

# Von Willebrand Factor in relation to coronary plaque burden and presence of high risk lesions on intravascular ultrasound and cardiovascular outcome

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## ABSTRACT

**Objective:** High VWF plasma levels are associated with an increased risk of coronary artery disease. It has been suggested that the increase of VWF levels is partly due to endothelial dysfunction and atherosclerosis. Our aim was to investigate the association between coronary plaque burden, the presence of high-risk coronary lesions as measured by intravascular ultrasound virtual histology (IVUS-VH) and VWF levels. In addition, we studied the association between VWF levels and 1-year cardiovascular outcome.

**Methods:** Between 2008 and 2011, IVUS-VH imaging of a non-culprit coronary artery was performed in 581 patients undergoing coronary angiography for acute coronary syndrome (ACS) (n= 318) or stable angina pectoris (SAP) (n= 263). Arterial blood was sampled prior to the coronary angiography. VWF antigen (VWF:Ag) levels were measured using ELISA (n= 577).

**Results:** Patients with ACS had significantly higher VWF:Ag levels than SAP patients (median 1.73 IU/ml [IQR 1.27-2.31] vs. 1.26 IU/ml [0.93-1.63],  $p < 0.001$ ). High coronary plaque burden was associated with higher VWF:Ag levels ( $\beta = 0.12$ ,  $p = 0.027$ ) in SAP patients, but not in ACS patients. In ACS patients, VWF:Ag levels were associated with 1-year MACE (HR 4.14 per SD increase of lnVWF:Ag, 95% CI 1.47-11.6), whereas in SAP patients VWF:Ag levels predicted 1-year all-cause death and hospitalisation for ACS (HR 7.07 95% CI 1.40-35.6).

**Conclusions:** Coronary plaque burden was associated with VWF:Ag levels in SAP patients undergoing coronary angiography. In ACS and SAP patients, high VWF levels are predictive of adverse cardiovascular outcome and death during 1-year follow-up.

## INTRODUCTION

Von Willebrand Factor (VWF) is a multimeric protein that plays a crucial role in primary hemostasis by mediating platelet adhesion and aggregation (1). VWF is produced by endothelial cells and megakaryocytes and stored in Weibel-Palade bodies in the endothelium and alpha-granules of platelets. VWF plasma levels are increased at moments of endothelial damage and are a marker of endothelial dysfunction (2).

It is well known that high VWF levels are associated with an increased risk of coronary heart disease and ischemic stroke in the general population (3-8). However, the underlying mechanisms of this association are still unclear. As high VWF levels are seen in situations with endothelial dysfunction, which is an important early process in atherosclerosis development, it has previously been suggested that VWF has a pathogenic role in atherosclerosis. This hypothesis is supported by results from animal studies (9-11). However, studies in patients with type 3 von Willebrand disease, characterized by a total deficiency of VWF in the circulation, revealed no reduction in atherosclerotic lesions (12-14). The role of VWF in the development of atherosclerosis in humans is therefore still unresolved. In a recent study, we observed a strong association between the extent of atherosclerosis, measured by the calcification volume in the aortic arch and carotid arteries, and VWF levels in ischemic stroke patients (15). Because VWF also plays a pivotal role in platelet aggregation and thrombus formation, these high VWF levels may further increase the risk of coronary events in patients with high risk atherosclerotic lesions.

Intravascular ultrasound (IVUS) can accurately quantify coronary atherosclerosis (16, 17). A previous study in 697 patients with an acute coronary syndrome at inclusion showed that half of the incident recurrent cardiovascular events occurred in patients with non-culprit lesions present at baseline, assessed by IVUS imaging (18). High-risk coronary lesions that are predictive for events include lesions with a plaque burden of at least 70%, a minimal luminal area of 4.0 mm<sup>2</sup> or less or the presence of IVUS virtual histology (VH)-derived thin-cap fibroatheroma lesions (VH-TCFA) (18).

In order to gain further insight into the relationship between VWF levels and cardiovascular outcome, the aim of the present study was to investigate the associations of coronary plaque burden, and the presence of high-risk coronary lesions as assessed by virtual histology intravascular ultrasound (VH-IVUS) with VWF levels, as well as to investigate the association of VWF with 1-year cardiovascular outcome in patients with coronary artery disease (CAD).

## METHODS

### Study population

The design of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described in detail elsewhere (19). In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for an acute coronary syndrome (ACS) or stable angina pectoris (SAP) have been included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands.

The ATHEROREMO-IVUS study was approved by the medical ethics committee of the Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all included patients. This study is registered in ClinicalTrials.gov, number NCT01789411.

### Von Willebrand Factor measurement

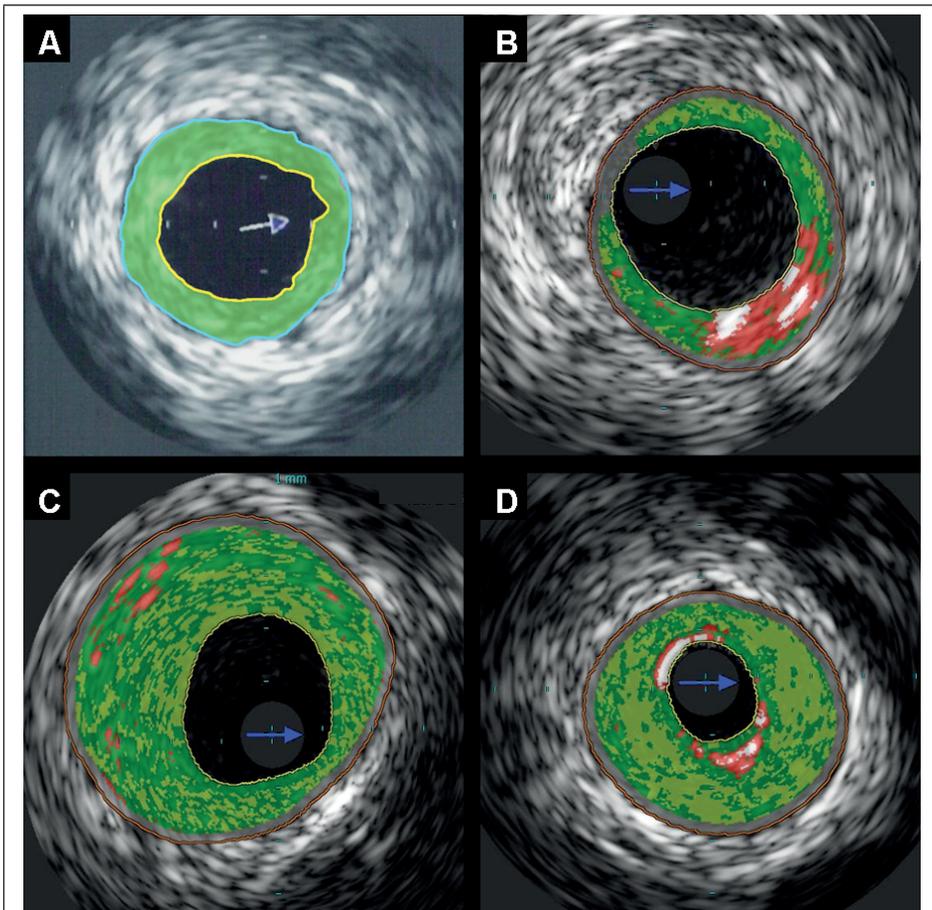
Blood samples were drawn from the arterial sheath prior to the coronary angiography procedure. The blood samples were transported to the clinical laboratory of the Erasmus MC for further processing and storage at temperature of  $-80^{\circ}\text{C}$  within 2 hours after blood collection. VWF antigen (VWF:Ag) levels were determined (N=577) using citrate blood with an in-house ELISA using rabbit anti-human VWF antibodies (DakoCytomation, Glostrup, Denmark) for catching and tagging. Reference standard plasma was calibrated against the international standard (Cryocheck Reference, Kordia, Leiden, The Netherlands) and was used as a calibrator. The intra- and inter-assay coefficients of variation were 2.6% and 4.7%.

### Intracoronary ultrasound imaging

Following the standard coronary angiography procedure, IVUS imaging of a non-culprit coronary artery was performed. Selection of the non-culprit vessel was predefined in the study protocol. The order of preference for selection of the non-culprit vessel was: 1. left anterior descending (LAD) artery; 2. right coronary artery (RCA); 3. left circumflex (LCX) artery. All IVUS data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS catheter (20 MHz). An automatic pull-back system was used with a standard pull back speed of 0.5 mm per second. The baseline IVUS images were sent to an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for baseline patient characteristics and clinical outcome data. The IVUS virtual histology analyses were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software.

The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Extent and phenotype of the atherosclerotic

plaque were assessed. Plaque burden was defined as plaque and media cross-sectional area divided by external elastic membrane cross-sectional area (Figure 1). A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Three types of high-risk lesions were identified: 1. Virtual histology-derived thin-cap fibroatheroma (VH-TCFA) lesion, defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen; 2. lesion with large plaque burden, defined as a lesion with a plaque burden of  $\geq 70\%$ ; 3. stenotic lesion, defined as a lesion with a minimal luminal area of  $\leq 4.0 \text{ mm}^2$  (Figure 1) (18, 20-22).



**Figure 1. Measurement of plaque burden and identification of high risk lesions with intravascular ultrasound virtual histology**

A: Plaque burden is defined as plaque and media cross-sectional area (green) divided by external elastic membrane cross-sectional area (contoured in blue). B: Thin-cap fibroatheroma lesion, defined as a lesion with presence of >10% confluent necrotic core (red) in direct contact with the lumen. White indicates dense calcium, light green indicates fibrofatty tissue, and dark green indicates fibrous tissue. C: Lesion with plaque burden of  $\geq 70\%$ . D: Lesion with a minimal luminal area of  $\leq 4.0 \text{ mm}^2$ .

## Clinical endpoints

Clinical follow-up started at inclusion and lasted 1 year. Post-discharge survival status was obtained from municipal civil registries. Post-discharge rehospitalizations were prospectively assessed during follow-up. Questionnaires focusing on the occurrence of major adverse cardiac events (MACE) were sent to all living patients. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contracted for additional information whenever necessary.

The primary endpoint was MACE, defined as all-cause mortality, ACS or unplanned coronary revascularization. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction (STEMI), non-STEMI or unstable angina pectoris in accordance with the guidelines of the European Society of Cardiology (23). Unplanned coronary revascularization was defined as unplanned repeat PCI (either culprit or non-culprit coronary artery) or coronary artery bypass grafting (CABG). The secondary endpoint was defined as the composite of all-cause mortality or ACS. The endpoints were adjudicated by a clinical event committee that had no knowledge of the VWF:Ag levels and IVUS data.

## Statistical analysis

The distributions of the continuous variables, including VWF levels and the IVUS parameters, were tested for normality by visual examination of the histogram. Normally distributed continuous variables are presented as mean  $\pm$  standard deviation (SD). Non-normally distributed continuous variables are presented as median and interquartile range (IQR). VWF levels were not normally distributed and were therefore natural logarithmically (ln) transformed (lnVWF:Ag), where after a normal distribution was acquired. Categorical variables are presented as numbers and percentages. We examined associations of plaque burden and presence of high-risk coronary lesions with VWF:Ag levels. VWF:Ag levels and plaque burden were divided into tertiles. To test for linear association, we used linear regression analyses with continuous ln-transformed VWF:Ag level as dependent variable. In multivariable analyses, the covariates age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking and history of myocardial infarction were considered as established cardiovascular risk factors and as potential confounders, and were therefore entered into the full model.

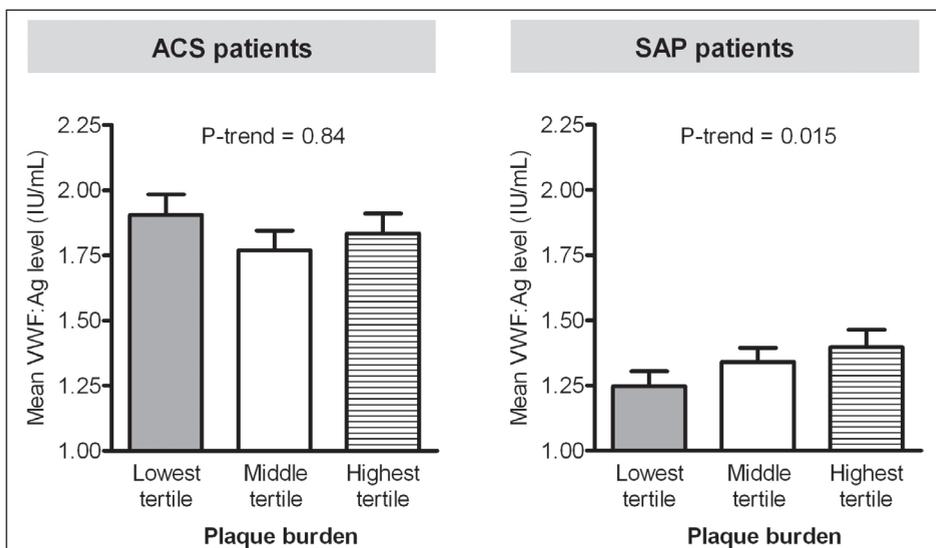
Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. Cumulative event rates were estimated according to the Kaplan-Meier method. Cox proportional hazards regression analyses were performed to evaluate the associations between VWF:Ag levels and study endpoints. Analyses were adjusted for age, gender and plaque burden. The final results are presented as crude and adjusted hazard ratios (HR) with 95% confidence interval (95% CI).

We a priori expected that there might be heterogeneity in effect estimates between patients with ACS and patients with stable angina pectoris, since VWF:Ag levels are known to be elevated in the acute phase of an ACS (24, 25). Therefore, all statistical analyses were performed separately for patients with ACS and patients with stable angina pectoris at inclusion. Data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

## RESULTS

In total 577 patients were included, 315 had an ACS and 262 had a SAP. Patients had a mean age of 61.5 years and 75% were men (Table 1). Over half of the patients had single vessel disease. SAP patients had a higher prevalence of cardiovascular risk factors than ACS patients. ACS patients were more likely to smoke. ACS patients had significantly higher VWF:Ag levels than patients with SAP (median 1.73 IU/ml [IQR 1.27-2.31] vs. 1.26 IU/ml [0.93-1.63],  $p < 0.001$ ) (Table 1).

Plaque burden was significantly higher in SAP patients than in ACS patients ( $39.7 \pm 11.0\%$  vs.  $36.9 \pm 11.8\%$ ,  $p = 0.005$ ). In SAP patients, higher plaque burden was associated with higher VWF:Ag levels (P for trend 0.015) (Figure 2). Also after adjustment for



**Figure 2. Coronary plaque burden of imaged coronary segment in relation to Von Willebrand Factor levels**

Mean  $\pm$  standard error VWF:Ag levels per tertile coronary plaque burden.

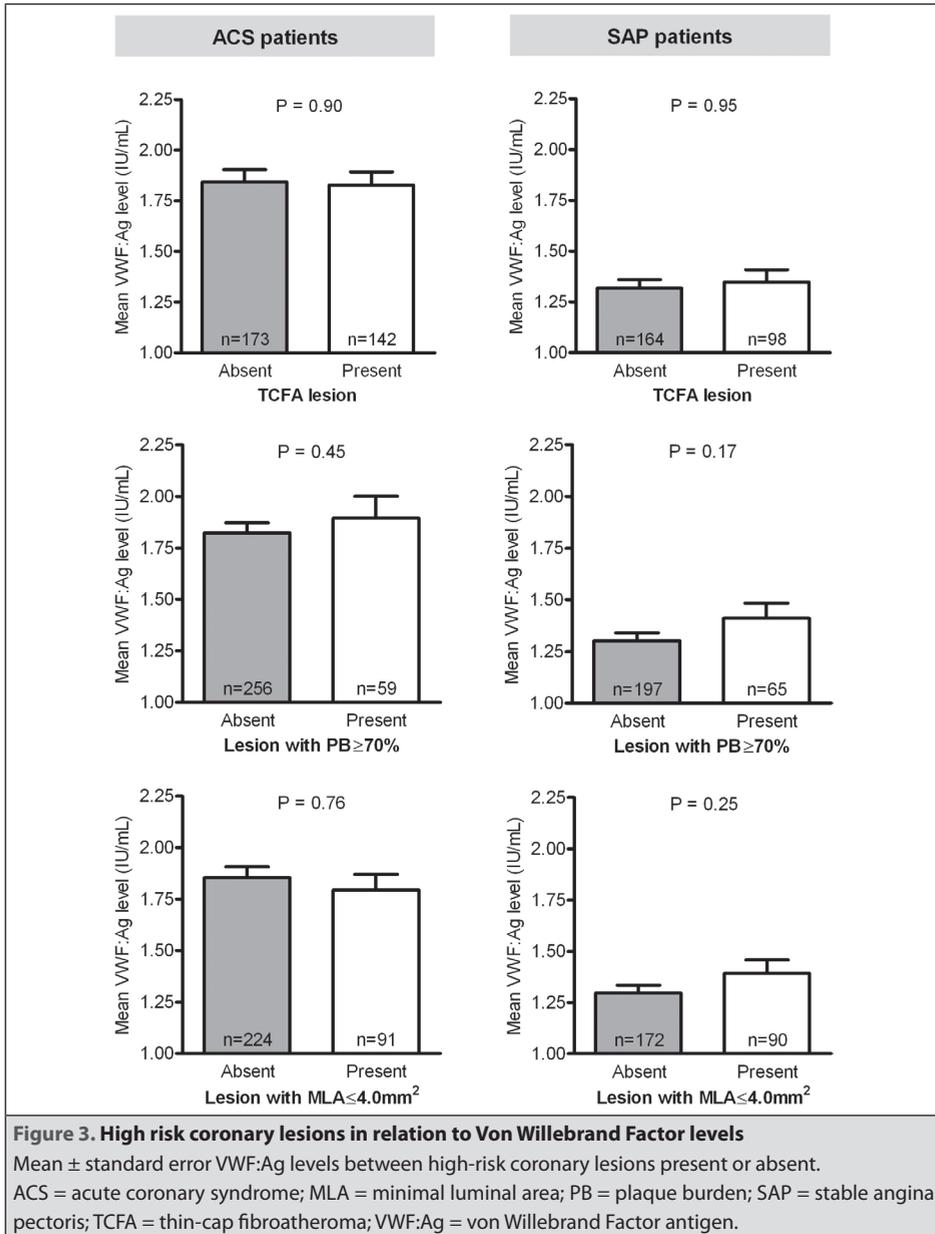
ACS = acute coronary syndrome; SAP = stable angina pectoris; VWF:Ag = von Willebrand Factor antigen.

<b>Table 1. Baseline characteristics</b>		
	<b>ACS patients (n=315)</b>	<b>SAP patients (n=262)</b>
<b>Patient characteristics</b>		
Age, years	59.7 ± 11.8	63.6 ± 10.2
Men, n (%)	232 (73.7)	203 (77.5)
Diabetes mellitus, n (%)	40 (12.7)	59 (22.5)
Hypertension, n (%)	138 (43.8)	161 (61.5)
Hypercholesterolemia, n (%)	139 (44.1)	180 (68.7)
Smoking, n (%)	117 (37.1)	50 (19.1)
Positive family history, n (%)	145 (46.0)	155 (59.2)
Previous MI, n (%)	80 (25.4)	104 (39.7)
Previous PCI, n (%)	57 (18.1)	128 (48.9)
Previous CABG, n (%)	7 (2.2)	11 (4.2)
Previous stroke, n (%)	11 (3.5)	15 (5.7)
Peripheral artery disease, n (%)	12 (3.8)	24 (9.2)
History of renal insufficiency, n (%)	13 (4.1)	19 (7.3)
History of heart failure, n (%)	6 (1.9)	13 (5.0)
Von Willebrand Factor, IU/mL	1.73 [1.27-2.31]	1.26 [0.93-1.63]
<b>Procedural characteristics</b>		
Coronary artery disease		
No significant stenosis, n (%)	18 (5.7)	25 (9.5)
1-vessel disease, n (%)	174 (55.2)	133 (50.8)
2-vessel disease, n (%)	88 (27.9)	78 (29.8)
3-vessel disease, n (%)	35 (11.1)	26 (9.9)
PCI performed, n (%)	293 (93.0)	214 (81.7)
<b>IVUS segment characteristics</b>		
Imaged coronary artery		
Left anterior descending, n (%)	120 (38.1)	88 (33.6)
Left circumflex, n (%)	110 (34.9)	84 (32.1)
Right coronary artery, n (%)	85 (27.0)	90 (34.4)
Segment length, mm	44.1 [33.0-54.3]	44.3 [34.3-57.2]

Data are presented as mean ± standard deviation or as median [interquartile range].

ACS = acute coronary syndrome; CABG = coronary artery bypass grafting; MI = myocardial infarction; PCI = percutaneous coronary intervention; SAP = stable angina pectoris.

established cardiovascular risk factors in multivariable analysis, higher plaque burden remained associated with higher VWF:Ag levels ( $p = 0.027$ ) in patients admitted with SAP. In ACS patients, the coronary plaque burden was not associated with VWF:Ag levels ( $P$  for trend 0.84). VWF:Ag levels were not significantly different between patients with and without high risk coronary lesions in both ACS and SAP patients (Figure 3).



For 575 (99.7%) patients the vital status at 1-year follow-up could be acquired, and the response rate to the questionnaires that were sent to all living patients was 93.4%. After 1 year of follow-up, 55 patients (9.6%) had experienced a MACE (Table 2). The cumulative Kaplan-Meier incidences of the 1-year MACE was 8.3% for patients with ACS, and 11.1% for patients with SAP. The risk of all-cause death and ACS was significantly associated

**Table 2. Number of patients with incident major adverse cardiac events**

Number of patients	ACS patients (n=315)	SAP patients (n=262)
Composite of major adverse cardiac events	26	29
Death from any cause	13	4
Definite cardiac or unexplained sudden death	6	2
Acute coronary syndrome	7	7
Myocardial infarction	4	3
Unplanned coronary revascularization	6	18
Composite of death or acute coronary syndrome	20	11

ACS = acute coronary syndrome; SAP = stable angina pectoris.

with higher VWF:Ag levels in both ACS patients (HR 7.45, 95% CI 2.15-25.9, P=0.002) and patients with SAP (HR 7.07 95% CI 1.40-35.6, P=0.018). Additional adjustment for plaque burden did not affect the risk estimate for all-cause death and ACS in ACS patients (HR 4.13 95% CI 1.47-11.6), while the risk in SAP patients was slightly lower (HR 4.05 95% CI 0.88-18.7). Higher VWF:Ag levels were also significantly associated with a higher incidence of MACE in ACS patients (HR 4.14, 95% CI 1.47-11.6, P=0.007), but not in patients with SAP (HR 1.31, 95% CI 0.52-3.29, p=0.57) (Table 3, Figure 4). Additional adjustment for plaque burden did not change the results.

**Table 3. Associations between von Willebrand Factor level and cardiovascular outcome**

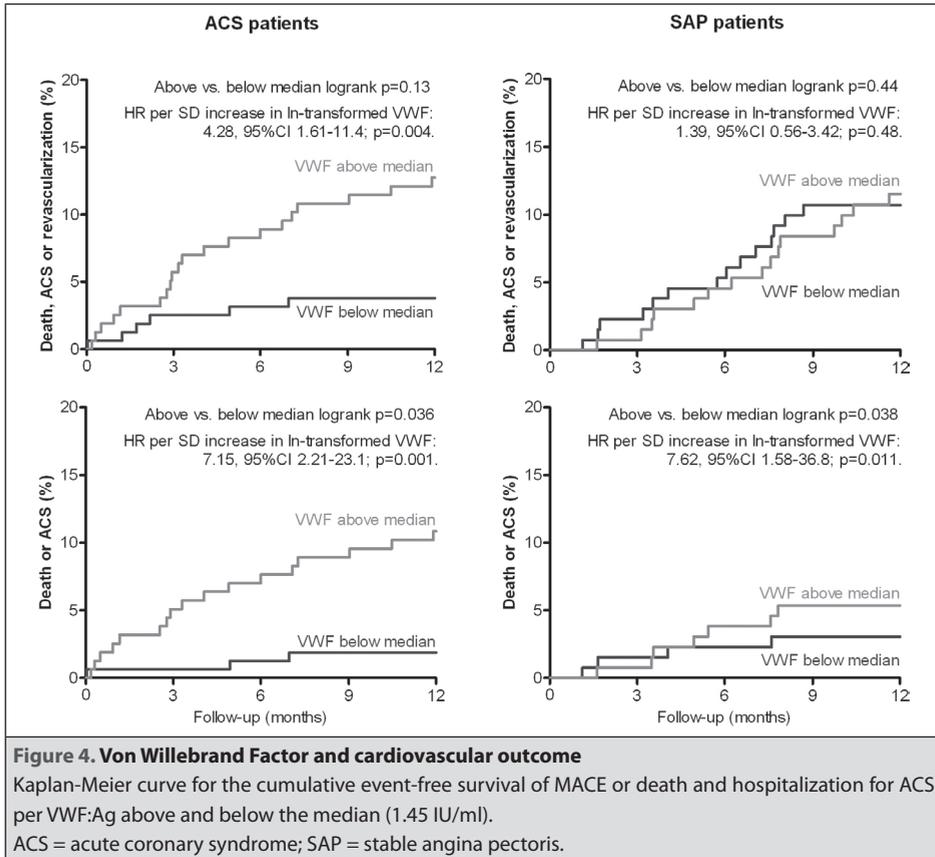
	ACS patients		SAP patients	
	HR (95%CI)*	P	HR (95%CI)*	P
<b>MACE</b>				
Unadjusted	4.28 (1.61-11.4)	0.004	1.39 (0.56-3.42)	0.48
Adjusted for age and gender	4.14 (1.47-11.6)	0.007	1.31 (0.52-3.29)	0.57
Adjusted for age, gender and plaque burden	4.13 (1.47-11.6)	0.007	1.08 (0.43-2.70)	0.87
<b>Composite of death or ACS</b>				
Unadjusted	7.15 (2.21-23.1)	0.001	7.62 (1.58-36.8)	0.011
Adjusted for age and gender	7.45 (2.15-25.9)	0.002	7.07 (1.40-35.6)	0.018
Adjusted for age, gender and plaque burden	7.65 (2.16-27.2)	0.002	4.05 (0.88-18.7)	0.073

\* Hazard ratio per SD increase in ln-transformed Von Willebrand Factor level.

ACS = acute coronary syndrome; MACE = major adverse cardiac event; SAP = stable angina pectoris.

## DISCUSSION

This is the first study that has investigated the association between invasive measured coronary atherosclerosis by VH-IVUS and VWF:Ag levels. We have shown that patients with an ACS have significantly higher VWF levels than patients with SAP. In patients with



SAP, coronary plaque burden was positively associated with VWF:Ag levels. In addition, high VWF:Ag levels were associated with death and ACS at 12 months follow up and this was also observed for all MACE in patients with ACS.

The exact pathophysiologic role of VWF in cardiovascular disease has not been elucidated yet. First, it has been hypothesized that VWF may play a causal role in the development of atherosclerosis, thereby increasing the risk of CAD. This was suggested by animal studies with VWF deficient mice, which showed less development of atherosclerosis (9-11). However, human studies, for instance in patients with type 3 von Willebrand disease who have a complete deficiency of VWF, could not confirm these findings (12, 14). However, these patients may incidentally receive VWF concentrates and some use prophylaxis at regular basis and are therefore not completely VWF deficient. It is now suggested that the association between atherosclerosis and VWF is mainly driven by the fact that VWF is a marker of endothelial damage, which is also observed in atherosclerosis (26, 27).

In this study we found that patients with ACS had significantly higher VWF:Ag levels compared with SAP patients, which is in line with a previous study (24). The finding that

plaque burden was associated with VWF:Ag levels in SAP patients confirms our previous findings that VWF is associated with the extent of atherosclerosis. In our previous study in ischemic stroke patients, we observed that a higher calcification volume in the aortic arch and carotid arteries was associated with higher VWF:Ag levels (15). The fact that there was no association between plaque burden and VWF:Ag levels in ACS patients might be explained by the strongly increased VWF:Ag levels in these patients due to an acute phase response, which is well known for VWF (2, 25).

We observed no association between several types of high-risk coronary lesions, including thin-cap fibroatheroma lesions, lesions with plaque burden  $\geq 70\%$  or lesions with a minimal luminal area  $\leq 4.0\text{mm}^2$  and VWF:Ag levels. High risk lesions are precursors of plaque rupture and may thereby account for the occurrence of coronary thrombi (18, 22, 28). Our results suggest that although VWF is associated with the extent of atherosclerosis, it is not associated with the phenotypic more vulnerable atherosclerotic lesions and might be more involved in stable atherosclerosis. However, a previous mice study showed, by molecular imaging, that activated VWF was found in atherosclerotic disease with high risk features (29). This difference might be explained by the VWF measurement, as only locally activated VWF was measured in the mice study and in our study we measured circulating VWF:Ag plasma levels. In addition, a difference in the pathophysiologic mechanism of destabilising the plaque between mice and human could also influence the results (30-33).

Our data on the association between VWF:Ag levels and MACE in ACS patients strengthens findings of previous studies suggesting that VWF has a predictive role in cardiovascular outcome (34-39). These results were not affected by additional adjustment for plaque burden, suggesting a role for VWF in cardiovascular outcome. In SAP patients, we found an association between high VWF levels and risk of death or ACS. After additional adjustment for plaque burden the association was not significant anymore in SAP patients, which may be explained by the small sample size, resulting in reduced power. These data suggest that the high VWF levels observed in ACS patients, the most severe CAD patients, at inclusion predict MACE at follow-up. However, in the definition of MACE unplanned revascularisation was included which may be considered as a weaker end-point and could therefore have influenced the adverse outcome risk (40). Overall these data supports the role for VWF in the prognosis of patients with a CAD, independent of plaque burden.

There are some limitations of this study. First, blood was sampled in the acute phase at the moment of the coronary angiography. This may explain the higher VWF:Ag levels in ACS patients compared with SAP patients. Therefore, this could have influenced our results. However, we separated the ACS and SAP patients for all analyses. Secondly, a single non-culprit coronary vessel was imaged in this study. This may have led to an underestimation of the association between the presence of high risk lesions in the overall

coronary tree and VWF:Ag levels. However, a previous study have shown that culprit and non-culprit lesions were equally related to MACE (18). In addition, the spatial resolution of IVUS-VH (150  $\mu\text{m}$ ) is insufficient to exactly replicate histopathologic definitions of a thin fibrous cap (<65  $\mu\text{m}$ ) (41). Therefore, IVUS-VH tends to overestimate the number of thin-cap fibroatheroma lesions. Nevertheless, the presence of VH-TCFA lesions has been shown to carry prognostic information (18, 22). Finally, due to the cross-sectional design our data are not able to distinguish whether VWF is causal or a marker of atherosclerosis.

In conclusion, the extent of coronary atherosclerosis is associated with VWF:Ag levels in SAP patients undergoing coronary angiography, but not in ACS patients which might be explained by the acute phase response. High VWF:Ag levels have a predictive role for adverse cardiovascular outcome, and also for MACE in ACS patients, independent of plaque burden.

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