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ORIGINAL ARTICLE

Brachial artery endothelial function predicts platelet function in control subjects and in patients with acute myocardial infarction†

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Abstract

Platelet activation occurs in an endothelium-dependent flow-mediated dilation (FMD) impairment environment. The aim of this study was to explore the association between platelet reactivity and brachial artery FMD in individuals without established cardiovascular disease (controls) and acute myocardial infarction (AMI) patients. We prospectively assessed brachial artery FMD in 151 consecutive subjects, 104 (69%) controls, and 47 (31%) AMI patients; 115 (76%) men, mean age 53 ± 11 years. Following overnight fasting and discontinuation of all medications for ≥ 12 h, percent change in brachial artery FMD (%FMD) and endothelium-independent, nitroglycerin-mediated vasodilation (%NTG) were assessed. Platelet aggregation was assessed by conventional aggregometry, and platelet adhesion and aggregation under flow conditions by cone-and-plate(let) technology (Impact-R). Smoking, diabetes, and hypertension were more common in AMI compared to control subjects ($p < 0.01$ for all). Furthermore, aspirin, clopidogrel, beta-blockers, angiotensin-converting enzyme inhibitors, and statin administration were more common in AMI compared to controls ($p < 0.01$ for all). %FMD but not %NTG was significantly lower in AMI patients compared to controls ($10.2 \pm 4.2\%$ vs. $15.4 \pm 4.4\%$; $p < 0.001$ and $17.2 \pm 3.9\%$ vs. $18.0 \pm 3.7\%$, $p = 0.803$, respectively). %FMD was significantly and inversely associated with all platelet functions tests ($p < 0.001$) in all study participants. In a multivariate logistic regression (unadjusted and adjusted for age, gender, smoking status, diabetes mellitus, hypertension, hypercholesterolemia, overweight, family history, and concomitant medications), %FMD remained the best predictor of platelet function, irrespective of group allocation (AMI patients or controls). In conclusion, FMD is inversely correlated to platelet reactivity in both controls and AMI patients.

Keywords: *Endothelium, platelets, myocardial infarction, nitric oxide, endothelial function*

Introduction

Endothelial function, defined as the sum of auto-, para-, and endocrine interactions of endothelial cells, counteracts platelet function and vascular inflammation, and inhibits vascular cell proliferation and vasoconstriction [1]. The vascular endothelium inhibits platelet adhesion and, to a lesser extent, platelet aggregation, by the release of nitric oxide (NO) *via* increased cytosolin levels of soluble cyclic guanosine monophosphate [2–6]. Furthermore, the magnitude of platelet inhibition correlates with the endothelial vasodilatory effect, which is reduced in patients with atherosclerosis and endothelial dysfunction [7]. It has been demonstrated that

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endothelial dysfunction reduces the bioavailability of NO, which plays a key role in the regulation of vascular and perivascular homeostasis, including platelet function [8]. Thus, while sustaining vascular damage, endothelial cells group with their fellow platelets toward boosting the very same thrombotic state they oppose in healthy subjects, thereby laying the foundation for what is termed endothelial dysfunction [9]. Although platelet activation plays a major role in the pathogenesis of atherothrombotic events, only few data exist regarding the predictors and modulators of platelet reactivity both in healthy subjects and in patients with acute atherothrombotic events. Thus, clarification of these predictors might improve our understanding of the atherothrombotic process and potentially improve the prevention and treatment of such events. We hypothesized that endothelial function may play an important role in determining platelet reactivity in both healthy and acute myocardial infarction (AMI) patients.

The aim of this study, therefore, was to explore the association of platelet and endothelial function assessed by brachial artery flow-mediated dilation (FMD) in individuals without established cardiovascular disease (controls) and in AMI patients.

Methods

Study design and population

We prospectively recruited consecutive subjects without established cardiovascular disease (controls) and patients with AMI. The control subjects included individuals with no history of chest pain or myocardial infarction, coronary artery bypass grafting surgery, coronary angiography with angioplasty and/or stenting, cerebrovascular accident or peripheral vascular disease, and who had normal electrocardiograms (ECGs), and echocardiography. The AMI patients included those hospitalized with a diagnosis of AMI $\leq$4 days, with typical chest pain lasting >20 min at rest, ECG changes compatible with either ST-segment or non ST-segment elevation and elevated cardiac troponin I. All AMI patients were treated with aspirin given as a loading dose of 300 mg and 100 mg/day thereafter, and with clopidogrel given as a loading dose of 600 mg and 75 mg/day thereafter. All AMI patients underwent percutaneous coronary intervention (PCI) $\leq$24 h.

Exclusion criteria included atrial fibrillation, sinus bradycardia (heart rate <50 bpm) without a pacemaker, sick sinus syndrome, second or third degree atrio-ventricular block, intolerance to nitrates, chronic or acute heart failure, renal failure with serum creatinine >1.5 mg dl^{-1}, history of drug or alcohol abuse, chronic liver disease, or refusal to sign the informed consent form.

Following an overnight fast and discontinuation of all medications for $\geq$12 h, brachial artery reactivity testing, ECG and echocardiographic assessment, and blood tests for measurements of platelet function, lipids, blood cell count, electrolytes, fasting glucose, homocysteine, asymmetric dimethylarginine (ADMA), and high-sensitivity C-reactive protein (hs-CRP), were performed. The blood samples, except those for platelet function, were centrifuged immediately for 15 min at 3000 min^{-1}. The sera were stored at -20°C, and tested at the end of the study. Blood samples for platelet function were assessed immediately after the blood was drawn. All blood samples were evaluated in the same laboratory and by the same operator who was blinded to the patients' clinical status and endothelial function results. The hospital review board approved the study, and all participants gave written informed consent.

Endothelial function assessment

Vascular function protocol. Endothelial function in the form of endothelium-dependent FMD in the brachial artery was measured, as previously described [10–16]. Briefly, FMD was assessed by a single ultrasonographer blinded to the subjects' clinical status (i.e. either controls or AMI patients) and platelet function results. The test was performed in the subject's left arm while in a recumbent position in a quiet, temperature-controlled room (22°C) after a 10-min equilibration period. Using a 15-6 MHz linear array (15-6 L HP) ultrasound (HP SONOS 7500 cv system, Agilent Technologies, Inc., Andover, MA, USA), the brachial artery was longitudinally imaged approximately 5 cm proximal to the antecubital crease. An ECG was monitored continuously and blood pressure was taken in the left arm each minute throughout the study.

Study phases.

Endothelium-dependent FMD

Following a 2-min baseline period, a longitudinal image of 3 cm of vessel without color flow was obtained and frozen for 5 s. The image was then unfrozen and switched to a pulse wave Doppler for 5 s at a sweep speed of 50 mm s^{-1}. A pneumatic tourniquet (Hokanson, AG101, Bellevue, Washington, DC, USA), placed around the left upper arm proximal to the target artery (upper-arm occlusion), was inflated after the baseline phase to 50 mmHg above the subject's systolic blood pressure (or until no blood flow was observed in the brachial artery by Doppler probe), and held for 5 min. Increased flow was subsequently induced by sudden cuff deflation, followed by a continuous scan at deflation, at 20, 40, 60, 90, and 120 s, with

frozen and Doppler measurements recorded at similar intervals from baseline.

Nitroglycerin-induced (non-endothelium-dependent) vasodilatation

A second 2-min baseline resting scan was recorded to confirm vessel recovery 13 min after cuff deflation. Scanning was performed continuously for 5 min following administration of a sublingual nitroglycerin (NTG) tablet (Nitrostat, 0.4 mg, Park-Davis, New York, NY, USA).

Data analysis. Ultrasound images were recorded on an S-VHS videotape with a SLV-RS7 videocassette recorder (SONY, Japan). Brachial artery diameter was measured from the anterior to the posterior interface between the media and adventitia (''m line'') at a fixed distance. The mean diameter was calculated from four cardiac cycles synchronized with the R-wave peaks on the ECG. All measurements were calculated at end diastole to avoid possible errors resulting from variable arterial compliance. The internal diameter was calculated with PC Prosound software (USC, Los Angeles, CA, USA) using a Horita Data Translation Image Processing board (DT2862-60 Hz; Mission Viejo, CA, USA) [11]. %FMD and %NTG were expressed as the percent change relative to that at the initial resting scan. %FMD was computed from the formula [(maximum diameter − baseline diameter)/ baseline diameter × 100]. %FMD, using the maximal brachial artery diameter achieved after cuff deflation, was used as an index of endothelium-dependent dilation; percent dilatation obtained 5 min after the administration of nitroglycerin represented %NTG. FMD and NTG vasodilatory response measurements were carried out in a blind fashion. The intra-observer correlation coefficients for baseline and deflation diameters were 0.99. The absolute error between measurements ranged from 0–0.12 mm (for brachial artery diameter) and 0.02–2.98% (for FMD). The determination of endothelial function was performed in accordance with published guidelines [17].

Platelet function assessment

Blood for platelet reactivity was drawn with a loose tourniquet through a short venous catheter into 3.2% sodium citrate-containing tubes. Blood samples, drawn from the AMI patients on the fourth day after admission and from the control cohort during the endothelial function ambulatory study, were centrifuged ($140 \times g$ for 12 min), and the upper fraction was collected as platelet-rich plasma. The remaining blood was centrifuged again ($1660 \times g$ for 12 min) to obtain platelet-poor plasma. Two tests directed at platelet function measurements were included in this study: (1) platelet aggregation by conventional aggregometry; and (2) platelet function under flow conditions.

Platelet aggregation was evaluated by a turbidimetric PACKS-4 aggregometer (Helena Laboratories, Beaumont, Texas) using adenosine diphosphate (ADP, 5.5 μM) and arachidonic acid (AA, 1.6 mM) as platelet agonists. Platelet function under flow conditions was studied using the Impact-R® device (DiaMed, Cresier, Switzerland), based on the cone and plate(let) analyzer (CPA) technology [18]. Briefly, the whole blood sample (130 μL) was placed in a polystyrene well and subjected to flow ($1800\ s^{-1}$ for 2 min) using a rotating cone. The well was washed and stained with the May-Grünwald stain. Platelet deposition was evaluated by the Impact-R image analysis system. Platelet adhesion, reflected as surface coverage (SC, %) by adherent platelet particles including single platelets and aggregates, was measured. Platelet aggregation under flow conditions was evaluated as the mean size of surface-bound aggregates designated as aggregate-size (μm^2).

Statistical analysis

A power calculation assessment based on the correlation between endothelial function and platelet function was performed prior to starting the study. Using a conventional estimate of sample size and assuming a 5% relative difference provided a standardized difference of 0.45. Using a p-value of 0.05, and a power of 80%, the number of patients to be studied per group was estimated to be 30. It was concluded by Fisher Z approximation test that a total of 30 participants per group would suffice to estimate a correlation with 0.29 precision and a 95% confidence interval. Values are expressed as mean ± SD for continuous variables, and frequencies (%) for categorical variables. Distributions of continuous variables were assessed using the Kolmogorov–Smirnov test. Endothelial function parameter (%FMD) was further assessed dichotomously on the basis of its median value. Correlations between platelet function tests and %FMD were assessed using the Spearman correlation test. Comparisons between control subjects and AMI patients, as well as between the ≥ median %FMD and the < median %FMD groups were assessed using the independent t-test for continuous variables and χ^2 test for categorical variables. Multivariate analysis of platelet function tests (< and ≥ the median) was performed using the logistic regression model. Conventional confounders of platelet function tests were chosen for the final model based on the associations with %FMD. All statistical calculations were performed using the SPSS 17

Table I. Patient characteristics.

	Total ($n=151$)	Controls ($n=104$)	Acute MI ($n=47$)	p-Value
Age (y)	53 ± 11	51 ± 11	57 ± 11	0.002
Males	115 (76%)	75 (72%)	40 (85%)	0.083
Cardiovascular risk factors				
Smoking	29 (19%)	9 (9%)	20 (43%)	0.001
Diabetes mellitus	13 (9%)	5 (5%)	8 (17%)	0.010
Hypertension	39 (26%)	18 (17%)	21 (47%)	<0.001
Overweight (BMI $>25\,kg\,m^{-2}$)	102 (69%)	69 (66%)	33 (70%)	0.399
Hypercholesterolemia	79 (53%)	57 (55%)	22 (47%)	0.506
Family history	30 (20%)	49 (47%)	12 (27%)	0.020
Concomitant medications				
Aspirin	59 (39%)	12 (12%)	47 (100%)	<0.001
Clopidogrel	47 (31%)	0	47 (100%)	<0.001
Beta-blockers	27 (18%)	1 (1%)	26 (55%)	<0.001
Calcium channel blockers	2 (1%)	0	2 (4%)	0.554
Angiotensin-converting enzyme inhibitors	36 (24%)	0	36 (77%)	<0.001
Statins	51 (34%)	7 (7%)	44 (94%)	<0.001
Laboratory results				
Fasting glucose ($mg\,dl^{-1}$)[a]	96 ± 24	89 ± 13	110 ± 35	<0.001
Total cholesterol ($mg\,dl^{-1}$)	187 ± 38	194 ± 30	173 ± 48	0.002
LDL-C ($mg\,dl^{-1}$)	116 ± 27	120 ± 22	110 ± 35	0.037
Triglycerides ($mg\,dl^{-1}$)	134 ± 78	134 ± 80	134 ± 62	0.989
HDL-C ($mg\,dl^{-1}$)	45 ± 11	48 ± 11	39 ± 9	<0.001
Homocysteine ($\mu mol\,l^{-1}$)	13 ± 7	12 ± 7	14 ± 7	0.278
Hs-CRP ($mg\,L^{-1}$)	2.5 ± 1.9	1.4 ± 1.2	19.8 ± 8.6	<0.001
ADMA ($\mu mol\,l^{-1}$)	0.69 ± 0.22	0.67 ± 0.24	0.72 ± 0.20	0.057
Echocardiography				
Left ventricular ejection fraction (%)	55 ± 10	60 ± 5	46 ± 11	<0.001

Notes: BMI, body mass index; MI, myocardial infarction.
Values are presented as mean $\pm$ SD or absolute numbers (percent).
p-Value for comparing healthy subjects and myocardial infarction patients.
[a]Blood glucose levels were measured after a 12-h fast.

software (statistical package for social science, SPSS Inc.). $P<0.05$ represented statistical significance.

Results

Study population

The study cohort comprised 151 consecutive subjects, of whom 47 had AMI and 104 were without established cardiovascular disease (controls). Table I summarizes baseline characteristics and clinical features of the entire study population. Compared with controls, the AMI patients were older and more likely to be males. Smoking, diabetes, and hypertension were more common in the AMI patients, while a family history of premature coronary artery disease (CAD) was more common among the controls. Furthermore, aspirin, clopidogrel, beta-blockers, angiotensin-converting enzyme inhibitors, and statin administration were more common in the AMI patients compared to controls. While homocysteine levels were similar in both groups, fasting blood glucose, hs-CRP, and ADMA were higher in the AMI patients. Conversely, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were lower in the AMI patients compared to controls.

Endothelial function assessment

Although baseline brachial artery diameter was similar in both groups (AMI patients: 5.61 ± 0.83 mm; controls: 5.63 ± 1.01 mm, $p=0.922$), %FMD ($10.2\pm4.2\%$ vs. $15.4\pm4.4\%$, respectively, $p<0.001$) but not %NTG ($17.2\pm3.9\%$ vs. $18.0\pm3.7\%$, respectively, $p=0.803$) was significantly lower in the AMI patients compared to controls. No adverse effects were noted while performing brachial artery testing.

Platelet function measurements

As expected, both ADP- and AA-induced platelet aggregations were lower in the AMI patients who were treated with aspirin and clopidogrel. On the other hand, platelet adhesion, which is not influenced by anti-aggregate therapy and therefore reflects baseline platelet reactivity, was greater in the AMI patients compared to controls, as seen by

Table II. Platelet function test results.

	Controls ($n=104$)	AMI ($n=47$)	p-Value
Conventional aggregometry			
ADP-induced aggregation (%)	62 ± 28	48 ± 26	0.006
AA-induced aggregation (%)	79 ± 27	46 ± 33	<0.001
Platelet deposition under flow conditions			
Average size (μm^2)	38 ± 20	41 ± 24	0.340
SC (%)	9.8 ± 6.6	12.9 ± 9.2	0.020

Notes: ADP, adenosine diphosphate; AA, arachidonic acid. Values are presented as mean ± SD.

Table III. Correlation between platelet function and %FMD (age-adjusted).

	%FMD	
	r	p
ADP-induced platelet aggregation (%)		
Total	−0.50	<0.001
Control subjects	−0.63	<0.001
AMI patients	−0.53	<0.001
AA-induced platelet aggregations (%)		
Total	−0.53	<0.001
Control subjects	−0.50	<0.001
AMI patients	−0.82	<0.001
Aggregate size (μm^2)		
Total	−0.54	<0.001
Control subjects	−0.56	<0.001
AMI patients	−0.63	<0.001
SC (%)		
Total	−0.58	<0.001
Control subjects	−0.57	<0.001
AMI patients	−0.68	<0.001

Notes: %FMD, percent flow-mediated vasodilation; ADP, adenosine diphosphate; AA, arachidonic acid.

significantly higher SC under flow conditions ($p<0.001$) (Table II).

Association between platelet function and endothelial function

A significant inverse association was observed in both groups between %FMD and all measured platelet function parameters ($p<0.001$) (Table III). Thus, the greater the vasodilation in response to reactive hyperemia, the lower the platelet adhesion and aggregation, as assessed by both conventional aggregometry and under flow conditions.

To further determine the association of platelet function and %FMD, the study cohort was stratified into those with %FMD ≥ or < the median of 13.4% (Table IV). There were more males, hypertensives, overweight patients, and aspirin users in the group with < the median %FMD. Furthermore, HDL-C was lower, while serum homocysteine and hs-CRP were higher in the group with <, compared to those with ≥ the median %FMD. The most significant and prominent findings were the enhanced platelet function parameters in patients with <, compared to those with ≥ the median %FMD ($p<0.001$) (Figure 1).

Odds ratios (OR) for %FMD above the median by platelet function tests above median compared to below the median are presented in Table V.

Discussion

This study demonstrates a significant inverse association between two of the most influential parameters in cardiovascular health and disease: FMD and platelet function. A significant inverse correlation was demonstrated both in AMI patients and subjects without established cardiovascular disease (controls). Furthermore, in a multivariate logistic regression, endothelial function, assessed by brachial FMD, remained the best predictor of platelet function.

Prior studies have shown significant individual variability in platelet reactivity and in platelet response to anti-aggregatory therapy [19, 20]. It has been speculated that platelet hyper-aggregation confers increased risk for atherothrombotic events. Moreover, the variability in platelet inhibition in response to anti-aggregants in patients sustaining an AMI and/or undergoing PCI, has been established as an important independent predictor of thrombotic complications and ultimately of short- and long-term clinical outcomes [21–25]. Nevertheless, only few data exist regarding the regulation of platelet reactivity and its response to anti-aggregants. The results of this study suggest a potential mechanism which might partially explain the variability in platelet reactivity.

Furthermore, endothelial dysfunction has been shown to be an independent risk factor for the development and progression of cardiovascular disease, including AMI, stroke, and a need for urgent percutaneous transluminal coronary angioplasty [26–31], and the reverse association between endothelial function and platelet reactivity could contribute to the above-mentioned relationship between endothelial function and clinical outcomes.

The nature of the association between endothelial function and platelet reactivity is unclear. Blood platelets and endothelial cells might interact in two of the following ways:

(1) *via* an indirect, paracrine path, using blood-borne mediators and (2) *via* a direct path, in which close physical contact triggers an array of intracellular cascades that result in structural and/or functional changes within each or both cell types.

Table IV. Patient characteristics according to median %FMD.

	%FMD ≥ 13.4% ($n=76$)	%FMD < 13.4% ($n=75$)	p-Value
Age (*y*)	51 ± 9	55 ± 1	0.008
Males	49 (64%)	66 (88%)	0.001
AMI	16 (21%)	31 (41%)	0.009
Cardiovascular risk factors			
Smokers	16 (21%)	13 (17%)	0.356
Diabetes mellitus	4 (5%)	9 (12%)	0.140
Hypertension	14 (19%)	25 (34%)	0.036
Overweight (BMI > 25 kg m^{-2})	46 (61%)	56 (76%)	0.060
Hypercholesterolemia	35 (47%)	44 (60%)	0.118
Family history	30 (40%)	31 (42%)	0.868
BMI (kg m^{-2})	26 ± 4	28 ± 3	0.022
Concomitant medications			
Aspirin	12 (16%)	47 (63%)	0.006
Beta-blockers	13 (17%)	14 (19%)	0.843
Angiotensin-converting enzyme inhibitors	14 (18%)	22 (29%)	0.116
Diuretics	3 (4%)	4 (5%)	0.715
Statins	21 (28%)	30 (42%)	0.086
Blood tests			
Platelets (number μl^{-1})	259 090 ± 68 072	251 330 ± 84 691	0.542
Creatinine (mg dl^{-1})	1.27 ± 0.50	1.49 ± 0.62	0.675
Glucose[a] (mg dl^{-1})	93 ± 23	99 ± 25	0.107
Total cholesterol (mg dl^{-1})	188 ± 31	186 ± 44	0.777
LDL-C (mg dl^{-1})	115 ± 22	117 ± 31	0.723
Triglycerides (mg dl^{-1})	120 ± 92	149 ± 145	0.148
HDL-C (mg dl^{-1})	48 ± 11	42 ± 10	0.001
Homocysteine (μmol l^{-1})	11 ± 4	14 ± 9	0.030
Hs-CRP (mg^{-} l^{-1})	5.9 ± 5.3	21.1 ± 18.8	0.012
Asymmetric dimethylarinine (μmol l^{-1})	0.65 ± 0.23	0.72 ± 0.24	0.064
Echocardiography			
Left ventricular ejection fraction (%)	57 ± 9	55 ± 11	0.386
Platelet function tests			
Conventional aggregometry			
Adenosine diphosphate aggregation (%)	46.6 ± 25.2	68.2 ± 26.3	<0.001
Arachidonic acid aggregation (%)	33.5 ± 25.5	69.1 ± 30.4	<0.001
Platelet function under flow conditions			
Aggregate size (μm^2)	28 ± 13	51 ± 22	<0.001
SC (%)	6.9 ± 3.5	14.8 ± 8.5	<0.001

Notes: BMI, body mass index.
Values are presented as mean ± SD or absolute numbers (percent).
[a]Blood glucose level was measured after a 12-h fast.

As demonstrated in previous investigations, any insult faced by either platelets or endothelium soon translates into homeostatic disruption in both of them, thereby creating a vicious cycle that ultimately ends in vascular damage [32, 33]. Therefore, based on the results of this study, enhanced platelet activity and endothelial dysfunction are closely related, and in effect, are two sides of the same fundamental disease.

Our study results concur with the results of Cowley et al. [34, 35] who found a similarity between platelet behavior and vascular tone, assessed by forearm blood flow (venous occlusion plethysmography), in response to cold stimulation in a group of volunteers who had not taken any drugs. In 26 normal volunteers, there was a close correlation between the maximum vasoconstrictor response to cold and the threshold concentration of sodium arachidonate that was needed to induce platelets from each individual to aggregate and to undergo a release reaction. Furthermore, infusion of epoprostenol (prostacyclin) in five volunteers altered both the maximum vasoconstrictor response to cold and the threshold concentration of sodium arachidonate needed to induce platelet aggregation in a manner corresponding to this relationship. Cowley et al. [34, 35] consequently found, that endothelial function, assessed by venous plethysmography had a reciprocal association with platelet reactivity.

Heitzer et al. [36] recently demonstrated that clopidogrel improves endothelial NO bioavailability and diminishes biomarkers of oxidant stress and

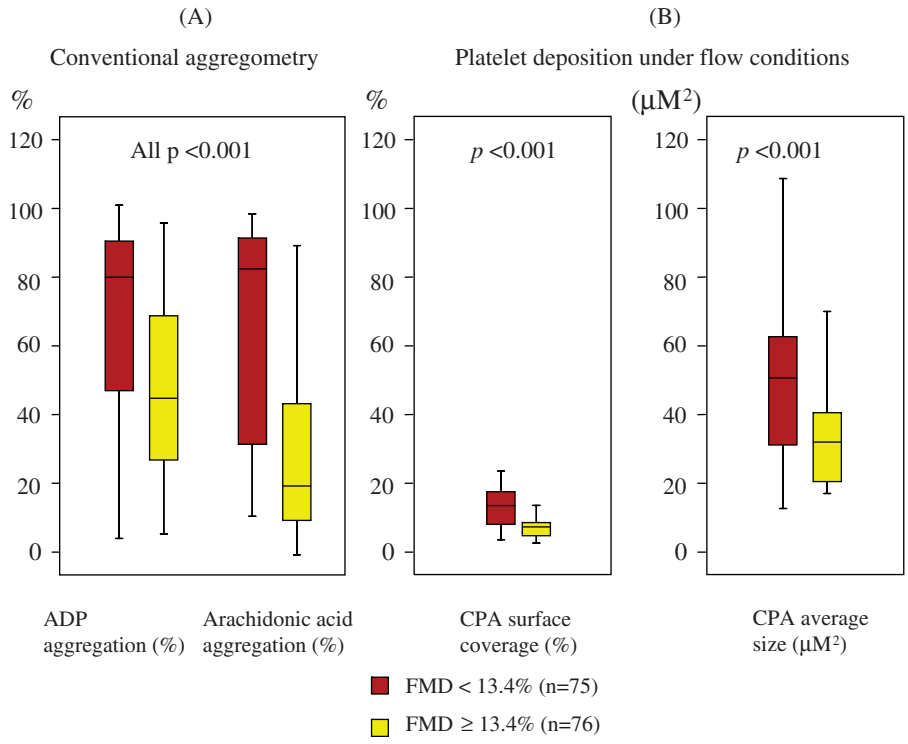

Figure 1. Platelet function parameters [(A) conventional aggregometry and (B) platelet deposition under flow conditions] as a function of endothelial function. All parameters of platelet function were significantly higher among participants with < the median FMD of 13.4% (in red), compared with those with ≥ the median (in yellow) ($p < 0.001$). ADP, adenosine diphosphate; CPA, cone and plate(let) analyzer; SC, surface coverage; FMD, flow-mediated dilation.

Table V. OR for %FMD above the median (of 13.4%) by platelet function parameters above *vs.* below the median.

	OR (95% CI) unadjusted	OR (95% CI) age and gender adjusted	OR (95% CI) multivariate adjusted[a]
Conventional aggregometry			
ADP-induced aggregations (>median of 58%)	3.42 (1.75–6.67)	3.34 (1.63–6.84)	5.36 (2.47–11.64)
AA-induced aggregations (>median of 45%)	7.20 (3.51–14.75)	7.53 (3.47–16.31)	7.35 (3.48–15.53)
Platelet deposition under flow conditions			
SC (>median of 9%)	11.98 (5.56–25.84)	11.16 (4.98–24.96)	12.66 (5.68–28.21)
Average size (>median of 34 μm^2)	6.75 (3.31–13.76)	6.97 (3.23–15.01)	7.89 (3.63–17.13)

Notes: AA, arachidonic acid; ADP, adenosine diphosphate; CI, confidence interval; FMD, flow-mediated dilation; OR, odds ratio; SC, surface coverage.

[a]Adjusted for age, gender, smoking status, diabetes mellitus, hypertension, hypercholesterolemia, overweight, family history, and concomitant medications.

inflammation in patients with symptomatic CAD, suggesting that beyond inhibition of platelet aggregation, adenosine phosphate receptor blockade may also have vasoprotective effects. Furthermore, Warnholtz et al. [37] recently demonstrated that clopidogrel dose-dependently improves endothelial dysfunction in stable CAD patients. Clopidogrel dose- and time-dependently inhibited the platelet ADP P2Y12 receptor without correlation with its stimulatory effects on FMD. In this study, however, although all AMI patients were treated with clopidogrel, %FMD was significantly worse among AMI patients compared to controls [17, 19, 29], whereas %NTG, which represents smooth muscle-dependent vasodilatation, was similar in both groups.

While ADP- and AA-induced platelet aggregations observed in this study were higher in the controls; platelet adhesion under flow conditions was higher in the AMI group. It seems that extensive exposure to anti-platelet therapy among AMI patients may be the cause of the lower aggregation values.

In this study, significantly higher levels of hs-CRP and ADMA in the AMI group compared to controls,

suggest that endothelial function serves as a marker of platelet function, and *vice versa*. Thus, it is reasonable to assume that this association is probably due to mere differences in vascular environment which are related to both platelets and endothelial cells.

Limitations

This study, however, suffers from a number of weaknesses: (1) endothelial function was assessed using a single technological approach without taking into account endothelial activation markers (such as endothelin, reactive oxygen species such as superoxide anions, RANTES and CD40L and IL-10), or oxidative stress status which could have provided a more comprehensive view of endothelium function; (2) the FMD method is prone to technical and biological variability, although such problems were minimized due to the skilled personnel who conducted the tests [8]; (3) inability to reach an absolute conclusion regarding women, due to the very high male percentage among the participants; (4) based on the current data we cannot establish a causal relationship. In light of these limitations, further studies are needed that will implement a wider range of endothelial function parameters and include larger samples with higher female-to-male ratios.

Conclusions

In conclusion, endothelial function, as assessed by brachial artery FMD, is inversely correlated to platelet reactivity in individuals without established cardiovascular disease as well as in AMI patients, suggesting that endothelial function may play a major role in determining platelet reactivity. Therefore, based on the results of this study, enhanced platelet activity and endothelial dysfunction are closely related, and in effect, are two sides of the same fundamental disease.

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