

# Elevated Plasma von Willebrand Factor Antigen and Activity Levels Are Associated With the Severity of Coronary Stenosis

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## Abstract

von Willebrand factor (VWF) acts as a bridge between platelets and the subendothelial matrix following vessel damage and plays a vital role in coronary artery disease (CAD). The aim of this study was to investigate the association between VWF and the severity of coronary stenosis quantified by the Gensini score in acute myocardial infarction (AMI), the most dangerous complication of CAD. Plasma VWF antigen (VWF: Ag) and VWF-collagen binding (VWF: CB) in normal controls ( $n = 123$ ) and in patients with AMI ( $n = 205$ ) were tested, and then the patients were divided based on Gensini scores. The levels of VWF: Ag and VWF: CB in patients with AMI were significantly higher than those in the control group ( $P < .001$ ). Plasma levels of VWF: Ag and VWF: CB were positively correlated with both Gensini score and the number of affected vessels. Both VWF: Ag and VWF: CB were independent factors for coronary stenosis, adjusting confounding factors. Thus, the levels of VWF: Ag and VWF: CB were positively correlated with the severity of coronary stenosis. Screening of VWF at time of AMI may have prognostic value in terms of the severity of coronary stenosis.

## Keywords

von Willebrand factor, collagen, coronary stenosis, acute myocardial infarction, risk factors

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## Introduction

Coronary artery disease (CAD), which is characterized by atherosclerotic stenosis and can result in myocardial ischemia, is a major cause of mortality and morbidity around the world.<sup>1</sup> There is evidence that atherosclerosis plays a crucial role in the occurrence of CAD. Damage to the inner layer of coronary arteries results in the recruitment of fatty deposits and carbohydrates, followed by the formation of arterial plaques. Rupture of the plaque and exposure of the endodermic matrix triggers the onset of coronary thrombosis. This results in artery stenosis and blockage, which can lead to acute myocardial infarction (AMI), the most dangerous complication of atherosclerotic plaque.<sup>2</sup>

In the last few decades, numerous traditional risk factors have been identified as predictors of cardiovascular disease (CVD).<sup>3</sup> Notably, the factor that causes thrombosis also plays a role in CAD. von Willebrand factor (VWF) is a multimeric glycoprotein that is present in blood plasma and plays a vital role during thrombosis.<sup>4</sup> It is stored in the  $\alpha$ -granules of

platelets and the Weibel-Palade bodies of endothelial cells in the form of ultra-large VWF (UL-VWF). The UL-VWF is converted into small, less active fragments by a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS13).<sup>5</sup> Damage to the vessel membrane results in high shear stress, and this change in blood flow can induce the binding of VWF to the platelet receptor GPIb-IX-V complex

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(via the A1 domain). Simultaneously, VWF binds endothelial collagen (via the A1 and A3 domains). Hence, VWF serves as a bridge between platelets and the subendothelial matrix. Furthermore, it acts as a marker of endothelial dysfunction, and VWF has negative correlation between brain-derived neurotrophic factor, a neurotrophin involved in angiogenesis and maintenance of endothelial integrity, in patients with stable CAD.<sup>6</sup>

Generally, VWF tests have been used to diagnose von Willibrand disease, which is characterized by quantitative deficiencies and/or qualitative defects in VWF.<sup>7</sup> These tests include the assessment of VWF antigen (VWF: Ag), VWF ristocetin cofactor activity assays, ristocetin-triggered platelet glycoprotein Ib binding assays, ristocetin-induced platelet agglutination assays, VWF-collagen binding assays (VWF: CB), and VWF-factor VIII binding assays. Animal studies have shown that atherosclerotic development is attenuated in VWF-deficient mice.<sup>8</sup> In hypercholesterolemic mice, ADAMTS13 regulates inflammatory plaque progression through a VWF-dependent mechanism.<sup>9</sup> Clinical studies have also indicated that elevated VWF levels are fundamentally associated with the occurrence of CAD,<sup>10</sup> while atherosclerotic plaque ulceration in the carotid arteries is not related to higher levels of VWF in patients with transient ischemic attack or ischemic stroke.<sup>11</sup> In our previous study, patients with AMI not only had higher levels of VWF: Ag but also had significantly increased numbers of UL-VWF multimers.<sup>12</sup> Studies have reported that coronary plaque burden is associated with VWF: Ag levels in patients with stable angina pectoris (SAP), and plasma levels of VWF were significantly higher in patients with angiographic no-reflow but not in those with electrocardiographic no-reflow.<sup>13</sup> Interestingly, VWF: Ag levels have not been shown to differ between sexes with regard to the severity of stable CAD, as quantified by SYnergy between percutaneous coronary intervention with TAXus and cardiac surgery (SYNTAX) score.<sup>14</sup>

Previous investigations have mainly focused on the risk of occurrence and prognosis value of plasma VWF in AMI,<sup>10,13</sup> and researchers also demonstrated the functional importance between osteoprotegerin and VWF: CB in an atherothrombosis-prone biological environment.<sup>15</sup> However, the relationship between VWF (especially VWF activity for evaluating endothelial dysfunction) and the severity of coronary stenosis in patients with AMI remains unclear.

## Materials and Methods

### Patients

This study was approved by the ethics committee of the First Affiliated Hospital of Soochow University. All study procedures were executed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients prior to inclusion in the study.

This study was a cross-sectional study. A total of 205 patients with AMI who underwent coronary angiography on admission were recruited from the First Affiliated Hospital of

Soochow University. The diagnosis of AMI conformed to American College of Cardiology (ACC)/American Heart Association (AHA) guidelines for the management of patients with ST-segment elevation myocardial infarction.<sup>16</sup> Exclusion criteria included serious heart failure, liver or renal insufficiency, valvular heart disease, myocarditis, other inflammation (such as pneumonia), and malignant tumors based on history, clinical evaluation, or investigations. Data regarding medical history, such as age, sex, hypertension, diabetes mellitus, and hyperlipidemia, were collected from included patients. An additional 123 healthy patients, who did not have hyperlipidemia, diabetes, and hypertension, were included as normal controls. These control specimens were collected in the same time period as the AMI group and were matched on sex and age with AMI group.

### Sample Preparation and Processing

Plasma collection and VWF detection by enzyme-linked immunosorbent assay (ELISA) were prepared and processed as described previously.<sup>17</sup> Whole blood samples from patients were collected within 24 hours of admission before coronary angiography. The normal range of VWF: Ag for healthy individuals was 30% to 200%, while there was currently no normal range for VWF: CB test.

### Coronary Angiographic Data and Gensini Score

Coronary artery disease was defined as the presence of stenosis (>50% of the luminal diameter) in the major coronary arteries, including the left main system (LMS), left anterior descending artery (LAD), circumflex artery (CX), and right coronary artery (RCA). Coronary angiography was carried out by 2 experienced interventional cardiologists, and the Gensini score was calculated to evaluate the severity of coronary stenosis, as described previously.<sup>18</sup> The Gensini score refers to the narrowing of the lumen of coronary arteries: 1 for 1% to 25% narrowing, 2 for 26% to 50% narrowing, 4 for 51% to 75% narrowing, 8 for 76% to 90% narrowing, 16 for 91% to 99% narrowing, and 32 for complete occlusion. A coefficient was defined according to the coronary artery and its location of stenosis: 5 for LMS lesions, 2.5 for proximal LAD, 1.5 for middle LAD lesions, 1 for distal LAD, 2.5 for proximal CX lesions, 1 for mid/distal CX and RCA lesions, and 0.5 for all other artery branches. The Gensini score was shown as a summation of multiplication by narrowing score and stenosis location (The total score of each branch blood vessel = scores of the stenosis degree × weight coefficient). In this study, patients with AMI were classified into 3 groups: G1 for Gensini scores below 50, G2 for scores between 50 and 80, and G3 for scores over 80. In addition, single-vessel disease was defined as the presence of stenosis in only 1 major coronary artery with >50% stenosis (main vessel and/or the underlying major branch of the lesion), and double-vessel disease was defined as the presence of stenosis in 2 major coronary arteries simultaneously. Stenosis in the LMS was also classified as double-vessel disease.

**Table 1.** Baseline Characteristics of Controls and Patients With AMI (Mean  $\pm$  SD).

Characteristic	Control	AMI	P Value
Age, years	57.96 $\pm$ 13.64	58.99 $\pm$ 11.57	.852
Gender, M/F	104/19	173/32	.969
Smoking, n (%)	61 (50.83%)	118 (57.56%)	.161
SBP, mm Hg	123.28 $\pm$ 13.15	122.94 $\pm$ 24.12	.571
DBP, mm Hg	80.25 $\pm$ 7.54	76.49 $\pm$ 15.19	.004
Glu, mmol/L	5.56 $\pm$ 1.17	7.18 $\pm$ 2.98	<.001
TG, mmol/L	1.59 $\pm$ 1.14	1.88 $\pm$ 1.34	.003
HDL-C, mmol/L	1.53 $\pm$ 0.37	1.09 $\pm$ 0.32	<.001
LDL-C, mmol/L	2.86 $\pm$ 0.83	2.85 $\pm$ 0.86	.968
UA, $\mu$ mol/L	322.41 $\pm$ 65.69	352.62 $\pm$ 93.69	.002
Cr, $\mu$ mol/L	64.21 $\pm$ 16.72	75.65 $\pm$ 25.42	<.001
White blood cell, $10^9/L$	6.14 $\pm$ 1.61	10.63 $\pm$ 3.28	<.001
Platelet, $10^9/L$	233.89 $\pm$ 58.96	217.15 $\pm$ 54.72	.024

Abbreviations: AMI, acute myocardial infarction; Cr, creatinine; DBP, diastolic blood pressure; Glu, fasting glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCI, percutaneous coronary intervention; SBP, systolic blood pressure; SD, standard deviation; TG, total triglycerides; UA, uric acid.

Finally, triple-vessel disease was defined as stenosis in 3 major coronary arteries.

### Statistical Analysis

Quantitative variables are shown as the mean  $\pm$  standard deviation, whereas categorical variables are expressed as numbers and percentages (%). The Mann-Whitney *U* test or Student *t* test,  $\chi^2$  test, and Kruskal-Wallis *H* were used to analyze differences between controls and patients with AMI as appropriate. The Spearman correlation coefficient was used to analyze the relationship between VWF levels (VWF: Ag and VWF: CB) and other parameters (Gensini score and the number of implicated vessels). Univariate analysis and multivariate logistic regression analysis were used to evaluate for independent factors of coronary stenosis. All statistical analyses were executed using SPSS 19.0 (IBM SPSS Statistics 19.0, Chicago, Illinois), and plots were drawn in GraphPad Prism 5.0 (GraphPad Software, San Diego, California). *P* < .05 was considered to be statistically significant.

## Results

### Baseline Characteristics

Baseline demographics and clinical characteristics are shown in Table 1. Overall, 328 patients were included (control, *n* = 123; patients with AMI, *n* = 205). The average age of patients with AMI was 58.99  $\pm$  11.57 years, and 173 (84.39%) patients were male. Of the 205 patients, 11 (5.37%), 43 (20.98%), 112 (54.63%), 18 (8.78%), 24 (11.71%), and 28 (13.66%) patients had a history of hyperlipidemia, diabetes, hypertension, stroke, percutaneous coronary intervention (PCI), and statin therapy, respectively. Levels of glucose, triglycerides (TG), uric acid, creatinine, and white blood cells

(within 24 hours of admission) were significantly higher in patients with AMI than in controls (all *P* < .05). In contrast, the levels of high-density lipoprotein (HDL) and platelets were lower in patients with AMI. No significant differences in systolic blood pressure and low-density lipoprotein levels were observed between controls and patients with AMI.

### Plasma Levels of VWF in Patients With AMI

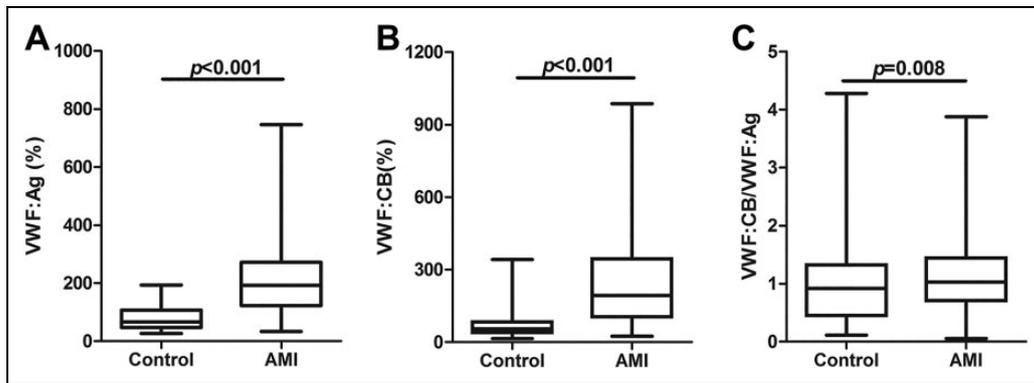
Plasma levels of VWF: Ag and VWF: CB were detected using ELISA. The level of VWF: Ag in patients with AMI was significantly higher than that in the control group (219.87%  $\pm$  1.32% vs 78.90%  $\pm$  0.38%; *P* < .001). Similar results were achieved for plasma VWF: CB levels and VWF: CB/VWF: Ag (257.58%  $\pm$  2.18% vs 68.40%  $\pm$  0.48%, *P* < .001; 1.17  $\pm$  0.66 vs 1.12  $\pm$  0.98, *P* = .008, respectively; Figure 1). Furthermore, plasma levels of VWF: Ag and VWF: CB were also analyzed based on the blood collection time during the acute status of AMI. Each group was divided into 3 groups based on different time range within parentheses: (1) time from onset to clinical intervention (1-4, 5-8, and 9-24 hours; Figure 2A and D), (2) time after surgery (1-5, 6-15, and 16-24 hours; Figure 2B and E), (3) time from onset to postoperative blood collection (4-10, 11-23, and 24-42 hours; Figure 2C and F). Data showed that there were no significant differences in plasma level of VWF: Ag no matter which acute status of AMI; however, there was significant difference in plasma level of VWF: CB. In addition, VWF: CB level of intermediate group was higher than the other time group during the acute status of AMI (Figure 2D-F).

### Analysis of Plasma VWF Levels in Patients With AMI Based on Different Risk Factors of CVD

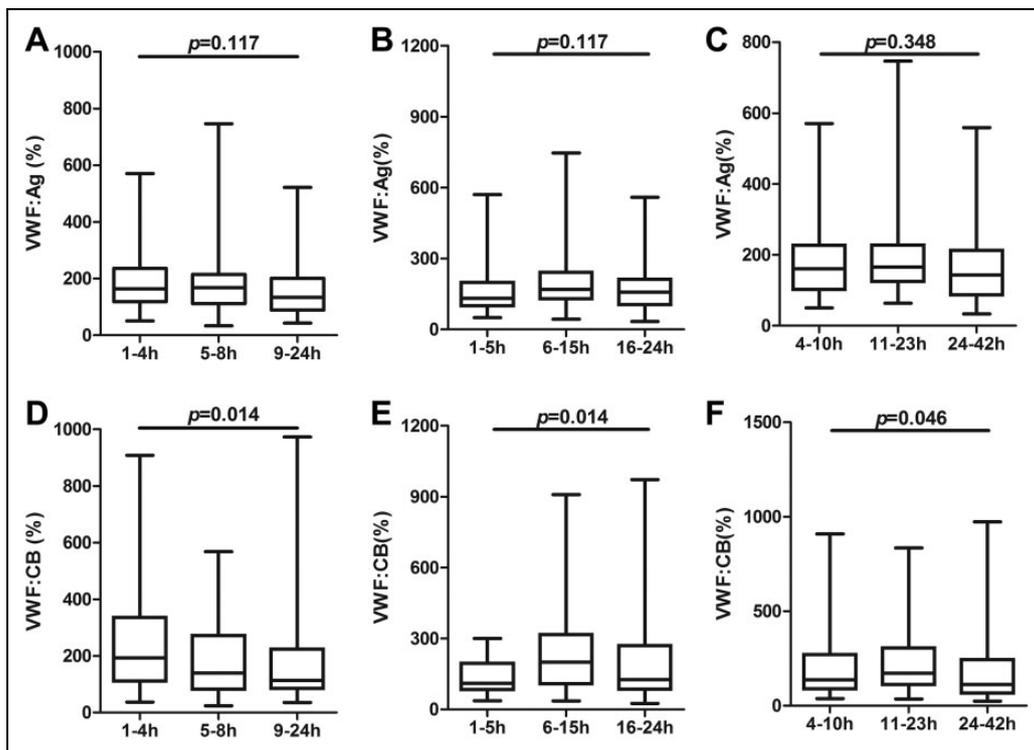
Patients with AMI were classified into different subgroups according to baseline characteristics and CVD-related comorbidities. Notably, the level of VWF: CB in younger patients ( $\leq$ 50 years) was lower than that in patients >50 years old (*P* = .042), and females had higher levels of VWF: Ag (*P* = .001). Patients with a history of hypertension had significantly lower levels of VWF: Ag compared to those without a history of hypertension (*P* = .041). Furthermore, patients with a history of stroke had higher levels of VWF: Ag, VWF: CB, and VWF: CB/VWF: Ag than those without a history of stroke (*P* = .011, <.0001, and .007, respectively). Levels of VWF were similar between patients with and without a history of PCI (Table 2).

### Analysis of Plasma VWF Levels in Patients With AMI Based on Different Severities of Coronary Stenosis

Patients were divided into 4 groups according to the severity of coronary stenosis. Both plasma VWF: Ag and VWF: CB levels were gradually enhanced as the severity of coronary stenosis increased (*P* < .001; Figure 3A). Notably, plasma VWF: Ag levels in G2 and G3 groups were higher than those in the G1 group. Similar findings were observed for plasma VWF: CB



**Figure 1.** Analysis of plasma VWF level in patients. Comparison of VWF: Ag levels (A), VWF: CB levels (B), and their ratio (VWF: CB–VWF: Ag; C) in controls (n = 123) and patients with AMI (n = 205)  $P < .05$ . AMI indicates acute myocardial infarction; VWF, von Willebrand factor; VWF: Ag, von Willebrand factor antigen; VWF: CB, VWF-collagen binding assays.



**Figure 2.** Analysis of plasma level of VWF: Ag (A-C) and VWF: CB (D-E) based on the blood collection time during the acute status of AMI (n = 124)  $P < .05$ . AMI indicates acute myocardial infarction; VWF, von Willebrand factor; VWF: Ag, von Willebrand factor antigen; VWF: CB, VWF-collagen binding assays.

levels in the analysis of the number of implicated vessels ( $P < .001$ ; Figure 3E). Plasma VWF: Ag levels in the single-vessel disease group, double-vessel disease group, and triple-vessel disease group were higher than those in the control group. Plasma VWF: Ag levels in the single-vessel disease group were lower than those in the triple-vessel disease group ( $P = .023$ ; Figure 3D). However, in comparison to controls, VWF: CB/VWF: Ag was higher in G1, G3, and double-vessel disease groups (Figure 3C and F). The plasma VWF levels of each group are presented in Supplementary Table S1.

#### Correlation Among Plasma VWF Levels, Gensini Score, the Number of Implicated Vessels, and Inflammatory Markers

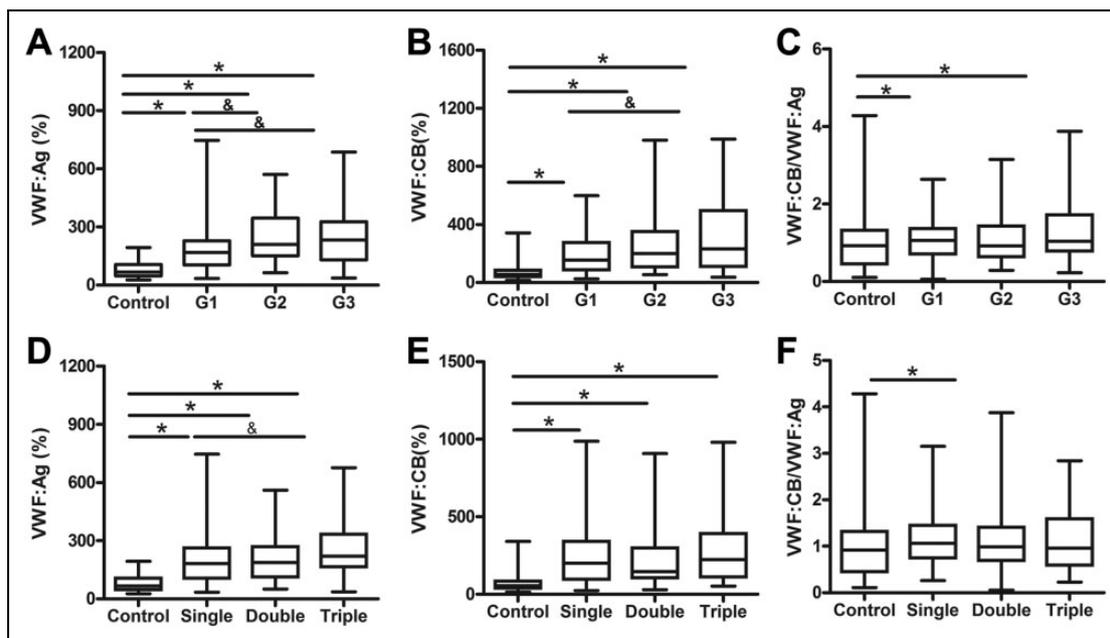
Plasma VWF: Ag and VWF: CB levels are positively correlated with the Gensini score in patients with AMI ( $r = 0.257$ ,  $P < .0001$ ;  $r = 0.228$ ,  $P = .001$ , respectively). In addition, there was no significant correlation between VWF: CB and the number of implicated vessels, while VWF: Ag was positively correlated with the number of implicated vessels ( $r = 0.143$ ,

**Table 2.** Demographics and Some Clinical Characteristics of Patients With AMI (Mean ± SD).

Demographics	n	VWF: Ag, %	VWF: CB, %	VWF: CB/VWF: Ag
<b>Age</b>				
≤50 years	46	191.51 ± 1.15	207.57 ± 1.75	1.11 ± 0.67
>50 years	159	228.09 ± 1.35	272.04 ± 2.27 <sup>a</sup>	1.19 ± 0.66
<b>Gender</b>				
Male	173	205.86 ± 1.23	239.12 ± 1.96	1.17 ± 0.62
Female	32	295.63 ± 1.49 <sup>a</sup>	357.33 ± 2.98	1.21 ± 0.85
<b>Hypertension</b>				
Nonhypertension	93	233.91 ± 1.18	282.16 ± 2.21	1.22 ± 0.72
Hypertension	112	208.22 ± 1.41 <sup>a</sup>	237.16 ± 2.14	1.13 ± 0.61
<b>Hyperlipidemia</b>				
Nonhyperlipidemia	194	219.39 ± 1.29	260.07 ± 2.16	1.18 ± 0.67
Hyperlipidemia	11	228.45 ± 1.76	213.50 ± 2.53	1.03 ± 0.59
<b>Diabetes</b>				
Nondiabetes	162	215.27 ± 1.32	254.95 ± 2.20	1.18 ± 0.66
Diabetes	43	237.21 ± 1.30	267.46 ± 2.13	1.15 ± 0.66
<b>Smoking</b>				
Nonsmoking	87	240.17 ± 1.38	272.22 ± 2.35	1.13 ± 0.67
Smoking	118	204.91 ± 1.25 <sup>a</sup>	246.78 ± 2.05	1.21 ± 0.66
<b>History of Stroke</b>				
Nonstroke	187	213.24 ± 1.30	233.54 ± 1.92	1.11 ± 0.59
Stroke	18	288.77 ± 1.32 <sup>a</sup>	507.25 ± 3.09 <sup>a</sup>	1.77 ± 1.00 <sup>a</sup>
<b>History of PCI</b>				
Non-PCI	173	218.67 ± 1.28	259.99 ± 2.20	1.18 ± 0.64
PCI	32	226.37 ± 1.50	244.51 ± 2.10	1.16 ± 0.80

Abbreviations: AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; SD, standard deviation; VWF, von Willebrand factor; VWF: Ag, VWF antigen; VWF: CB, VWF-collagen binding.

<sup>a</sup>P < .05.



**Figure 3.** Comparison of plasma VWF: Ag and VWF: CB levels in patients with AMI based on different subgroups. A to C, Plasma VWF levels in controls and patients with AMI. Patients with AMI were classified into different groups according to the Gensini score. D to F, Plasma VWF levels in controls and patients with AMI. Patients with AMI were classified into different groups according to the number of implicated vessels. \*P < .05 versus controls. &P < .05 versus G1 or single-vessel disease group. AMI indicates acute myocardial infarction; VWF, von Willebrand factor; VWF: Ag, von Willebrand factor antigen; VWF: CB, VWF-collagen binding assays.

**Table 3.** Univariate and Multivariable Analysis for Independent Factors of Coronary Stenosis.

Variable	Unadjusted					Adjusted <sup>a</sup>				
	B	Wals	OR	95% CI	P	B	Wals	OR	95% CI	P
VWF: Ag	0.030	70.786	1.030	1.023-1.037	<.01	0.031	63.003	1.032	1.024-1.040	<.01
VWF: CB	0.022	52.016	1.022	1.016-1.028	<.01	0.021	45.761	1.021	1.015-1.027	<.01
VWF: CB/VWF: Ag	0.077	0.277	1.080	0.812-1.437	.598	0.025	0.024	1.025	0.753-1.394	.876

Abbreviations: CI, confidence interval; LDL, low-density lipoprotein; OR, odds ratio; TC, total cholesterol; TG, triglyceride; VWF, von Willebrand factor; VWF: Ag, VWF antigen; VWF: CB, VWF-collagen binding.

<sup>a</sup>Adjusted for age, gender, TC, LDL, and TG level.

$P = .041$ ). However, there was also no significant correlation between VWF and inflammatory markers, including white blood cell, absolute neutrophil count, and hypersensitive C-reactive protein (the hypersensitive C-reactive protein and common C-reactive protein belong to the same class of proteins, but hypersensitive C-reactive protein is named due to the more sensitive detection method; data are shown in Supplementary Table S2, and raw data regarding the inflammatory markers in Supplementary Table S3).

### Univariate and Multivariable Analysis for Independent Factors of Coronary Stenosis

To evaluate the independent predictors for the presence of coronary stenosis, univariate and multivariable logistic regression analyses were performed to assess contribution of VWF to the presence of CAD. In an unadjusted model, plasma VWF: Ag and VWF: CB levels were independently correlated with the existence of CAD (odds ratio [OR]:1.030, 1.022, respectively, all  $P < .01$ ). After adjusting for confounding factors such as age, gender, and serum TC, low-density lipoprotein (LDL) and TG levels and plasma VWF: Ag and VWF: CB levels remained independently correlated with the existence of CAD (OR: 1.032, 1.021, respectively, all  $P < .01$ ; Table 3). Thus, VWF: Ag and VWF: CB were independent factors for coronary stenosis. However, VWF: CB/VWF: Ag was not independent factor for coronary stenosis, no matter whether adjusted confounding factors (Table 3;  $P > .05$ ).

## Discussion

To our knowledge, this is the first study to investigate the value of plasma VWF levels in predicting the severity of coronary atherosclerosis, as quantified by the Gensini score, in patients with AMI. Our results show that both plasma VWF: Ag and VWF: CB levels were significantly elevated in patients with AMI compared to controls. This was in line with previous findings.<sup>10,11</sup> In this study, the levels of serum TG and HDL were significantly higher and lower, respectively, in patients with AMI than those in controls. Plasma VWF self-association under high shear stress has crucial effect on the complex signal pathways of platelet activation.<sup>19</sup> Federici has reported that VWF self-association under shear stress can be modulated by the HDL and apolipoprotein A-I complex, with significant

reduction in the length and thickness of VWF fibers.<sup>20</sup> Thus, we hypothesize that a lower level of HDL may contribute to the increase in plasma VWF levels in patients with AMI. Furthermore, studies have shown that a low level of ADAMTS13 is associated with an increased risk of myocardial infarction.<sup>21</sup> The ADAMTS13 binds to the A2 domain of VWF and cleaves UL-VWF into smaller, less adhesive forms. Whether HDL and ADAMTS13 have synergistic or other effects in regulating thrombosis remains unclear.

Many parameters can affect plasma VWF levels and activity.<sup>4</sup> The timing of measurement of VWF: Ag and VWF: CB during the acute status of AMI is one major issue. We analyzed the levels of VWF: Ag and VWF: CB in different time group and found VWF: Ag was not affected by time, while there was significant difference in plasma level of VWF: CB. This may be due to clinical measures taken after the onset of AMI, which led to changes in endothelial function. In this study, patients with a history of hypertension had lower VWF: Ag levels than those in patients without hypertension. Hypertension can induce changes in systemic blood flow and generate high shear, which then exposes more VWF sites that can be cleaved by ADAMTS13.<sup>4,5</sup> Our findings that older patients with AMI had increased VWF: CB levels compared to younger patients suggest that older patients have higher VWF activity. Previously, Cowman et al reported that age and gender significantly impacts on platelet interactions with VWF.<sup>22</sup> A second myocardial infarction has been shown to occur earlier in women than in men (16 vs 33 months,  $P < .001$ ).<sup>23</sup> There was also an increase in VWF levels in female patients with AMI in our study. Overall, these data have demonstrated that women carry a higher risk factor burden and may require more aggressive and prompt secondary prevention.

Studies utilizing clinical and animal models have shown that VWF-deficient mice have significantly smaller infarctions.<sup>8</sup> In addition, the risk of myocardial infarction is increased when plasma levels of VWF are high and levels of ADAMTS13 are low.<sup>10,21</sup> In the present study, we reported that patients with AMI with a history of stroke had significantly higher VWF: Ag and VWF: CB levels than those without a history of stroke. Moreover, there were no significant differences in VWF: Ag levels, VWF: CB levels, and VWF: CB/VWF: Ag between patients having AMI with and without a history of PCI. Previous studies have suggested that the synergistic action of VWF and VWF-bound microvesicles promotes systemic vascular

leakage and coagulation.<sup>24</sup> Our study demonstrates that patients with a history of stroke had an elevated risk of AMI; this may be attributed to the pivotal role of platelet-derived VWF in stroke. In addition, atherosclerosis is an inflammatory disease,<sup>25</sup> and inflammation has been shown to cause thrombosis via VWF-mediated mechanisms.<sup>26</sup> However, there was no significant correlation between VWF and hypersensitive C-reactive protein. The study found that the VWF level of patients with AMI after reperfusion therapy generally rises around 24 hours after the onset, peaks at 48 to 72 hours, then returns to the basal level on the 14th day.<sup>27</sup> One explanation for a lack of correlation between VWF and hypersensitive C-reactive protein could be the asynchronous time process. Hence, there was no evidence showing that patients with AMI should lower VWF through anti-inflammatory agents. We hypothesize that VWF may be an acute-phase response protein in the onset of AMI. Moreover, patients with a history of PCI should be made aware of their high risk of recurrent MI regardless of VWF levels.

This study focused on exploring the link between VWF: Ag or VWF: CB and coronary artery stenosis. Plasma levels of VWF: Ag and VWF: CB in AMI patients group were higher than those in the control group, and levels increased gradually according to the severity of coronary stenosis. Further analysis also revealed significant differences referring to VWF: Ag and VWF: CB levels between groups with different Gensini scores. Similar results were found when analyzing VWF levels with regard to the number of implicated vessels. Furthermore, univariate and multivariable analysis results demonstrated VWF: Ag and VWF: CB were independent factors for coronary stenosis. Acute myocardial infarction results from the rupture or erosion of an atherosclerotic plaque, and thrombus formation plays a vital role in the pathophysiology of myocardial infarction. von Willebrand factor has been shown to modulate intimal hyperplasia and stimulate the proliferation of mouse aortic smooth muscle cells via a dose–response effect.<sup>28</sup> von Willebrand factor can also be synthesized by vascular endothelial cells<sup>4</sup>; we hypothesized that secreted VWF may modulate the physiological activity of the endothelium via a negative feedback mechanism. Kaikita et al found that the coronary thrombi derived from patients with AMI consisted of platelets, fibrin, and inflammatory molecules, and VWF was prominently localized to the site of platelet accumulation and fibrin deposition.<sup>29</sup> This suggested that VWF is involved in the formation of thrombi in patients with AMI. To further explore the potential role of VWF in coronary artery stenosis, we analyzed whether plasma VWF levels were associated with the Gensini score. Our results demonstrate that VWF: Ag and VWF: CB were positively correlated with the severity of coronary stenosis. Methia et al reported that atherosclerosis was attenuated in the aortas of VWF-deficient mice that were treated with a diet rich in saturated fat and cholesterol.<sup>8</sup> However, the exact pathophysiological role of VWF in CVD has not been clarified. Sonneveld et al reported that patients with acute coronary syndrome (ACS) had significantly higher VWF: Ag levels than patients with SAP, and high coronary plaque burden was

associated with higher VWF: Ag levels in patients with SAP. Nevertheless, the correlation between coronary plaque burden and VWF: Ag was not found in patients with ACS. Several reasons may explain why our results differed from those reported by Sonneveld et al: (1) VWF levels can be affected by many different factors, and variations in the characteristics of controls between studies may contribute to differences in results, which may be the reason for the low OR value analyzing independent factors of coronary stenosis in our study; (2) different methods were used to measure coronary plaques (intravascular ultrasound vs coronary angiography), and there may be a reaction between the vascular endothelium and radiographic contrast media; and (3) only a single nonculprit coronary vessel was included in Sonneveld et al's study, whereas stenosis of the overall coronary tree was calculated by the Gensini score. Thus, the previous study may underestimate the role of VWF in coronary plaque. In brief, more clinical data of patients with AMI should be collected to analyze the value of VWF in risk assessment, and prospective study rather than retrospective study may be more suitable. Given the limitations of our retrospective study, we have established a database in collecting data prospectively. Notably, VWF propeptide (VWFpp) can be secreted together with VWF: Ag upon endothelial cell activation and is not influenced by blood type.<sup>30</sup> Thus, VWFpp may be a potential biomarker that is superior to VWF: Ag in assessing the severity of coronary stenosis. Although our study showed a positive correlation of plasma VWF: Ag and VWF: CB levels with the Gensini score, there is more to learn about the possible role of VWF in the development of AMI. Hopefully, our results will provide researchers with further direction in studying the hemostatic mechanism of AMI.

There are several limitations in this study. First, this article was a cross-sectional study. Hence, specific details were lacking in our data (such as blood group, ADAMTS 13 detection), which may pose a bias. Second, dynamic detection of VWF at the time of MI may be necessary. Third, this study demonstrated rosuvastatin could increase LDL extracellular vesicles coagulation protein VWF in patients with subclinical atherosclerosis while not in plasma.<sup>31</sup> However, we were limited in terms of the correlation between statin therapy and VWF activity. Finally, we only included patients with AMI and healthy patients. Patients presented to the emergency department in who AMI was ruled out should be included as another group. Joint analysis of the differences and correlations between the 3 groups may reach more meaningful conclusions.

## Conclusion

In conclusion, the present study shows that plasma VWF: Ag and VWF: CB levels were increased in patients with AMI and tended to be enhanced in groups with higher Gensini scores. Additionally, we provided evidence that plasma VWF: Ag and VWF: CB levels were positively correlated with the Gensini score evaluating coronary stenosis in patients with AMI. These

findings suggest that VWF screening at time of AMI may have prognostic value in terms of the severity of coronary stenosis.

### Authors' Note

B.Y. and Y.Z. were primarily in charge of study design. B.Y. and Q.W. executed the experiments. L.X. and C.R. provided material support and contributed to the study design. W.D., S.Z., C.G., and T.H. analyzed and interpreted the data. B.Y. wrote the manuscript. L.X. and Y.Z. revised and finalized the discussion. All authors read and approved the final manuscript. All relevant data within this article will be made available. Any additional information is available from the corresponding author upon reasonable request.

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### Supplemental Material

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