Von Willebrand Factor, ADAMTS13, and the Risk of Mortality
The Rotterdam Study

Michelle A.H. Sonneveld, Oscar H. Franco, M. Arfan Ikram, Albert Hofman, Maryam Kavousi, Moniek P.M. de Maat, Frank W.G. Leebeek

Objective—Von Willebrand Factor (VWF) is a plasma protein that plays a major role in platelet adhesion and aggregation. Large VWF multimers are cleaved into smaller, less procoagulant forms by the metalloprotease ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13). Previous studies have shown that high VWF and low ADAMTS13 levels are associated with cardiovascular disease, but whether these factors are associated with mortality is unclear. Our aim is to establish the association between VWF antigen (VWF:Ag) levels, ADAMTS13 activity, and mortality.

Approach and Results—We included 6130 participants of the Rotterdam study, a population-based cohort study among individuals aged ≥55 years. We determined the association between ADAMTS13 activity, VWF:Ag levels, and all-cause and cardiovascular mortality by Cox proportional hazard regression analysis. During a median follow-up time of 11.3 years and a total of 90635 person years, 1868 of the 6130 individuals died (30.5%), of whom 442 (23.7%) died because of cardiovascular disease. In individuals with low ADAMTS13 activity, the risk of cardiovascular mortality (hazard ratio, 1.46; 95% confidence interval, 1.09–1.96) was higher than that in individuals with high ADAMTS13 activity. The risk of cardiovascular mortality (hazard ratio, 1.29; 95% confidence interval 0.98–1.70) was higher in individuals with the highest VWF:Ag levels than in those with the lowest levels. In individuals with both low ADAMTS13 activity and high VWF:Ag levels, the risk of cardiovascular mortality was even higher (hazard ratio, 1.73 95% confidence interval, 1.28–2.35).

Conclusions—In this large prospective cohort study, ADAMTS13 activity and VWF:Ag levels are both associated with an increased risk of all-cause and cardiovascular mortality. (Arterioscler Thromb Vasc Biol. 2016;36:2446-2451. DOI: 10.1161/ATVBAHA.116.308225.)

Key Words: cardiovascular disease ▪ disintegrins ▪ stroke ▪ thrombospondin ▪ von Willebrand Factor

Von Willebrand Factor (VWF) is a multimeric glycoprotein that plays a major role in platelet adhesion and aggregation.1 Large VWF multimers are highly procoagulant and are cleaved by its metalloprotease ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13) in smaller, less procoagulant forms.2 In case of a deficiency of ADAMTS13, large VWF multimers are not cleaved, resulting in more high-molecular VWF multimers in the circulation. This is characteristic for thrombotic thrombocytopenic purpura,3 in which patients experience microangiopathies, kidney failure, and neurological symptoms because of the formation of microthrombus in the circulation.

Cardiovascular disease (CVD) is one of the leading causes of death in the Western world.4 We recently showed in the Rotterdam study that high VWF and low ADAMTS13 levels are associated with an increased risk of CVD, including coronary heart disease and ischemic stroke.5–8 Although VWF and ADAMTS13 levels are associated with CVD, it is not yet clear whether these factors are associated with mortality.

Some prospective studies have shown an association between VWF levels and mortality.9–13 Most of these studies have shown a significant association between high VWF levels and a worse cardiovascular health,9,10,12,13 but almost all studies have focused on the cardiac-specific mortality,9–11 and only 2 studies have focused on the total cardiovascular mortality because of coronary heart disease or stroke.12,13 Studies on
Nonstandard Abbreviations and Acronyms

| ADAMTS13 | a disintegrin and metalloprotease with thromboSpondin motif repeats 13 |
| CI | confidence interval |
| COPD | chronic obstructive pulmonary disease |
| CVD | cardiovascular disease |
| HR | hazard ratio |
| VWF | von Willebrand factor |
| VWF:Ag | von Willebrand factor antigen |

the association between ADAMTS13 and all-cause mortality are lacking.

We, therefore, aimed to investigate the association between VWF levels, ADAMTS13 activity, and all-cause mortality. We further examined the association between VWF levels, ADAMTS13 activity, and cause-specific mortality, in particular mortality because of CVD, cancer, and chronic obstructive pulmonary disease (COPD).

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Briefly, this study is part of the Rotterdam study, a prospective population-based cohort study among individuals aged ≥55 years who are all living in Ommoord, a suburb of Rotterdam, The Netherlands. We included all participants who were alive at baseline and of whom blood was sampled (n=6511). Information on mortality was reported through general practitioner files and municipal records and coded according to the International Classification of Diseases, Tenth Revision. VWF antigen (VWF:Ag) levels were determined with an in-house ELISA. Polyclonal rabbit antihuman VWF antibodies were used for catching antigen (VWF:Ag) levels were determined with an in-house ELISA. ADAMTS13 activity (HR, 1.46; 95% CI, 1.09–1.96). The lowest ADAMTS13 and the highest VWF quartiles were both associated with the lowest event-free survival after adjustment for age and sex (Figure).

In individuals with both high VWF:Ag levels and low ADAMTS13 activity (n=448), the risk of all-cause mortality was strongly increased compared with individuals with both low VWF:Ag levels and high ADAMTS13 activity (Table 4). Of these 448 individuals, 247 individuals (55.1%) died during follow-up compared with 22.6% in the group individuals with low VWF:Ag levels and high ADAMTS13 activity. Individuals with both high VWF levels and low ADAMTS13 activity had a 58% increased risk of all-cause mortality (HR, 1.58; 95% CI, 1.36–1.84). Also, the risk of cardiovascular mortality was increased most strongly in individuals with both low ADAMTS13 activity and high VWF levels (HR, 1.73; 95% CI, 1.28–2.35). The risks of cancer-related mortality and COPD-related mortality were both increased with the combination of low ADAMTS13 activity and high VWF levels.

Results

In total, 6130 individuals were included in this study. The mean age of the included individuals was 69±8.0 years, and 57% were female individuals (Table 1). The median VWF:Ag level was 1.19 IU/mL (interquartile range: 0.92–1.58 IU/mL), and the mean ADAMTS13 activity was 91.8±17.6%. During the median follow-up time of 11.3 years and a total of 90365 person years, 1868 (30.5%) individuals died, 442 of them (23.7%) because of CVD, cancer, and chronic obstructive pulmonary disease (COPD).

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>N=6130</th>
<th>Total Cohort, n (%) or Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>68.9±8.0</td>
</tr>
<tr>
<td>Female sex</td>
<td>3520 (57.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1057 (17.4)</td>
</tr>
<tr>
<td>Current</td>
<td>3019 (49.6)</td>
</tr>
<tr>
<td>Antithrombotic medication</td>
<td>1194 (20.4)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1406 (24.1)</td>
</tr>
<tr>
<td>Lipid-reducing agents</td>
<td>785 (13.4)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>623 (10.3)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.8±1.0</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>143±21</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>77±11</td>
</tr>
<tr>
<td>Blood group O</td>
<td>2360 (45.3)</td>
</tr>
<tr>
<td>ADAMTS13 activity, %</td>
<td>91.76±17.59</td>
</tr>
<tr>
<td>VWF:Ag level, IU/mL, median (IQR)</td>
<td>1.19 (0.92–1.58)</td>
</tr>
</tbody>
</table>

Data are presented as n (%) or mean±SD, unless for VWF:Ag levels for which median (IQR) values are shown.

ADAMTS13 indicates a disintegrin and metalloprotease with thromboSpondin motif repeats 13; BMI, body mass index; HDL, high-density lipoprotein; IQR, interquartile range; and VWF:Ag, von Willebrand factor antigen.
albeit the results were not statistically significant (HR, 1.16; 95% CI, 0.84–1.60 and HR, 1.32; 95% CI, 0.54–3.22).

**Discussion**

In this large prospective cohort study with a median follow-up time of 11.3 years and a total of 90,635 person years, ADAMTS13 activity and VWF:Ag levels were associated with an increased risk of mortality and specifically cardiovascular mortality. These risks were independent of prevalent CVD and established cardiovascular risk factors.

We found an association between low ADAMTS13 activity and the risk of all-cause and cardiovascular mortality. Recently, we have shown that low ADAMTS13 activity is associated with an increased risk of ischemic stroke and coronary heart disease,\(^8,16,17\) but no studies on the association with mortality have been performed to date. We found that individuals with low ADAMTS13 activity (<80.7%) had a 1.46-fold higher risk of mortality than individuals with high ADAMTS13 activity. In individuals with the lowest ADAMTS13 activity, the risk estimates of all-cause and cardiovascular mortality (HR, 1.46 for both) were similar, suggesting that the risk of all-cause mortality might be driven by an increased risk of CVD.

We found an increased risk of all-cause mortality in individuals with the highest VWF:Ag levels. In addition, there was a borderline significant association between cardiovascular mortality and high VWF:Ag levels, although the risk estimate was higher compared with all-cause mortality. This

<table>
<thead>
<tr>
<th>ADAMTS13 Activity</th>
<th>Number of Cases/Total Number at Risk</th>
<th>Model 1 (HR (95% CI))</th>
<th>Model 2 (HR (95% CI))</th>
<th>Model 3 (HR (95% CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality (n=1868)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>679/1530</td>
<td>1.50 (1.30–1.73)</td>
<td>1.46 (1.26–1.69)</td>
<td>1.46 (1.26–1.69)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>490/1531</td>
<td>1.21 (1.04–1.40)</td>
<td>1.18 (1.02–1.38)</td>
<td>1.20 (1.03–1.39)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>412/1530</td>
<td>1.13 (0.97–1.32)</td>
<td>1.12 (0.96–1.31)</td>
<td>1.13 (0.97–1.31)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>287/1530</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
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<tr>
<td>Cardiovascular mortality (n=442)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>171/1514</td>
<td>1.41 (1.06–1.89)</td>
<td>1.46 (1.09–1.96)</td>
<td>1.46 (1.09–1.96)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>98/1525</td>
<td>0.92 (0.68–1.25)</td>
<td>0.93 (0.68–1.27)</td>
<td>0.95 (0.69–1.29)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>100/1525</td>
<td>1.05 (0.78–1.43)</td>
<td>1.07 (0.79–1.45)</td>
<td>1.07 (0.79–1.46)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>73/1523</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
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</tbody>
</table>

Model 1 adjusted for age and sex. Model 2 additionally adjusted for antithrombotic medication, hypertension, diabetes mellitus, lipid-reducing agents, BMI, smoking status, total cholesterol, HDL cholesterol, systolic blood pressure, and diastolic blood pressure. Model 3 additionally adjusted for prevalent cardiovascular disease. ADAMTS13 indicates a disintegrin and metalloprotease with thrombospondin motif repeats 13; BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; and HR, hazard ratio.

<table>
<thead>
<tr>
<th>VWF:Ag Levels</th>
<th>Number of Cases/Total Number at Risk</th>
<th>Model 1 (HR (95% CI))</th>
<th>Model 2 (HR (95% CI))</th>
<th>Model 3 (HR (95% CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality (n=1863)</td>
<td></td>
<td></td>
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<tr>
<td>Quartile 1</td>
<td>366/1558</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>414/1537</td>
<td>0.99 (0.86–1.14)</td>
<td>0.99 (0.86–1.14)</td>
<td>0.99 (0.86–1.14)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>451/1495</td>
<td>0.96 (0.84–1.11)</td>
<td>0.97 (0.84–1.11)</td>
<td>0.97 (0.84–1.11)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>632/1530</td>
<td>1.21 (1.06–1.38)</td>
<td>1.21 (1.06–1.38)</td>
<td>1.21 (1.06–1.38)</td>
</tr>
<tr>
<td>Cardiovascular mortality (n=439)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>80/1551</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>95/1526</td>
<td>1.03 (0.77–1.39)</td>
<td>1.04 (0.77–1.41)</td>
<td>1.03 (0.76–1.38)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>109/1486</td>
<td>1.04 (0.77–1.39)</td>
<td>1.04 (0.78–1.39)</td>
<td>1.05 (0.78–1.40)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>155/1523</td>
<td>1.30 (0.99–1.71)</td>
<td>1.29 (0.98–1.70)</td>
<td>1.29 (0.98–1.70)</td>
</tr>
</tbody>
</table>

Model 1 adjusted for age and sex. Model 2 additionally adjusted for antithrombotic medication, hypertension, diabetes mellitus, lipid-reducing agents, BMI, smoking, total cholesterol, HDL cholesterol, systolic blood pressure, and diastolic blood pressure. Model 3 additionally adjusted for prevalent cardiovascular disease. ADAMTS13 indicates a disintegrin and metalloprotease with thrombospondin motif repeats 13; BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; VWF, von Willebrand factor; and VWF:Ag, von Willebrand factor antigen.
might be explained by the smaller number of cases in this subgroup. These results indicate that ADAMTS13 activity may play a more prominent role in CVD in our elderly study population. Previous studies using the Rotterdam study have already found a higher cardiovascular risk in individuals with a low ADAMTS13 activity than in those with a high VWF:Ag level.\textsuperscript{5,8,18} The association between VWF levels and cardiovascular mortality has been reported before.\textsuperscript{9–13} However, these studies only investigated cardiac-specific or cardiovascular mortality, and no studies are performed yet on the association with all-cause mortality and cause-specific mortality.

Compared with individuals with both low VWF levels and high ADAMTS13 activity, we found that individuals with a high VWF:Ag level and low ADAMTS13 activity had an increased risk of all-cause and cardiovascular mortality of $\leq 1.73$-fold, although this was only a small subgroup of individuals (24.1% and 7.3%, respectively). This finding suggests that VWF levels and ADAMTS13 activity could be independent risk factors and might have an additive effect, which was also shown before.\textsuperscript{8}

The mechanism by which low ADAMTS13 activity is associated with cardiovascular mortality was not addressed in this epidemiological study. It is well known that reduced levels of ADAMTS13 lead to less cleavage of ultralarge, highly active, VWF multimers.\textsuperscript{3} Especially in combination with high VWF:Ag levels, this may lead to a prothrombotic phenotype. Especially at sites of vascular damage, such as in atherosclerotic lesions, VWF is locally secreted from the endothelium as large multimers, and in combination with low ADAMTS13 levels, this may lead to thrombus formation and thrombus growth and arterial
thrombotic complications, which will eventually lead to an increase of cardiovascular mortality. It is evident that additional mechanistic studies are needed to strengthen this hypothesis.

The risks of cancer- and COPD-related mortality were not increased in individuals with either high VWF:Ag levels or low ADAMTS13 activity, although we observed a borderline significant increased risk of cancer-related mortality in individuals with the lowest ADAMTS13 activity. Cancer and COPD are both common causes of mortality. Previous studies have shown a relation between high VWF levels and cancer,\textsuperscript{19–22} COPD,\textsuperscript{23,24} or inflammation,\textsuperscript{25} and also, an association between ADAMTS13 and inflammation was shown before.\textsuperscript{26–28} This would suggest that there may be an association between high VWF levels, low ADAMTS13 activity, and all-cause or cardiovascular mortality. However, the lack of this association in our study suggests that ADAMTS13 activity and VWF levels are influenced by other mechanisms such as inflammation in patients with COPD or cancer, and their effect might be explained by alternative factors.

To date, this study investigated the association between VWF:Ag levels, ADAMTS13 activity, and the risk of all-cause and cardiovascular mortality in a population that is representative for the Dutch population. We had a long follow-up time of 11.3 years in which many individuals died (30.5%). However, only 59 participants died because of COPD-related mortality, which was too small to have sufficient power to evaluate the association. Moreover, VWF levels and ADAMTS13 activity could have been influenced by an already existing disease at the moment of blood sampling. Therefore, we excluded all individuals who died within the first 3 years after blood sampling to reduce this influence. Our study was performed in a population of predominantly whites aged ≥55 years who live in a middle-income district of Rotterdam. This limits the generalizability of our results, especially for younger individuals.

In conclusion, we observed in this large prospective cohort study with a long follow-up time an association between high VWF levels, low ADAMTS13 activity, and all-cause or cardiovascular mortality. This risk increased ≤1.73-fold in individuals with both high VWF and low ADAMTS13 levels.

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### Disclosures

F.W.G. Leebeek has received unrestricted research grants of CSL Behring and Baxter, unrelated to this study, in the past and is a consultant for UniQure. O.H. Franco works in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec, Ltd) Metagenics, Inc, and AXA. The other authors report no conflicts.

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Highlights

- Low ADAMTS13 activity and high von Willebrand factor antigen levels are both associated with an increased risk of all-cause and cardiovascular mortality.

- Individuals with both low ADAMTS13 activity and high von Willebrand factor antigen levels have the highest risk of all-cause and cardiovascular mortality.

- ADAMTS13 activity and von Willebrand factor antigen levels are not statistically significantly associated with chronic obstructive pulmonary disease–related or cancer-related mortality.
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The association between VWF:Ag levels, ADAMTS13 activity and cardiovascular mortality.

6130 individuals of the Rotterdam Study, a population based cohort study in individuals of 55 years and older were followed for mortality until January 1, 2012. Over a median follow-up time of 11.3 years a total of 442 individuals died due to cardiovascular disease. The risk of cardiovascular mortality was higher in individuals with the highest VWF:Ag levels than in those with the lowest levels (HR 1.29, 95% CI 0.98 - 1.70). The risk of cardiovascular mortality was also higher in individuals with the lowest ADAMTS13 activity than in individuals with the highest ADAMTS13 activity (HR 1.46, 95% CI 1.09 - 1.96). A Kaplan Meier curve shown in the figure illustrates the association between ADAMTS13 activity levels and cardiovascular mortality.
Materials and Methods

Study design and study population

This study is part of the Rotterdam Study, a prospective population-based cohort study among individuals of 55 years and older who are all living in Ommoord, a suburb of Rotterdam, The Netherlands. Of the 10,215 eligible individuals, 7,983 agreed to participate at the start of the study in 1990 (RS-1). In 1999 the study was extended with 3,011 individuals (out of 4,472 invitees) who moved into the study district or became 55 years since the start of the study. All participants were asked to visit the study center every 3 to 4 years to assess established cardiovascular risk factors. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Population Studies Act: Rotterdam Study. Written informed consent was obtained from all participants. For this study, we used the data of the participants in the third examination of the original cohort (RS-I-3) and the first examination of the extended cohort (RS-II-1). We included all participants who were alive at baseline and of whom blood was sampled (N= 6511). Patients who died within 3 years after blood sampling were excluded from the analyses (N= 381) as an already existing disease at moment of blood sampling may have influenced ADAMTS13 activity and VWF levels.

Von Willebrand Factor and ADAMTS13 measurements

VWF antigen (VWF:Ag) levels were determined with an in-house ELISA. Polyclonal rabbit anti-human VWF antibodies were used for catching and tagging (DakoCytomation, Glostrup, Denmark). ADAMTS13 activity was measured in a kinetic assay with the previously described Fluorescence Resonance Energy Transfer Substrate VWF 73 (FRETS-VWF73). All samples were measured against a reference curve of serial dilutions of normal human plasma (Cryocheck normal reference plasma, Presizion Biologic, Dartmouth, USA).

Assessment of mortality
Information on mortality was continuously reported through automatic linkage of general practitioner files. Additionally, municipal records were checked on a monthly basis. Information on cause of death was obtained from general practitioners and hospital records. Research physicians reviewed all information and coded all events according to the *International Classification of Diseases, 10th edition (ICD-10)*. Deaths due to cardiovascular disease were coded as I21 or I64. A consensus panel adjudicated the final cause of death according to ICD-10 codes using standardized definitions \(^3\). If the cause of death was coded as C01-C97, the cause of death was labeled as cancer related mortality. Chronic Obstructive Pulmonary Disease (COPD) related mortality was coded as J43-J44. Participants were followed from date of entry in the study until date of death, lost to follow-up or January 1\(^{st}\), 2012, whichever came first.

**Baseline characteristics**

Of all participants, data was collected by structured interviews and physical examination. In a subset of participants blood was sampled. Blood pressure was measured as the mean of two readings using a random-zero sphygmomanometer in sitting position. The use of antihypertensive drugs was defined as the use of antihypertensive medication indicated for the treatment of high blood pressure (≥ grade 1 hypertension according to World Health Organization Criteria \(^4\)). Antithrombotic medication was defined as the use of vitamin K antagonists, platelet aggregation inhibitors, and direct thrombin inhibitors. Diabetes mellitus was defined as fasting serum glucose level ≥ 7.0 mmol/L and/or the use of blood glucose lowering medication \(^5\). Lipid lowering agents was defined as the use of statins. Total cholesterol and high-density lipoprotein cholesterol were measured using an automated enzymatic procedure. Body mass index was calculated as the weight (in kilograms) divided by the square of the height (in meters). Smoking status was defined as current, former or no smoking at baseline.

**Statistical analysis**
Mean and standard deviation or counts and percentages were used to describe baseline characteristics. Log minus Log plots were drawn to determine proportional hazards assumption. Cox proportional hazard regression analysis was used to assess the association between ADAMTS13, VWF and mortality. For these analyses quartiles of ADAMTS13 activity and VWF:Ag levels were used. A Kaplan Meier curve was constructed using ADAMTS13 and VWF quartiles adjusted for age and sex. For the combination of ADAMTS13 activity and VWF levels, we used the 25 percentile for ADAMTS13 and the 75 percentile as cut-off for VWF. All analyses were adjusted for age and sex and additionally for use of antithrombotic agents, antihypertensives, diabetes mellitus, lipid reducing agents, BMI, current smoking, total cholesterol, HDL cholesterol, systolic blood pressure and diastolic blood pressure and finally additionally adjusted for prevalent cardiovascular disease. Missing values of all these covariates (0-4.7%) were imputed five times using a multiple imputation method including all covariates. Statistical analyses were performed on all five datasets and pooled into one final result using SPSS software. Data were analyzed using SPSS version 21 (SPSS 21.0 IBM, Somers, NY, USA). All statistical tests were two-tailed and a p value of <0.05 was considered statistically significant.
References


