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# Shear-Induced Platelet Aggregation Increases in Patients with Proximal and Severe Coronary Artery Stenosis

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**Summary**

*Background:* Shear stress generated in stenosed arteries promotes platelet thrombi formation at the stenosed sites by accelerating the binding of von Willebrand factor (vWF) to platelets. Shear-induced platelet aggregation (SIPA) has been studied in acute coronary syndromes, but not in chronic coronary disease.

*Hypothesis:* We investigated the effect of both the site and severity of coronary stenosis on SIPA in patients with chronic coronary artery disease.

*Methods:* Shear-induced platelet aggregation was measured using platelet-rich plasma in 49 patients (41 men and 8 women; mean age 61 ± 10 years) with coronary artery disease to evaluate the association between the extent of SIPA and coronary angiographic findings. Stenoses > 75% were considered severe. In all, 62 healthy individuals (54 men and 18 women; mean age 45 ± 7 years) served as controls. The correlation between SIPA and the site and severity of the coronary lesion, and parameters of coagulation and fibrinolysis were evaluated.

*Results:* Shear-induced platelet aggregation was increased in the stenosis group (69.0 ± 10.6%) compared with the controls (57.7 ± 10.3%, $p < 0.0001$). Patients with severe stenosis in the proximal segments had significantly increased SIPA ($p < 0.0001$) and vWF larger multimer concentration ($p < 0.0001$) compared with the control group. A significant correlation existed between SIPA and the vWF larger multimer concentration in all subjects studied ($r = 0.422$, $p < 0.0001$).

*Conclusions:* Shear-induced platelet aggregation is increased in patients with severe stenosis of the proximal coronary arteries and correlates with plasma concentrations of vWF larger multimers, suggesting that severe stenosis in the proximal segments is not only associated with an increased risk of significant myocardial ischemia, but may also generate high shear stress in the stenosed artery and increase plasma vWF larger multimers, thereby promoting the formation of platelet thrombi.

**Key words:** stenosis, coronary disease, von Willebrand factor, platelet

## Introduction

Platelet activation and subsequent platelet adhesion and aggregation play a role in the initiation and exacerbation of acute coronary syndromes, including acute myocardial infarction (MI).[1–6] Shear stress has been considered an important promoter of adhesion and cohesion of platelets to endothelial cells activated by atherosclerosis.[7] The shear stress generated when blood flows through a vessel increases as blood flows rapidly through a stenosed artery,[8] which accelerates thrombus formation by activating platelets and inducing platelet adhesion and aggregation.[8–10]

Most studies of platelet function, coagulation, and fibrinolysis in patients with ischemic heart disease have focused on acute coronary syndromes.[1, 2, 11–15] However, no study has examined angiographic findings and hematologic parameters to determine which characteristics place patients with stable ischemic heart disease at risk of transition to acute coronary syndrome.

We developed a shear-induced platelet aggregometer to measure shear-induced platelet aggregation (SIPA).[16] The device is useful for examining platelet aggregation after activation by shear stress in stenosed arteries.

The objective of the present study was to investigate the effect of the site and severity of coronary stenoses on SIPA in patients with stable ischemic heart disease who underwent coronary angiography.

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## Methods

### Study Population

In all, 49 patients with coronary artery disease (26 with prior MI and 23 with stable angina pectoris), presenting to the Kyorin University Hospital for routine coronary angiography from May to December in 1997, were enrolled in the study. The subjects included 41 men and 8 women (mean age 61.2 ± 0.3 years, range 34–80). Patients with acute MI or unstable angina pectoris were excluded from the study since elevated plasma catecholamines in these patients are known to affect SIPA. Patients were included 3 months after the onset of MI, and those with unstable angina pectoris were eligible if angina was stabilized for at least 3 months. Sixty-two healthy subjects (54 men and 8 women; mean age 44.7 ± 7.0 years, range 34–59) without a history of ischemic heart disease or a positive treadmill test served as controls. All subjects gave informed consent prior to participation in this study. The study was approved by the Institutional Review Committee of Kyorin University Hospital, and procedures were followed in accordance with institutional guidelines.

### Preparation of Platelet-Rich Plasma

Venous blood (3.8% citrate/blood 1:9) was collected from all subjects and centrifuged at 100 g for 15 min at 22˚C. Platelet-rich plasma was prepared from the supernatant, and the remaining blood was centrifuged at 3000 g for 20 min to obtain platelet-poor plasma. The platelet count in the platelet-rich plasma was determined using a Coulter counter (S-Plas IV, Coulter Electronics, Miami, Fla., USA) and the platelet concentration was adjusted to $150 \times 10^9$/liter with platelet-poor plasma. Acetylsalicylic acid (81 mg/day), which reportedly does not affect SIPA,[17] was given to all subjects. Ticlopidine, which is known to inhibit SIPA, was prohibited during the 2 weeks prior to blood collection.

### Measurement of Shear-Induced Platelet Aggregation

Shear-induced platelet aggregation was measured according to a method described previously.[16] Briefly, 400 µl of platelet-rich plasma was applied to the surface of a polymethylmethacrylate plate and exposed to varying degrees of shear stress. After an initial 15 s at 6 dyn/cm$^2$, the shear stress was maintained between 6 and 12 dyn/cm$^2$ for 90 s, then between 12 and 108 dyn/cm$^2$ for the next 120 s, and constant at 108 dyn/cm$^2$ for the last 90 s. A laser light with a wavelength of 633 nm was applied to the platelet-rich plasma, and the platelet aggregation was calculated according to the following formula:[18] platelet aggregation (%) = $\log(Ia/Ib)/(Ipp/Ib) \times 100$, where Ia and Ib indicate transmitted light intensity of the platelet suspension after and before the application of shear stress, respectively, and Ipp is the transmitted light intensity of the platelet-free suspending medium. Platelet aggregation is biphasic, with the first aggregation induced by low shear stress that is fibrinogen dependent, and the second aggregation induced by high shear stress that is von Willebrand factor (vWF) dependent. We compared the maximal value (%) of the high shear-induced platelet aggregation in this study.

### Parameters of Coagulation, Fibrinolysis, Platelet Activity, and Catecholamines

Platelet factor 4, β thromboglobulin, and vWF antigen were measured using an enzyme immunoassay (Asserachrom PF4, Asserachrom β-TG and Asserachrom vWF, Diagnostica Stago, Paris, France). Plasminogen activator inhibitor-1 was measured via enzyme immunoassay (H-5 PAI-1 ELISA kit, Monozyme, Hoersholm, Denmark). Thrombin-antithrombin III complex was assayed using an enzyme immunoassay (TAT kit, SRL, Tokyo, Japan). Plasmin-antiplasmin complex was determined by latex agglutination (LPIA ACE PPI, Yatron, Tokyo, Japan). Antiplasmin was measured by a functional assay (Testzyme S APL, Daiichi-Kagaku, Tokyo, Japan). Prothrombin fragment F1+2 was determined by enzyme immunoassay (Enzygnost F1+2, Dade Behring, Marburg, Germany). Fibrinogen was estimated by the thrombin clotting time (Fibrinogen a BMY, Diagnostica Stago). Epinephrine and norepinephrine were measured by high performance liquid chromatography and an assay kit (CA test TOSOH, Tosoh, Yamaguchi, Japan).

### von Willebrand Factor Larger Multimer

Analysis of the vWF larger multimer was performed using an SDS-agarose gel electrophoresis. Larger multimers were defined as bands above the 10th band from the bottom. The relative amount (%) of each band was measured using a densitometer (Advantec DM303, Nihon Denshi Kagaku, Tokyo, Japan). The total amount of the larger bands was calculated according to the following formula:

The total amount of the larger bands (%) = plasma vWF antigen (%; the antigen in the pooled plasma of normal subjects defined as 100%) × A/100, where A is the sum of the relative amounts of larger multimers (%).

### Coronary Angiography

Coronary angiography was performed in multiple views using the Judkins technique. Serial orthogonal coronary angiograms were obtained after maximal coronary vasodilation with intracoronary injection of isosorbide dinitrate. The location of the stenosis was determined according to the Coronary Artery Surgery Study (CASS) classification system.[19] The severity of coronary artery stenosis was measured by a caliper method, using a Cardio-500 (Fukuda Denshi Co., Ltd., Tokyo, Japan), and the severity of each stenosis was assessed according to the American Heart Association classification system.[20] Subjects were classified into two groups according to the degree of coronary stenosis: mild stenosis group (≤ 75% stenosis), and severe stenosis group (> 75% stenosis). Subjects were also classified as follows into two groups according to the location of the most severe stenosis: proximal stenosis group

(patients with CASS seg. 1, seg. 11, seg. 12, or seg. 18), and distal stenosis group (patients with the other distal segment lesions). Each patient was classified into one of four groups according to the location and stenosis of the most severe stenotic lesion: proximal-severe stenosis group, distal-severe stenosis group, proximal-mild stenosis group, and distal-mild stenosis group. Coronary angiograms were assessed independently by two experienced observers blinded to the clinical findings and SIPA results. In cases of discordant evaluations, the results were discussed and the difference reconciled.

### Statistical Analysis

Statistical analysis was performed using analysis of variance for comparison of SIPA and vWF larger multimers. Statistically significant differences among the five groups were determined by a one-way analysis of variance (ANOVA), followed by a Bonferroni method if the ANOVA probability value was $< 0.05$. Association between SIPA and age was evaluated using regression analysis. Association between SIPA and parameters of coagulation, fibrinolysis, and platelet activity was evaluated with Pearson's correlation coefficient. The Kruskal-Wallis test was used to compare clinical characteristics between the subgroups. A p value of $< 0.05$ was considered statistically significant.

## Results

### Shear-Induced Platelet Aggregation in Patients with Ischemic Heart Disease and Healthy Controls

Shear-induced platelet aggregation was increased significantly in the patients with ischemic heart disease (69.0 ± 10.6%) compared with the healthy controls (57.7 ± 10.3%) ($p < 0.0001$). Since a significant difference was observed in the ages of the patient and healthy groups ($p < 0.0001$), simple regression analysis was performed using age as the independent variable and SIPA as the dependent variable. The regression was not significant, with a p value of 0.579 for the patient group and 0.642 for the healthy group. It was concluded that the two variables were not correlated, and that age does not affect the extent of SIPA.

### Coronary Artery Lesion and Shear-Induced Platelet Aggregation

There were no significant differences in SIPA between single- and multivessel disease ($p = 0.77$). There were no significant differences among the four angiographic groups of single-, double-, triple-vessel disease and normal coronary artery ($p = 0.80$).

Based on the site and severity of coronary stenosis, the subjects were divided into the following groups: mild stenosis in the distal segments (9 patients), mild stenosis in the proximal segments (17 patients), severe stenosis in the distal segments (10 patients), and severe stenosis in the proximal segments (13 patients). No significant difference existed among the four groups in age, gender, underlying disease (MI or angina pectoris), coronary risk factors (hypertension, diabetes mellitus, hyperlipidemia, and smoking), or medications (beta blockers, calcium antagonists, angiotensin-converting enzyme inhibitors, or nitrates) (Table I).

Comparison of SIPA in the five groups, including the healthy group, demonstrated that SIPA was greatest in the patients with severe stenosis in the proximal segments (76.1 ± 9.9%), followed by those with severe stenosis in the distal segments (67.11 ± 11.6%), mild stenosis in the proximal segments (66.01 ± 10.7%), mild stenosis in the distal segments (66.51 ± 6.6%), and the healthy group, which had the lowest

TABLE I Baseline characteristics of the patient groups

| Clinical factors | Group 1 (n = 9) | Group 2 (n = 17) | Group 3 (n = 10) | Group 4 (n = 13) |
|---|---|---|---|---|
| Age, years (mean ± SD) | 61.2 ± 12.1 | 63.5 ± 7.5 | 56.5 ± 10.5 | 61.9 ± 11.8 |
| Male gender, n (%) | 9 (100) | 15 (88) | 9 (90) | 8 (62) |
| Myocardial infarction, n (%) | 5 (56) | 10 (59) | 6 (60) | 4 (31) |
| Angina pectoris, n (%) | 4 (44) | 5 (29) | 4 (40) | 9 (69) |
| Coronary risk factors | | | | |
| Hypertension, n (%) | 2 (22) | 7 (41) | 6 (60) | 7 (54) |
| Diabetes mellitus, n (%) | 1 (11) | 8 (47) | 3 (30) | 5 (38) |
| Hyperlipidemia, n (%) | 4 (44) | 5 (29) | 3 (30) | 9 (69) |
| Smoking habit, n (%) | 7 (78) | 13 (76) | 9 (90) | 8 (62) |
| Medications | | | | |
| Beta blocker, n (%) | 1 (11) | 5 (29) | 4 (40) | 4 (31) |
| Calcium antagonist, n (%) | 5 (56) | 11 (65) | 5 (50) | 11 (85) |
| ACE inhibitor, n (%) | 1 (11) | 6 (35) | 4 (40) | 1 (8) |
| Nitrates, n (%) | 4 (44) | 7 (41) | 4 (40) | 7 (54) |

Group 1: Group with mild stenosis-distal segment, Group 2: Group with mild stenosis-proximal segment; Group 3: Group with severe stenosis-distal segment; Group 4: Group with severe stenosis-proximal segment.
*Abbreviations:* ACE = angiotensin-converting enzyme, SD = standard deviation.

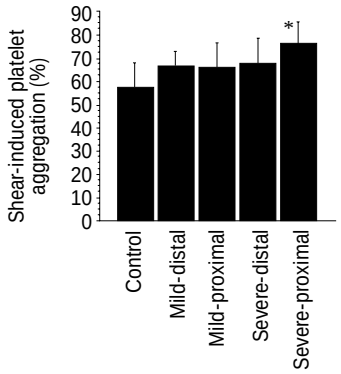


FIG. 1 Shear-induced platelet aggregation (SIPA) (%) for the patient and healthy groups: The graph displays the mean (%) and each bar represents 1 standard deviation. Shear-induced platelet aggregation was greatest in the patients with severe stenosis in the proximal segments (76.1 ± 9.9%, *: $p < 0.0001$), followed by those with severe stenosis in the distal segments (67.1 ± 11.6%), mild stenosis in the proximal segments (66.0 ± 10.7%), and mild stenosis in the distal segments (66.5 ± 6.6%). The controls had the lowest value (57.7 ± 10.3%). No significant difference existed between the patients with mild stenosis in the distal segments, mild stenosis in the proximal segments, or severe stenosis in the distal segments. The SIPA values in the healthy group were significantly lower than those in all the other groups. Control: healthy subjects; mild-distal: patients with mild stenosis in the distal segments; mild-proximal: patients with mild stenosis in the proximal segments; severe-distal: patients with severe stenosis in the distal segments; severe-proximal: patients with severe stenosis in the proximal segments.

value (57.71 ± 10.3%) ($p < 0.0001$, Fig. 1). The SIPA in the patients with severe stenosis in the proximal segments was greater than that in any of the other groups (vs. severe stenosis in the distal segments: $p = 0.0392$; vs. mild stenosis in the proximal segments: $p = 0.0088$; vs. mild stenosis in the distal segments: $p = 0.0334$; and vs. controls: $p < 0.0001$). No significant difference in SIPA was noted between the patients with severe stenosis in the distal segments, mild stenosis in the proximal segments, and mild stenosis in the distal segments. Shear-induced platelet aggregation values in the healthy group were significantly lower than those in all the other groups (vs. severe stenosis in the proximal segments: $p < 0.0001$, vs. severe stenosis in the distal segments: $p = 0.0081$; vs. mild stenosis in the proximal segments: $p = 0.0037$; and vs. mild stenosis in the distal segments: $p = 0.0172$).

### Relationship between Parameters of Coagulation, Fibrinolysis, and Platelet Activity, and Catecholamines (Table II)

No significant correlation was observed between SIPA and parameters of coagulation (vWF, fibrinogen, thrombin-antithrombin III complex, and prothrombin fragment F1 + 2), fibrinolysis (plasminogen activator inhibitor I, antiplasmin, and plasmin-antiplasmin complex), or platelet activity (β-thromboglobulin and platelet factor 4). Similarly, no correlation existed between epinephrine or norepinephrine, parameters of sympathetic activity, and SIPA.

### von Willebrand Factor Larger Multimer Analysis (Fig. 2)

The plasma concentration (%) of vWF larger multimers was greatest in the patients with severe stenosis in the proximal segments (85.4 ± 43.0%), followed by those with mild stenosis in the proximal segments (59.91 ± 33.3%), mild stenosis in the distal segments (52.8 ± 35.8%), severe stenosis in the distal segments (46.8 ± 17.2%), and the healthy group, which had the lowest value (38.4 ± 20.1%) ($p < 0.0001$). The vWF larger multimer concentration in the patients with severe stenosis in the proximal segments was significantly greater than that in the other four groups (vs. severe stenosis in the distal segments: $p = 0.0016$; vs. mild stenosis in the proximal segments: $p = 0.0240$; vs. mild stenosis in the distal segments: $p = 0.0109$; and vs. the healthy group: $p < 0.0001$). No significant difference in vWF larger multimer concentration was ob-

TABLE II Association between shear-induced platelet aggregation and other hematologic parameters

| Parameter | n | Mean ± SD | Correlation coefficient | p Value |
|---|---|---|---|---|
| vWF antigen (%) | 49 | 158 ± 55 | 0.159 | 0.228 |
| Fibrinogen (mg/dl) | 49 | 331 ± 126 | −0.137 | 0.301 |
| Thrombin-antithrombin III complex (ng/ml) | 49 | 7 ± 10 | −0.063 | 0.648 |
| Prothrombin fragment F1+2 (nmol/l) | 49 | 1 ± 0.4 | −0.028 | 0.838 |
| Plasminogen activator inhibitor-1(ng/ml) | 49 | 199 ± 93 | 0.176 | 0.194 |
| Antiplasmin (%) | 49 | 102 ± 11 | −0.048 | 0.726 |
| Plasmin-antiplasmin complex (μg/ml) | 49 | 1 ± 0.3 | 0.009 | 0.949 |
| Beta thromboglobulin (ng/ml) | 46 | 116 ± 96 | −0.108 | 0.491 |
| Platelet factor 4 (ng/ml) | 43 | 58 ± 63 | −0.077 | 0.623 |
| Epinephrine (pg/ml) | 49 | 78 ± 62 | −0.038 | 0.806 |
| Norepinephrine (pg/ml) | 49 | 418 ± 213 | 0.007 | 0.963 |

Data are displayed as mean ± SD.

*Abbreviations:* SD = standard deviation, vWF = von Willebrand factor.

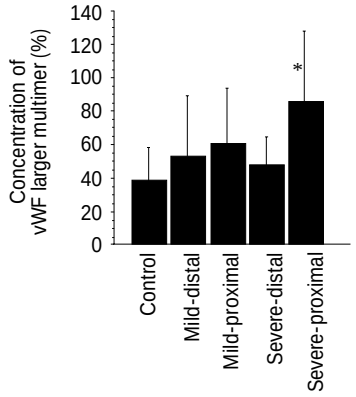


FIG. 2 von Willebrand factor (vWF) larger multimer concentration (%) for the patient and healthy groups: The graph displays the mean (%) and each bar represents 1 standard deviation. The vWF larger multimer concentration was greatest in patients with severe stenosis in the proximal segments (85.4 ± 43.0%, *: $p < 0.0001$), followed by those with mild stenosis in the proximal segments (59.9 ± 33.3%), mild stenosis in the distal segments (52.8 ± 35.8%), and severe stenosis in the distal segments (46.8 ± 17.2%). The controls had the lowest value (38.4 ± 20.1%). No significant difference existed between the patients with mild stenosis in the proximal segments, mild stenosis in the distal segments, and severe stenosis in the distal segments. Group definitions as in Figure 1.

served among patients with mild stenosis in the proximal segments, those with mild stenosis in the distal segments, and those with severe stenosis in the distal segments. The vWF larger multimer concentration in the healthy group was significantly lower than that in the patients with severe stenosis in the proximal segments or mild stenosis in the proximal segments ($p < 0.0001$ and $p = 0.0094$, respectively).

A significant correlation did exist between SIPA and vWF larger multimer concentration in the patients and controls as a whole ($r = 0.422$, $p < 0.0001$) (Fig. 3).

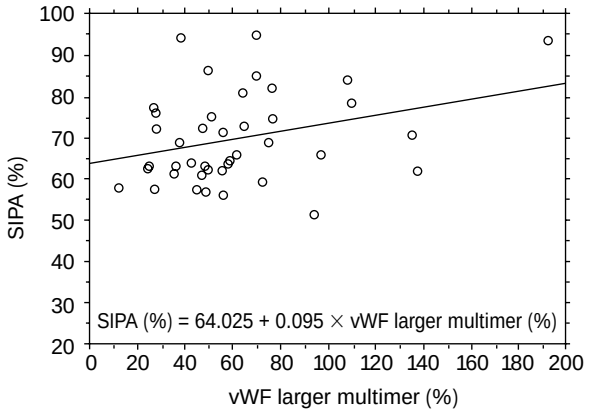


FIG. 3 Correlation between shear-induced platelet aggregation (SIPA) and von Willebrand factor (vWF) larger multimer concentration in the patient and healthy groups as a whole. A positive correlation was observed ($r = 0.422$, $p < 0.0001$).

## Discussion

### Coronary Angiography Findings in the Chronic Phase and Shear-Induced Platelet Aggregation

Exposure of tissue factor and collagen on the endothelium following plaque rupture results in platelet activation and the formation of platelet thrombi in acute coronary syndrome.[1, 2, 21, 22] In contrast, the role of platelets in the chronic phase of ischemic heart disease is not yet understood. The results of the present study demonstrate that SIPA increases appreciably in patients with chronic ischemic heart disease compared with healthy individuals. Shear-induced platelet aggregation was greatest in patients with severe stenosis in the proximal segments. Although a previous study observed an increase in SIPA in patients with acute MI,[23] ours is the first study to demonstrate a relationship between coronary angiography findings and SIPA in ischemic heart disease in the chronic phase.

An important finding of the present study is that vWF-dependent platelet aggregation is increased in chronic ischemic heart disease as well as in acute coronary syndromes. Our results also suggest that patients with severe stenosis in the proximal segments are at increased risk of exacerbation of stenotic lesions due to the formation of platelet thrombi.

### Shear Stress and Shear-Induced Platelet Aggregation

The shear-induced platelet aggregometer[16] is a modified cone-and-plate viscometer that induces platelet aggregation by applying shear stress in the absence of agonists. It is useful not only for examining SIPA by subjecting resting platelets and plasma proteins to shear stress, but also for evaluating interactions between plasma proteins and platelets already activated by shear stress in stenosed arteries in vivo. A previous study using the device has reported that SIPA was accelerated after exercise in patients with angina pectoris,[24] but did not evaluate the patients on the basis of coronary angiography findings.

The shear stress generated in an artery is proportional to blood flow, and inversely proportional to the third power of the internal diameter of the artery.[25] Severe stenosis in the proximal segments should therefore result in extremely high shear stress. The increase in SIPA observed ex vivo may be attributable to the application of shear stress to platelets, plasma proteins, or endothelial cells due to severe stenosis in the proximal segments in vivo.

High SIPA is dependent on vWF and platelet glycoproteins GPIb-IX and GPIIb-IIIa.[16] Previous reports have demonstrated that elevated plasma vWF concentration is an independent coronary risk factor,[26] and that aurintricarboxylic acid, which inhibits binding of GPIb-IX and vWF,[27] monoclonal antibodies,[28] and vWF fragments,[29] and reduces thrombus formation in the coronary arteries of animal models. These experimental and clinical findings indicate that vWF and the platelet glycoproteins GPIb-IX and GPIIb-IIIa play an important role in atherosclerosis and arterial thrombus formation in vivo. To determine the effect of shear stress on platelets, plasma pro-

teins, and endothelial cells, we examined the correlation between SIPA and parameters of plasma activity, plasma fibrinogen concentration, plasma vWF concentration, the results of vWF larger multimer analysis, and parameters of coagulation and fibrinolysis.

**The Effect of Shear Stress on Platelet Activity**

Although it has been reported that shear stress causes activation of the platelet glycoprotein GPIIb-IIIa,[7] the effect of shear stress on GPIIb-IIIa could not be evaluated in the present study, since we did not measure GPIIb-IIIa activity. Instead, we measured β-thromboglobulin and platelet factor 4, which are constituents of the α-granule, as markers of platelet secretion. However, no correlation existed between these parameters and SIPA. This supports a previous suggestion[30] that platelet activation and aggregation caused by shear stress are not accompanied by the release of α-granules.

**The Effect of Shear Stress on Plasma Proteins (Coronary Angiography Findings and von Willebrand Factor Larger Multimer Concentration)**

Low SIPA is fibrinogen dependent and high SIPA is vWF dependent.[16] In the present study, fibrinogen concentration did not correlate with high SIPA. A previous study showed that the vWF concentration was elevated in acute MI and correlated with SIPA.[23] In the present study, no correlation was observed between SIPA and vWF concentration in the patient and healthy groups overall, but a correlation was present between SIPA and vWF larger multimer concentration (Fig. 3). Our study also showed that the vWF larger multimer concentration was greatest in the patients with severe stenosis in the proximal segments, the group in which SIPA was increased the most.

A positive correlation between SIPA and vWF larger multimer concentration has also been reported in patients with stable angina pectoris after treadmill exercise test,[24] and in cerebral ischemia.[17] This study is the first to correlate SIPA, vWF larger multimer concentration, and angiography findings, demonstrating that both SIPA and the vWF larger multimer concentration are elevated in patients with severe stenosis in the proximal segments. This suggests that plasma vWF larger multimer concentration increases as a result of an unknown mechanism and may stimulate SIPA.

**The Effect of Shear Stress on Vascular Endothelial Cells**

Although the mechanism responsible for the increase in vWF larger multimer concentration seen in the present study is unclear, shear stress on stenosed arteries may act on the endothelial cells to promote the release of vWF larger multimers.[31] Shear stress generated in stenosed arteries may act on endothelial cells to stimulate vWF larger multimer secretion and increase the plasma vWF larger multimer concentration, thereby further precipitating platelet aggregation.

How vWF larger multimers induce greater platelet aggregation than smaller multimers under high shear stress is unknown. A study using atomic force microscopy demonstrated that vWF underwent a shear stress-induced conformational transition from a globular state to an extended chain conformation.[32] This conformational change may increase the number of sites available to interact with platelets. The number of the interaction sites should increase with the multimer size, thus making larger multimers more potent to support higher SIPA.

**Shear-Induced Platelet Aggregation and Coagulation and Fibrinolysis**

In an acute coronary syndrome, the progression of the extrinsic coagulation pathway in the coronary endothelium stimulates platelet activation through thrombin formation.[21, 22] In our patients, thrombin-antithrombin III complex and prothrombin fragment F1 + 2 did not correlate with SIPA, suggesting that thrombin generation is not involved in the acceleration of SIPA in the chronic phase of ischemic heart disease. Similarly, SIPA did not correlate with antiplasmin, plasmin-antiplasmin complex, or fibrin degradation product, thus excluding the involvement of fibrinolysis.

Epinephrine has also been reported to stimulate SIPA;[33] however, we found no correlation between SIPA and plasma epinephrine concentration, removing the possibility that platelet aggregability was stimulated by increased sympathetic activity.

**Clinical Implications**

Coronary angioplasty ameliorates coronary stenosis, restores coronary blood flow, and decreases the risk of coronary ischemia. However, angioplasty appears to do more than restore coronary flow: our results suggest that angioplasty might decrease shear stress generated in stenosed arteries, reduce the release of vWF larger multimers from endothelial cells, and suppress the formation of platelet thrombi. The reduction of coronary stenosis by coronary intervention should therefore be considered for patients with severe stenosis in the proximal segments who are stable with medical treatment. Further prospective studies of the effects of coronary intervention on SIPA and vWF larger multimer concentration are therefore needed.

**Study Limitations**

This was a study of a relatively small number of patients. A significant correlation existed between SIPA and the vWF larger multimer concentration, though relatively weak. A larger study would be needed to confirm the relation between SIPA and coronary stenosis, and between SIPA and vWF larger multimers. The present study focused on platelet aggregation but did not investigate platelet adhesion, another important function of platelets. The vWF has also been reported to play a role in platelet adhesion under high shear stress,[34] emphasizing the need to study shear-induced platelet adhesion in ischemic heart disease.

## Conclusions

Shear-induced platelet aggregation increases in stable patients with coronary stenoses and is particularly increased in patients with severe stenosis in the proximal segments. This increase correlates with plasma elevations of vWF larger multimers, which may play a role in enhanced platelet aggregation and thrombi formation.

## References


1. Fuster V, Badimon L, Badimon JJ, Chesebro JH: The pathogenesis of coronary artery disease and the acute coronary syndromes (I). *N Engl J Med* 1992;326:242–250
2. Fuster V, Badimon L, Badimon JJ, Chesebro JH: The pathogenesis of coronary artery disease and the acute coronary syndromes (II). *N Engl J Med* 1992;326:310–318
3. Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J: Risk of thrombosis in human atherosclerotic plaques: Role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J* 1993;69:377–381
4. Falk E, Shah PK, Fuster V: Coronary plaque disruption. *Circulation* 1995;92:657–671
5. Willerson JT: Conversion from chronic to acute coronary heart disease syndromes. Role of platelets and platelet products. *Tex Heart Inst J* 1995;22:13–19
6. Neumann FJ, Marx N, Gawaz M, Brand K, Ott I, Rokitta C, Sticherling C, Meinl C, May A, Schomig A: Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. *Circulation* 1997;95:2387–2394
7. Holme PA, Orvim U, Hamers MJAG, Solum NO, Brosstad FR, Barstad RM, Sakariassen KS: Shear-induced platelet activation and platelet microparticle formation at blood flow conditions as in arteries with a severe stenosis. *Arterioscl Thromb Vasc Biol* 1997;17: 646–653
8. Gibson MC, Diaz L, Kandarpa K, Sacks FM, Paternak RC, Sandor T, Feldman C, Stone PH: Relation of vessel wall shear stress to atherosclerosis progression in human coronary arteries. *Arterioscl Thromb* 1993;13:310–315
9. Folts J: An in vivo model of experimental arterial stenosis, intimal damage, and periodic thrombosis. *Circulation* 1991;83(suppl IV): IV3–14
10. Taeymans Y, Théroux P, Lesperance J, Waters D: Quantitative angiographic morphology of the coronary artery lesions at risk of thrombotic occlusion. *Circulation* 1992;85:78–85
11. Kapiotis S, Jilma B, Quenhenberger P, Ruzicka K, Handler S, Speiser W: Morning hypercoagulability and hypofibrinolysis: Diurnal variations in circulating activated factor VII, prothrombin fragment F1 + 2, and plasin-plasmin inhibitor complex. *Circulation* 1997;96:19–21
12. Patrono C, Renda G: Platelet activation and inhibition in unstable coronary syndromes. *Am J Cardiol* 1997;80:17E–20E
13. Ghaisas NK, Shahi CN, Foley B, Goggins M, Crean P, Kelly A, Kelleher D, Walsh M: Elevated levels of circulating soluble adhesion molecules in peripheral blood of patients with unstable angina. *Am J Cardiol* 1997;80:617–619
14. Moreno PR, Bernardi VH, Lopez-Cuellar J, Murcia AM, Palacios IF, Gold HK, Mehran R, Sharma SK, Nemerson Y, Fuster V, Fallon JT: Macrophages, smooth muscle cells, and tissue factor in unstable angina. Implications for cell-mediated thrombogenicity in acute coronary syndromes. *Circulation* 1996;94:3090–3097
15. Fuster V, Badimon L, Cohen M, Ambrose JA, Badimon JJ, Chesebro J: Insights into the pathogenesis of acute ischemic syndromes. *Circulation* 1988;77:1213–1220
16. Ikeda Y, Handa M, Kawano K, Kamata T, Murata M, Araki Y, Anbo H, Kawai Y, Watanabe K, Itagaki I, Sakai K, Ruggeri ZM: The role of von Willebrand factor and fibrinogen in platelet aggregation under varying shear stress. *J Clin Invest* 1991;87:1234–1240
17. Uchiyama S, Yamazaki M, Maruyama S, Handa M, Ikeda Y, Fukuyama M, Itagaki I: Shear-induced platelet aggregation in cerebral ischemia. *Stroke* 1994;25:1547–1551
18. Ikeda Y, Handa M, Kamata T, Kawano K, Kawai Y, Watanabe K, Kawakami K, Sakai K, Fukuyama M, Itagaki I, Yoshioka A, Ruggeri ZM: Transmembrane calcium influx associated with von Willebrand factor binding to GPIb in the initiation of shear-induced platelet aggregation. *Thromb Haemost* 1993;69:496–502
19. The Principal Investigators of CASS and Associates: The National Heart, Lung, and Blood Institute Coronary Artery Surgery Study (CASS). *Circulation* 1981;63(suppl I):I-1–8
20. AHA Committee Report: A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for grading of coronary artery disease, Council on Cardiovascular Surgery, American Heart Association. News from the American Heart Association. *Circulation* 1975;51:5–40
21. Davies MJ, Thomas AC: Plaque fissuring: The cause of acute myocardial infarction, sudden ischemic death, and crescendo angina. *Br Heart J* 1985;53:363–373
22. Fuster V, Stein B, Ambrose JA, Badimon L, Badimon JJ, Chesebro JH: Atherosclerotic plaque rapture and thrombosis. Evolving concepts. *Circulation* 1990;82(suppl II):II-47–59
23. Goto S, Sakai H, Goto M, Ono M, Ikeda Y, Handa S, Ruggeri ZM: Enhanced shear-induced platelet aggregation in acute myocardial infarction. *Circulation* 1999;99:608–613
24. Tokuue J, Hayashi J, Hata Y, Nakahara K, Ikeda Y: Enhanced platelet aggregability under high shear stress after treadmill exercise in patients with effort angina. *Thromb Haemost* 1996;75:833–837
25. Goldsmith HL, Turitto VT: Rheological aspects of thrombosis and haemostasis: Basic principles and applications. *Thromb Haemost* 1986;55:415–435
26. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW: Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med* 1995; 332:635–641
27. Strony J, Phillips M, Brands D, Moake J, Adelman B: Aurintricarboxylic acid in a canine model of coronary artery thrombosis. *Circulation* 1990;81:1106–1114
28. Badimon L, Badimon J, Chesebro H, Fuster V: Inhibition of thrombus formation: Blockade of adhesive glycoprotein mechanisms versus blockade of the cyclooxygenase pathway (abstr). *J Am Coll Cardiol* 1988;11:30A
29. Yao S, Ober JC, Garfinkel LI, Hagay Y, Ezov N, Ferguson J, Anderson HV, Panet A, Gorecki M, Buja LM, Willerson JT: Blockade of platelet membrane glycoprotein Ib receptors delays intracoronary thrombogenesis, enhances thrombolysis, and delays coronary artery reocclusion in dogs. *Circulation* 1994;89:2822–2828
30. Okada M, Murata M, Suzuki H, Kitaguchi T, Handa M, Watanabe K, Sakai K, Tanoue K, Ikeda Y: Von Willebrand factor binding to glycoprotein Ib/IX induces platelet shape change under high shear stress conditions—an electron microscopic study. *Thromb Haemost* 1997;78:(suppl):644
31. Moake JL, Turner NA, Stathopoulos NA, Nolasco LN, Hellums JD: Involvement of large plasma von Willebrand factor (vWF) multimers and unusually large vWF forms derived from endothelial cells in shear stress-induced platelet aggregation. *J Clin Invest* 1986;78:1456–1461
32. Siedlecki CA, Lestini BJ, Kottke-Marchant K, Eppel SJ, Wilson DL, Marchant RE: Shear-dependent changes in the three-dimensional structure of human von Willebrand factor. *Blood* 1996;88: 2939–2950
33. Goto S, Ikeda Y, Murata M, Handa M, Takahashi E, Yoshioka A, Fujimura Y, Fukuyama M, Handa S, Ogawa S: Epinephrine augments von Willebrand factor-dependent shear-induced platelet aggregation. *Circulation* 1992;86:1859–1863
34. Savage B, Saldivar E, Ruggeri ZM: Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell* 1996;84:289–297