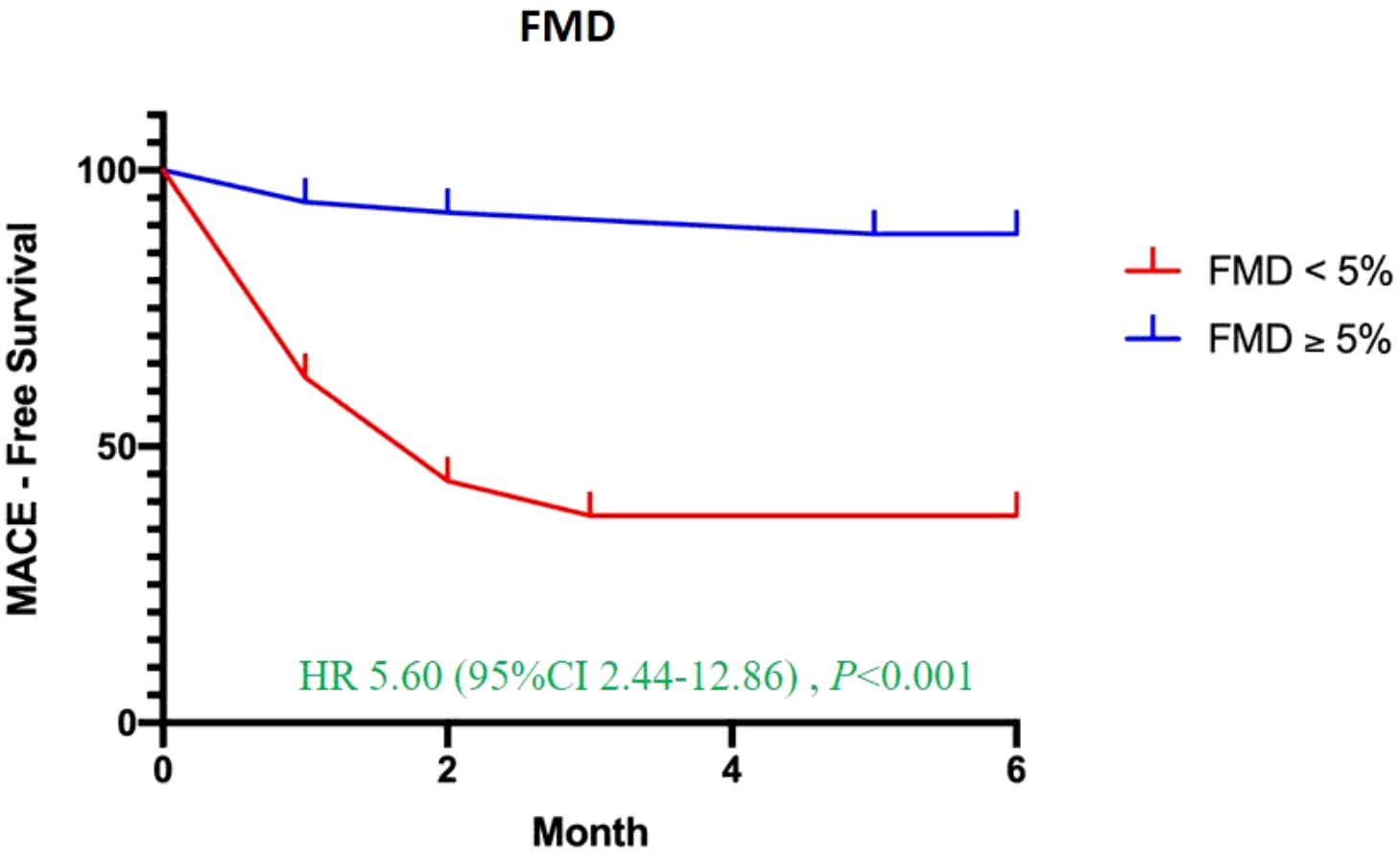


Figure 3

MACE-free survival curve based on %FMD variable