Atherosclerosis 290 (2019) 31–36

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Von Willebrand factor antigen levels predict major adverse cardiovascular events in patients with carotid stenosis of the ICARAS study

Katarina D. Kovacevic[a,1], Florian J. Mayer[b,1], Bernd Jilma[a,*], Nina Buchtele[a], Georg Obermayer[b], Christoph J. Binder[b], Andrew D. Blann[c], Erich Minar[d], Martin Schillinger[d], Matthias Hoke[d]

[a] Medical University of Vienna, Clinical Pharmacology, Vienna, Austria
[b] Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria
[c] University of Birmingham, Institute for Cardiovascular Sciences, Birmingham, United Kingdom
[d] Medical University of Vienna, Department for Internal Medicine II, Vienna, Austria

HIGHLIGHTS

- The predictive value of VWF for MACE is unknown in patients with asymptomatic carotid stenosis.
- This large cohort study investigated the predictive value of VWF for MACE in 811 patients with carotid stenosis.
- VWF levels within the highest quartile were independently associated with a 2.1-fold increase in MACE.
- This cohort study in 811 patients with carotid stenosis showed that high VWF-levels predicted MACE, which may be useful for risk stratification.

ARTICLE INFO

Keywords:
MACE
Predictor
Risk
Stenosis
VWF

ABSTRACT

Background and aims: Von Willebrand factor (VWF) plays an important role in thrombogenesis and mediates platelet adhesion particularly under high shear stress. Such conditions are generally found in stenotic arteries and can eventually cause myocardial infarction or stroke. We aimed to study whether levels of VWF antigen (VWF:Ag) predict future major adverse cardiovascular events (MACE) in patients suffering from carotid artery stenosis.

Methods: Patients with atherosclerotic carotid artery disease defined by the presence of nonstenotic plaques or any degree of carotid stenosis were prospectively enrolled. Concentrations of VWF were measured by enzyme immunoassay.

Results: VWF:Ag levels were more stable after 4 freeze-thaw cycles, when compared to VWF activity, and we showed similar concentrations of VWF in citrated plasma and serum (± 4%). Levels of VWF:Ag predicted future cardiovascular events in 811 patients with carotid stenosis independent of known cardiovascular risk factors. Patients with VWF:Ag concentrations in the 4th quartile had a 44% event rate after an average 3-year follow up and a hazard ratio of 2.15 (95% confidence interval 1.46–3.16; $p < 0.001$).

Conclusions: High concentrations of VWF:Ag predict major cardiovascular events in patients with carotid stenosis, and given their high event rate may be useful for risk stratification of such patients.

1. Introduction

Von Willebrand factor (VWF) is a multimeric glycoprotein which is synthesized by vascular endothelial cells and megakaryocytes [1] and is essential for primary haemostasis [2]. On the one hand, high shear stress can lead to VWF abnormalities and bleeding [3–7], and this process appears to be dependent on the ADAMTS-13 [8]. On the other hand, the central role of von Willebrand factor in thrombosis is particularly apparent in blood flow conditions with high shear stress, such as found in capillaries under physiologic conditions or in stenosed arteries [2]. VWF mediates the first step in platelet thrombus formation, i.e. adhesion of platelets in the bloodstream to the luminal surface of blood vessels at sites of vascular injury under conditions of moderate to high shear force [9–16]. One of the main functions of VWF is binding to

* Corresponding author. Department of Clinical Pharmacology, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.
E-mail address: bernd.jilma@meduniwien.ac.at (B. Jilma).
[1] contributed equally.

https://doi.org/10.1016/j.atherosclerosis.2019.09.003
Received 11 January 2019; Received in revised form 26 July 2019; Accepted 12 September 2019
Available online 17 September 2019

other proteins, including glycoprotein Ib and collagen. As has recently been reviewed in detail [17], the VWF-GPIb axis contributes to the risk of transient ischemic attack (TIA)/stroke in patients with cerebrovascular disease [18–22].

VWF levels were found to be associated with early markers of atherosclerosis in otherwise healthy individuals [23] and various epidemiological studies have shown a positive correlation between levels of VWF and risk of coronary heart disease and stroke [19,24–27]. Concordantly, platelet aggregation under high shear rates is enhanced by increased plasma VWF levels in patients with acute myocardial infarction when compared with controls [28,29]. Finally, genetic studies have demonstrated that VWF is not only a biomarker for atherosclerosis, but is actually involved in the pathogenesis of stroke [30].

It is unknown whether VWF can predict cardiovascular outcomes in patients with subclinical carotid atherosclerosis. Given the large body of evidence suggesting a role of VWF in arterial thrombosis, we hypothesized that VWF antigen concentrations are a predictor of cardiovascular outcome in patients with carotid atherosclerosis. Therefore we also hypothesized that VWF levels predict the occurrence of major adverse cardiovascular events in patients suffering from carotid artery stenosis.

2. Materials and methods

2.1. Study designs and patients

Inclusion and exclusion criteria of the ICARAS design have been published elsewhere [31]. In brief, subjects with atherosclerosis in their carotid arteries, who, at the time of screening were clinically asymptomatic, were enrolled between March 2002 and March 2003. Prevalent carotid atherosclerosis was defined as the presence of either non-stenotic plaques or any degree of carotid stenosis. Patients underwent duplex ultrasound investigations of the extracranial carotid arteries. Patients were excluded if any of the following factors were present: active infectious or inflammatory disease, symptomatic carotid artery stenosis necessitating revascularisation therapy, recent operations or endovascular interventions (recent is classified as within the last 14 days), bilateral carotid occlusions, with bilateral stent implantation or bilateral carotid endarterectomy, myocardial infarction (MI), coronary revascularisation, stroke, or peripheral vascular surgery during the preceding 6 months. The rationale behind this was the assumption that acute cardiovascular events may affect laboratory measures and thus it may reflect rather the acute situation than the chronic nature of atherosclerosis [31]. The study complied with the Declaration of Helsinki and was approved by the institutional review board of the Medical University of Vienna. All patients gave their written informed consent. Definitions have been published previously, for all the clinical data (diabetes mellitus, arterial hypertension, hyperlipidemia etc.) [31].

2.2. ICARAS VWF substudy

811 samples of 1268 prospectively collected samples, stored at −80 °C, were available from Caucasian patients with carotid atherosclerosis. 203 patients (16%) were lost to clinical follow-up and of 254 patients no serum samples were available. The 457 patients who had to be excluded did not significantly differ from the patients who were included in terms of baseline and demographic parameters (age, sex, frequency of atherothrombotic risk factors, cardiovascular comorbidities, patients' medical history, family history, results of health assessments and physical examinations, and degree of carotid stenosis; data not shown). Patients were evaluated by duplex sonography and followed up clinically for a median of 3.1 years in order to quantify the occurrence of major adverse cardiovascular events (MACE), a composite of stroke (n = 43), myocardial infarction (n = 35), percutaneous coronary intervention (n = 59), coronary artery bypass graft (n = 35) and death (n = 96). MACE was defined as the primary study end point.

2.3. Loss of VWF antigen vs. activity after multiple freeze/thaw cycles

The analyst was blinded to all clinical and ultrasound data. The ELISA was performed following the manufacturer's protocol. We compared two different commercially available ELISA-kits, one measuring VWF antigen levels and one measuring VWF activity (both REAADS from Corgenix, Haemochrom Diagnostica GmbH, Essen, FRG) [32], using plasma and serum samples from three healthy volunteers. We froze samples and subjected them to five freeze-thaw cycles. After freezing the samples in the freezer at −20°, samples were thawed at 37 °C in a water bath incubator for 20 min before analyzing VWF antigen and VWF activity with ELISA assay. The same samples were used in both ELISAs. The median coefficient of variation for inter- and intra-assay variability was less than 5%.

2.4. Plasma vs. serum VWF levels

As only serum samples were available, we compared VWF antigen levels between citrated plasma and serum in order to confirm that serum is suitable as demonstrated previously [33]. We used samples of patients with atherosclerosis (coronary, carotid, peripheral arteries, MI, stroke, critical limb ischaemia) and an ESR (erythrocyte sedimentation rate) < 20 mm/h (to exclude the cofounder of gross inflammation) and of healthy controls.

Additionally, plasma VWF antigen levels were measured in healthy volunteers for the purpose of another study at our department. Baseline values were 97% (95% CI 69-125) which was in agreement with our results and we used these values as normal VWF antigen range.

2.5. Statistical methods

Statistics were calculated as previously described [34]. To obtain clinically useful measures, levels of VWF were categorized in quartiles. Continuous data are presented as median and interquartile range (range from the 25th to the 75th percentile). Discrete data are given as counts and percentages. Analysis of variance and the $\chi 2$ test were used for comparisons between quartiles and Spearman correlation coefficients (R_s) for comparison of discrete data, as appropriate. The log-rank test was used for comparison between groups. Event-free survival probabilities were estimated using the Kaplan–Meier method. Univariable and multivariable Cox proportional hazards models were applied to assess the association between levels of VWF levels and the occurrence of MACE. The multivariable model included the following variables: age (years), sex (male/female), history of myocardial infarction (binary), history of stroke (binary), peripheral arterial disease (binary), body mass index (kg/m2), hypertension (binary), diabetes mellitus (binary), serum creatinine (mg/dL), glycohemoglobin A1 (%), levels of triglycerides (mg/dL), total cholesterol levels (mg/dL), low density lipoprotein cholesterol levels (mg/dL), and statin treatment (binary) (Supplementary Table 5). The selection of the variables was defined *a priori* and is based on current guidelines for cardiovascular risk prediction. All the variables listed above were included in every multivariable Cox proportional hazard model used for this study. Results of the Cox models are presented as hazard ratios (HR; 95% confidence interval [CI]). We assessed the overall model fit using Cox–Snell residuals.

We also tested the proportional hazard assumption for all covariates using Schoenfeld residuals (overall test) and the scaled Schoenfeld residuals (variable-by-variable testing). Interactions between VWF and the degree of carotid artery stenosis were tested by entering interaction terms in the Cox proportional hazard regression models. A 2-sided p value of < 0.05 was considered significant. All calculations were performed with SPSS (version 20.0 SPSS Inc) and the STATA11 software package (StataCorp) for Windows.

3. Results

3.1. Pre-study experiments

Measuring VWF activity and VWF antigen level after multiple freeze-thaw cycles, we found an average decrease in VWF activity of 19% compared to less than 1% decrease of VWF antigen levels (Supplementary Table 1). As the patient samples may have been exposed to similar cycles, the VWF antigen ELISA was selected as the appropriate measurement.

Before performing the main analyses we investigated possible differences in concentrations of VWF antigen between citrated plasma and serum. While atherosclerotic patients had ~30% higher VWF levels than controls irrespective of the matrix (Supplementary Table 2), there were no differences in the levels of VWF antigen between serum and citrate plasma ($p = 0.752$), and there was an excellent correlation between VWF antigen levels measured in these two matrices in the patient group (r = 0.98) and also in the control group (r = 0.95).

3.2. VWF antigen levels and MACE

Baseline demographic data and clinical characteristics of 811 patients with carotid stenosis are given in Table 1. Von Willebrand Factor antigen concentrations according to the degree of carotid artery stenosis are given in the Supplementary Table 3. The number of patients who suffered a major adverse cardiovascular event (MACE) during a median follow up of 3.1 years (IQR 2.5–3.5) was 235, which comprises 22.1% of the entire study population. A total of 43 cases of stroke, 32 cases of myocardial infarction, 59 cases of percutaneous coronary intervention, 35 cases of the coronary artery bypass graft and 96 cases of death were recorded.

The Cox regression model (Table 2) showed that independent predictors of outcome were high levels of VWF, previous myocardial infarction, family history of cardiovascular disease and high levels of highly sensitive-C reactive protein (hs-CRP). Patients within the highest quartile of VWF antigen levels had a 2.15-fold 95% confidence interval 1.46–3.16; $p < 0.001$ higher risk for MACE as compared to those in the first quartile (Fig. 1). A sensitivity analysis was performed including discrete VWF antigen levels as a continuous variable rather than quartiles, which showed the same effect (Supplementary Table 4). Although there was obviously a substantial overlap, patients with a higher degree of carotid stenosis had on average ~10% higher VWF levels than those with less carotid narrowing (Table 3; plaques only, or < 30% stenosis). This resulted in a relatively weak correlation between VWF levels and the degree of stenosis (Spearman ranks correlation test: Rs = 0.08; $p = 0.024$).

Table 1
Demographic characteristics of patients.

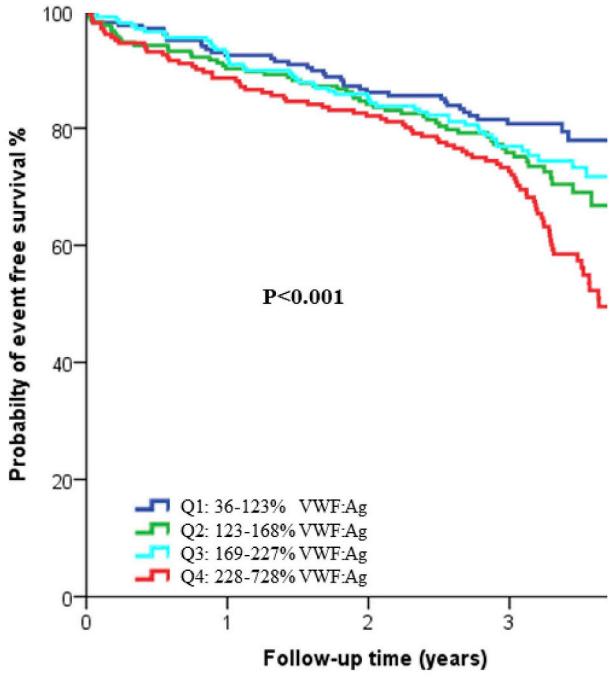
Variable	
Age, years	69.0 (61.2–76.2)
Female gender	322 (38.5%)
Hypertension	570 (68.1%)
Family history of cardiovascular disease	457 (54.6%)
Previous peripheral arterial disease	366 (43.7%)
Current smokers	217 (25.9%)
Previous myocardial infarction	204 (24.4%)
Diabetes	194 (23.2%)
Previous stroke	130 (15.5%)
Body Mass Index (kg/m^2)	26.1 (24.0–28.7)
HbA1c% (glycated haemoglobin)	6.0 (5.6–6.6)
Triglycerides (mg/dl)	147 (107–216)
Total cholesterol (mg/dl)	205 (175–236)
Low Density Lipoprotein- cholesterol (mg/dl)	118 (94–146)
Highly sensitive C- reactive protein (mg/dl)	0.29 (0.14–0.64)
Serum creatinine (mg/dl)	1.06 (0.93–1.23)
VWF antigen level (%)	168 (122–227)

*Data presented as counts (%) or as median values and 25th and 75th percentiles for continuous values. VWF, von Willebrand Factor.

Table 2
Independent predictors of major adverse coronary events in patients with carotid stenosis.

Independent predictors of outcome	Hazard ratio	95% CI	Significance
VWF antigen levels in the 4th quartile	2.15	1.46–3.16	< 0.001
Highly sensitive- C reactive protein[a]	1.26	1.07–1.46	0.004
Family history of cardiovascular disease	1.42	1.09–1.85	0.011
Previous myocardial infarction	1.47	1.08–1.98	0.013
Diabetes mellitus type 2	1.48	1.04–2.11	0.030

[a] Hazard ratio per 1 mg/dl increase in C reactive protein. VWF, von Willebrand Factor.



No. at risk/No. of events within the following year

Q1	206/15	180/12	161/9	110/3
Q2	206/20	182/12	162/14	107/12
Q3	198/14	183/15	167/15	111/6
Q4	201/23	178/13	165/18	119/34

Fig. 1. Cardiovascular event free survival rate according to quartiles of VWF antigen level.

Table 3
Von Willebrand Factor antigen and hazard ratios for major adverse coronary events.

VWF Ag group	VWF concentration %	Hazard ratio	95% CI
1st quartile	36–123%	1.00	
2nd quartile	123–168%	1.49	0.99–2.24
3rd quartile	169–227%	1.25	0.82–1.90
4th quartile	228–728%	2.15	1.46–3.16

*Patients were stratified by the quartiles of VWF antigen concentration, hazard ratio and confidence interval. VWF, von Willebrand Factor.

4. Discussion

Whether VWF is a predictor for major adverse cardiovascular events (MACE) in patients with carotid stenosis has not previously been investigated. Our study shows that VWF levels within the highest quartile are associated with a 2.1-fold increase in events. The incidence of

MACE was 22% after a median follow up of three years in our study. This rather high incidence rate can be explained by the inclusion and exclusion criteria. As patients with previous cardio- or cerebrovascular disease were not excluded, this could be the reason for this high incidence rate. Demographic characteristics of our patients showed a high rate of hypertension, previous myocardial infarction, peripheral arterial disease and diabetes, which are all risk factors for MACE and a likely explanation for coronary events happening not in close proximity of the carotid stenosis.

A recent study also looked at the incidence of MACE in subjects with asymptomatic mild and moderate carotid artery stenosis [35]. The incidence of MACE in that study was substantially lower when compared with our study. This was likely due to their exclusion of patients with previous cardiovascular disease.

In the general population VWF levels are a weak predictor of cardiovascular disease [36–38]. Nevertheless, results from studies in high-risk populations often show substantially stronger associations. The patients with the highest risk are likely those with acute coronary syndromes. Patients with VWF levels in the 4th quartile had a 6-fold higher rate of recurrent MACE, and fatal events (approximately ~10%) were only seen in the 3rd and 4th quartile of VWF-levels [39]. VWF levels also correlate with myocardial damage in acute coronary syndromes [40] and VWF antigen concentrations were an independent predictor of poor outcome after cardiopulmonary resuscitation and were associated with increased adverse outcome rates in patients with cardiac arrest risk scores of 2–3 [41].

4.1. VWF and stroke

Several studies showed an association between high VWF levels and stroke [18,42]. Similar to patients with cardiovascular disease, the association between VWF levels and stroke appears to be stronger in acute settings. The relative risk of ischemic stroke was highest in individuals in the upper quartile of VWF:Ag (odds ratio, 3.2; 95% CI, 1.4 to 7.5) compared with individuals in the lowest quartile [25]. Patients with a high VWF antigen level had a higher risk of unfavorable outcomes when compared with patients with a low VWF antigen level (hazard ratio: 2.15; 95% CI, 1.46–3.16) [43]. The hazard ratio for stroke was 1.79 (95% CI, 1.19–2.45) in those individuals with VWF levels in the 4th quartile of the population-based Rotterdam study which included approximately 6000 elderly subjects (aged ≥ 55years) [44].

4.2. VWF and atherosclerosis in stroke patients

An important indicator of atherosclerosis is calcification volume [45–47] which has been shown to correlate with VWF levels. Sonneveld et al. examined the association between the extent of atherosclerosis, determined by the calcification volume in both the aortic arch and the carotid arteries and VWF antigen levels in patients suffering from TIA or ischemic stroke [43]. Patients with calcifications in the aortic arch and/or carotid arteries had significantly higher VWF antigen levels compared with patients without calcifications in the aortic arch and/or carotid arteries [43]. Levels of VWF antigen linearly increased with increasing aortic calcification volume. We found a similar, but weaker correlation between levels of VWF antigen and the severity of carotid stenosis. The stronger correlation in Sonneveld's work may be attributed to their use of more sophisticated imaging procedures.

The role of VWF in atherosclerosis is controversial and was experimentally addressed in different animal models. First, atherogenesis was investigated in three different groups of pigs after diet-induced atherosclerosis. Atherosclerosis developed in both the control group and heterozygous VWF deficient pigs whereas the severe homozygous VWF deficient animals were resistant to atherosclerosis [48]. In contrast, another study did not find a protective role for VWF deficiency in pigs [49]. Yet, the same group reported that after diet-induced atherosclerosis, VWF deficient pigs failed to develop an occlusive arterial thrombosis [50]. Atherogenesis was examined after an atherogenic diet in two groups of mice, one lacking low-density lipoprotein receptor and VWF and the other one lacking low-density lipoprotein receptor but with normal VWF. Fatty streaks in the aortic sinus of mice lacking VWF were 40% smaller and contained fewer monocytes than in controls; further, the distribution of lesion in the aortas was different in different regions [51]. In addition, endothelial-cell derived VWF and platelet-derived VWF were investigated separately in genetically modified mice. Endothelial-cell derived VWF, but not platelet-derived VWF contributed to VWF-dependent atherosclerosis [52].

In contrast to this evidence that severe VWF deficiency protects against atherogenesis in animals, it seems harder to prove that in human studies. Evidently, data in humans are scarce and methodological weaknesses exist. Some studies indicate that VWF protects against atherosclerosis [23,53–56], others do not [57–59]. However, humans with severe VWD are commonly treated with VWF replacement therapy which could possibly influence the findings by neutralizing any protective effective of VWD [60]. In summary, VWF appears to have a role in atherosclerosis in different species although its contribution to arterial thrombosis may be better documented. In that regard recently developed VWF inhibitors may be interesting drug candidates [61,62].

Conditions like high shear forces [63] or inflammation [64] increase VWF secretion, so that one may hypothesise that these conditions and atherosclerosis could be possible reasons for increased VWF levels in our population. Increased production and secretion of VWF from endothelium in case of inflammation has been previously shown [65,66]. Nonetheless, the effects of inflammation on secretion and production by megakaryocytes has not been investigated, except for interleukin-11 (IL-11).

The explanation for that could be the difference in the amount of secreted VWF from endothelium and platelets. The VWF secretion is predominantly endothelium-dependent, whereas the contribution of platelets is limited for several reasons [64]. First, platelets do not have large deposits of VWF and second, platelet VWF is selectively released upon platelet activation [64]. Therefore, to significantly participate in increased VWF levels in inflammation, substantial platelet activation would be needed [64]. However, it has been shown that platelet VWF mRNA is up-regulated by IL-11 in dogs [67] and mice [68] which likely reflects enhanced VWF transcription in megakaryocytes too. Additionally, recombinant IL-11 increased VWF antigen and activity in patients with mild von Willebrand disease [69]. which could mean that VWF is secreted from platelets and megakaryocytes. So in principle at least some cytokines could stimulate VWF release from platelets and megakaryocytes and influence inflammation generating atherosclerosis in our population.

Before analyzing VWF antigen concentrations, we investigated the influence of up to four freeze-thaw cycles on laboratory results. The samples from the ICARAS study have been frozen and thawed at least once. Two previous studies have indicated that freezing and subsequent thawing does not influence the stability of VWF antigen in plasma samples [70] and we were able to confirm this. A further study indicated that the freeze-thaw cycle does not influence VWF activity in plasma samples [71], but we observed a 19% decrease in VWF activity after 4 freeze-thaw cycles. This difference in results could be assay dependent. Although 15 years elapsed between patient enrollment and measurements of VWF Ag, the measured levels are in accordance with the literature and expected for this patient group [17,72].

The samples available for this study were serum samples, and for this reason, we explored possible differences between VWF antigen in serum or plasma samples. One previous study showed no major differences in paired samples comparing plasma vs serum of 81 patients [33]. Our analyses showed that VWF levels in citrated plasma were comparable to those in serum. Therefore, serum appears to be an appropriate matrix, and serum was also used in a recent elegant study on the prognostic utility of VWF in amyloidosis [73]. A couple of previous studies also successfully investigated the concentration of VWF levels in serum samples [73,74].

4.3. Limitations

As described, patients included in this study suffered from carotid stenosis. Annual stroke rates in patients with carotid artery occlusion range from 0 to 5% [75,76]. In our study comprising 811 patients, there were not enough stroke cases to examine the relationship between VWF and stroke events. In addition, substantial proportions of our patients had previously suffered from cardiovascular events. In our study, only those who had suffered cardiovascular events in the last six months were excluded. Therefore, we cannot extrapolate our data to patients with “isolated” carotid stenosis. Additionally, VWF data were not adjusted for ABO blood groups, as we lacked information about the blood groups of the participants. Nevertheless, the differences in the MACE events between the blood groups are probably driven by the VWF levels. Moreover, high-molecular-weight VWF multimers were not investigated or measured.

4.4. Conclusion

High concentrations of VWF:Ag predict major cardiovascular events in patients with carotid stenosis, and given their high event rate may be useful for risk stratification of such patients.

Financial support

This study was supported by a grant from the Austrian Science Funds (FWF; grant# SFB54–P04).

Author contributions

K. D. Kovacevic performed the analyses and wrote the draft paper. F. J. Mayer did the statistical analyses and wrote the statistical part of the paper. M.Hoke, E. Minar, M. Schillinger, C. Binder were responsible for the conduct the main ICARAS study. N. Buchtele contributed to the idea, analysis of the literature and writing of the background, and G. Obermayer was responsible for sample handling, storage and analysis. A. Blann was responsible for the study and performed the analysis in the atherosclerotic patients validating the matrix. B. Jilma conceived the study, organized funding and contributed to the writing of the manuscript. All authors critically reviewed the manuscript and contributed to its content.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.09.003.

References

[1] M. Bryckaert, J.P. Rosa, C.V. Denis, P.J. Lenting, Of von Willebrand factor and platelets, Cell. Mol. Life Sci. 72 (2015) 307–326.
[2] Z.M. Ruggeri, J. Ware, von Willebrand factor, FASEB J. 7 (1993) 308–316.
[3] A. Vincentelli, S. Susen, T. Le Tourneau, I. Six, O. Fabre, F. Juthier, A. Bauters, C. Decoene, J. Goudemand, A. Prat, B. Jude, Acquired von Willebrand syndrome in aortic stenosis, N. Engl. J. Med. 349 (2003) 343–349.
[4] E. Van Belle, A. Rauch, F. Vincent, E. Robin, M. Kibler, J. Labreuche, E. Jeanpierre, M. Levade, C. Hurt, N. Rousse, J.B. Dally, N. Debry, J. Dallongeville, A. Vincentelli, C. Delhaye, J.L. Auffray, F. Juthier, G. Schurtz, G. Lemesle, T. Caspar, et al., Von Willebrand factor multimers during transcatheter aortic-valve replacement, N. Engl. J. Med. 375 (2016) 335–344.
[5] T. Le Tourneau, S. Susen, C. Caron, A. Millaire, S. Marechaux, A.S. Polge, A. Vincentelli, F. Mouquet, P.V. Ennezat, N. Lamblin, P. de Groote, E. Van Belle, G. Deklunder, J. Goudemand, C. Bauters, B. Jude, Functional impairment of von Willebrand factor in hypertrophic cardiomyopathy: relation to rest and exercise obstruction, Circulation 118 (2008) 1550–1557.
[6] J.J. Badimon, C.G. Santos-Gallego, Modulatory role of pulsatility on von Willebrand factor: implications for mechanical circulatory support-associated bleeding, J. Am. Coll. Cardiol. 71 (2018) 2119–2121.
[7] B. Steinlechner, M. Dworschak, B. Birkenberg, M. Duris, P. Zeidler, H. Fischer, L. Milosevic, G. Wieselthaler, E. Wolner, P. Quehenberger, B. Jilma, Platelet dysfunction in outpatients with left ventricular assist devices, Ann. Thorac. Surg. 87 (2009) 131–137.
[8] P. Jilma-Stohlawetz, P. Quehenberger, H. Schima, M. Stoiber, P. Knobl, B. Steinlechner, A. Felli, B. Jilma, Acquired von Willebrand factor deficiency caused by LVAD is ADAMTS-13 and platelet dependent, Thromb. Res. 137 (2016) 196–201.
[9] P. Andre, P. Hainaud, C. Bal dit Sollier, L.I. Garfinkel, J.P. Caen, L.O. Drouet, Relative involvement of GPIb/IX-vWF Axis and GPIIb/IIIa in thrombus growth at high shear rates in the Guinea pig, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 919–924.
[10] H. Choi, K. Aboulfatova, H.J. Pownall, R. Cook, J.F. Dong, Shear-induced disulfide bond formation regulates adhesion activity of von Willebrand factor, J. Biol. Chem. 282 (2007) 35604–35611.
[11] M. Galbusera, C. Zoja, R. Donadelli, S. Paris, M. Morigi, A. Benigni, M. Figliuzzi, G. Remuzzi, A. Remuzzi, Fluid shear stress modulates von Willebrand factor release from human vascular endothelium, Blood 90 (1997) 1558–1564.
[12] S. Goto, H. Sakai, M. Goto, M. Ono, Y. Ikeda, S. Handa, Z.M. Ruggeri, Enhanced shear-induced platelet aggregation in acute myocardial infarction, Circulation 99 (1999) 608–613.
[13] M. Li, S. Goto, H. Sakai, J.Y. Kim, N. Ichikawa, M. Yoshida, Y. Ikeda, S. Handa, Enhanced shear-induced von Willebrand factor binding to platelets in acute myocardial infarction, Thromb. Res. 100 (2000) 251–261.
[14] A.J. Reininger, H.F.G. Heijnen, H. Schumann, H.M. Specht, W. Schramm, Z.M. Ruggeri, Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress, Blood 107 (2006) 3537–3545.
[15] C.A. Siedlecki, B.J. Lestini, K.K. Kottke-Marchant, S.J. Eppell, D.L. Wilson, R.E. Marchant, Shear-dependent changes in the three-dimensional structure of human von Willebrand factor, Blood 88 (1996) 2939–2950.
[16] A. Le Behot, M. Gauberti, S. Martinez De Lizarrondo, A. Montagne, E. Lemarchand, Y. Repesse, S. Guillou, C.V. Denis, E. Maubert, C. Orset, D. Vivien, GpIbalpha-VWF blockade restores vessel patency by dissolving platelet aggregates formed under very high shear rate in mice, Blood 123 (2014) 3354–3363.
[17] N. Buchtele, M. Schwameis, J.C. Gilbert, C. Schorgenhofer, B. Jilma, Targeting von Willebrand factor in ischaemic stroke: focus on clinical evidence, Thromb. Haemost. 118 (2018) 959–978.
[18] T.N. Bongers, M.P.M. de Maat, M.L. van Goor, V. Bhagwanbali, H.H.D.M. van Vliet, E.B. Gomez Garcia, D.W.J. Dippel, F.W.G. Leebeek, High von Willebrand factor levels increase the risk of first ischemic stroke: influence of ADAMTS13, inflammation, and genetic variability, Stroke 37 (2006) 2672–2677.
[19] A.J. Catto, A.M. Carter, J.H. Barrett, J. Bamford, P.J. Rice, P.J. Grant, von Willebrand factor and factor VIII: C in acute cerebrovascular disease. Relationship to stroke subtype and mortality, Thromb. Haemost. 77 (1997) 1104–1108.
[20] F. Denorme, S.F. De Meyer, The VWF-GPIb axis in ischaemic stroke: lessons from animal models, Thromb. Haemost. 116 (2016) 597–604.
[21] Tobin WO, Kinsella JA, Kavanagh GF, O'Donnell JS, McGrath RT, Tierney S, Egan B, Feeley TM, Coughlan T, Collins DR, O'Neill D, Murphy SJX, Lim SJ, Murphy RP, McCabe DJH. Profile of von Willebrand factor antigen and von Willebrand factor propeptide in an overall TIA and ischaemic stroke population and amongst subtypes. J. Neurol. Sci.; 375: 404-410.
[22] P. Paulinska, A. Spiel, B. Jilma, Role of von Willebrand factor in vascular disease, Hämostaseologie 29 (2009) 32–38.
[23] J.A. Paramo, O. Beloqui, I. Colina, J. Diez, J. Orbe, Independent association of von Willebrand factor with surrogate markers of atherosclerosis in middle-aged asymptomatic subjects, J. Thromb. Haemost. 3 (2005) 662–664.
[24] M. Cortellaro, C. Boschetti, E. Cofrancesco, C. Zanussi, M. Catalano, G. de Gaetano, L. Gabrielli, B. Lombardi, G. Specchia, L. Tavazzi, et al., The PLAT Study: hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients. Principal results. PLAT Study Group. Progetto Lombardo Atero-Trombosi (PLAT) Study Group, Arterioscler. Thromb. 12 (1992) 1063–1070.
[25] T.N. Bongers, M.P. de Maat, M.L. van Goor, V. Bhagwanbali, H.H. van Vliet, E.B. Gomez Garcia, D.W. Dippel, F.W. Leebeek, High von Willebrand factor levels increase the risk of first ischemic stroke: influence of ADAMTS13, inflammation, and genetic variability, Stroke 37 (2006) 2672–2677.
[26] S.G. Thompson, J. Kienast, S.D. Pyke, F. Haverkate, J.C. van de Loo, Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group, N. Engl. J. Med. 332 (1995) 635–641.
[27] A.O. Spiel, J.C. Gilbert, B. Jilma, von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes, Circulation 117 (2008) 1449–1459.
[28] S. Goto, H. Sakai, M. Goto, M. Ono, Y. Ikeda, S. Handa, [Enhanced shear-induced platelet aggregation in acute myocardial infarction], J. Cardiol. 33 (1999) 102–103.
[29] S. Goto, H. Sakai, Y. Ikeda, S. Handa, Acute myocardial infarction plasma augments platelet thrombus growth in high shear rates, Lancet 349 (1997) 543–544.
[30] S.R. Williams, F.C. Hsu, K.L. Keene, W.M. Chen, G. Dzhivhuho, J.L. Rowles 3rd, A.M. Southerland, K.L. Furie, S.S. Rich, B.B. Worrall, M.M. Sale, Genetic drivers of von Willebrand factor levels in an ischemic stroke population and association with risk for recurrent stroke, Stroke 48 (2017) 1444–1450.
[31] M. Schillinger, M. Exner, W. Mlekusch, S. Sabeti, J. Amighi, R. Nikowitsch, E. Timmel, B. Kickinger, C. Minar, M. Pones, W. Lalouschek, H. Rumpold, G. Maurer, O. Wagner, E. Minar, Inflammation and carotid artery–risk for atherosclerosis study (ICARAS), Circulation 111 (2005) 2203–2209.

[32] P. Jilma-Stohlawetz, J.C. Gilbert, M.E. Gorczyca, P. Knobl, B. Jilma, A dose ranging phase I/II trial of the von Willebrand factor inhibiting aptamer ARC1779 in patients with congenital thrombotic thrombocytopenic purpura, Thromb. Haemost. 106 (2011) 539–547.
[33] A.D. Blann, J. Hopkins, J. Winkles, A.C. Wainwright, Plasma and serum von Willebrand factor antigen concentrations in connective tissue disorders, Ann. Clin. Biochem. 29 (Pt 1) (1992) 67–71.
[34] F.J. Mayer, M. Hoke, M. Schillinger, E. Minar, I. Arbesu, R. Koppensteiner, C. Mannhalter, Mean platelet volume predicts outcome in patients with asymptomatic carotid artery disease, Eur. J. Clin. Investig. 44 (2014) 22–28.
[35] H. Kwon, H.K. Kim, S.U. Kwon, S.W. Lee, M.J. Kim, J.W. Park, M. Noh, Y. Han, T.W. Kwon, Y.P. Cho, Risk of major adverse cardiovascular events in subjects with asymptomatic mild carotid artery stenosis, Sci. Rep. 8 (2018) 4700.
[36] A.R. Folsom, K.K. Wu, W.D. Rosamond, A.R. Sharrett, L.E. Chambless, Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study, Circulation 96 (1997) 1102–1108.
[37] A.M. Thogersen, J.H. Jansson, K. Boman, T.K. Nilsson, L. Weinehall, F. Huhtasaari, G. Hallmans, High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor, Circulation 98 (1998) 2241–2247.
[38] J. Danesh, J.G. Wheeler, G.M. Hirschfield, S. Eda, G. Eiriksdottir, A. Rumley, G.D. Lowe, M.B. Pepys, V. Gudnason, C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease, N. Engl. J. Med. 350 (2004) 1387–1397.
[39] I. Fuchs, M. Frossard, A. Spiel, E. Riedmuller, A.N. Laggner, B. Jilma, Platelet function in patients with acute coronary syndrome (ACS) predicts recurrent ACS, J. Thromb. Haemost. 4 (2006) 2547–2552.
[40] M. Frossard, I. Fuchs, J.M. Leitner, K. Hsieh, M. Vlcek, H. Losert, H. Domanovits, W. Schreiber, A.N. Laggner, B. Jilma, Platelet function predicts myocardial damage in patients with acute myocardial infarction, Circulation 110 (2004) 1392–1397.
[41] A. Geppert, G. Zorn, G. Delle-Karth, M. Koreny, P. Siostrzonek, G. Heinz, K. Huber, Plasma concentrations of von Willebrand factor and intracellular adhesion molecule-1 for prediction of outcome after successful cardiopulmonary resuscitation, Crit. Care Med. 31 (2003) 805–811.
[42] G. Licata, A. Tuttolomondo, D. Di Raimondo, S. Corrao, R. Di Sciacca, A. Pinto, Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke, Thromb. Haemost. 101 (2009) 929–937.
[43] M.A. Sonneveld, A.C. van Dijk, E.G. van den Herik, J.E. van Loon, L.M. de Lau, A. van der Lugt, P.J. Koudstaal, M.P. de Maat, F.W. Leebeek, Relationship of Von Willebrand Factor with carotid artery and aortic arch calcification in ischemic stroke patients, Atherosclerosis 230 (2013) 210–215.
[44] M.A. Sonneveld, M.P. de Maat, M.L. Portegies, M. Kavousi, A. Hofman, P.L. Turecek, H. Rottensteiner, F. Scheiflinger, P.J. Koudstaal, M.A. Ikram, F.W. Leebeek, Low ADAMTS13 activity is associated with an increased risk of ischemic stroke, Blood 126 (2015) 2739–2746.
[45] A.E. Odink, A. van der Lugt, A. Hofman, M.G. Hunink, M.M. Breteler, G.P. Krestin, J.C. Witteman, Risk factors for coronary, aortic arch and carotid calcification; the Rotterdam Study, J. Hum. Hypertens. 24 (2010) 86–92.
[46] S.E. Elias-Smale, R.V. Proenca, M.T. Koller, M. Kavousi, F.J. van Rooij, M.G. Hunink, E.W. Steyerberg, A. Hofman, M. Oudkerk, J.C. Witteman, Coronary calcium score improves classification of coronary heart disease risk in the elderly: the Rotterdam study, J. Am. Coll. Cardiol. 56 (2010) 1407–1414.
[47] K.R. Nandalur, E. Baskurt, K.D. Hagspiel, M. Finch, C.D. Phillips, S.R. Bollampally, C.M. Kramer, Carotid artery calcification on CT may independently predict stroke risk, AJR Am. J. Roentgenol. 186 (2006) 547–552.
[48] L. Badimon, P. Steele, J.J. Badimon, E.J. Bowie, V. Fuster, Aortic atherosclerosis in pigs with heterozygous von Willebrand disease. Comparison with homozygous von Willebrand and normal pigs, Arteriosclerosis 5 (1985) 366–370.
[49] T.C. Nichols, D.A. Bellinger, K.E. Davis, G.G. Koch, R.L. Reddick, M.S. Read, J. Rapacz, J. Hasler-Rapacz, K.M. Brinkhous, T.R. Griggs, Porcine von Willebrand disease and atherosclerosis. Influence of polymorphism in apolipoprotein B100 genotype, Am. J. Pathol. 140 (1992) 403–415.
[50] T.C. Nichols, D.A. Bellinger, D.A. Tate, R.L. Reddick, M.S. Read, G.G. Koch, K.M. Brinkhous, T.R. Griggs, von Willebrand factor and occlusive arterial thrombosis. A study in normal and von Willebrand's disease pigs with diet-induced hypercholesterolemia and atherosclerosis, Arteriosclerosis 10 (1990) 449–461.
[51] N. Methia, P. Andre, C.V. Denis, M. Economopoulos, D.D. Wagner, Localized reduction of atherosclerosis in von Willebrand factor-deficient mice, Blood 98 (2001) 1424–1428.
[52] P. Doddapattar, N. Dhanesha, M.R. Chorawala, C. Tinsman, M. Jain, M.K. Nayak, J.M. Staber, A.K. Chauhan, Endothelial cell-derived von Willebrand factor, but not platelet-derived, promotes atherosclerosis in apolipoprotein E-deficient mice, Arterioscler. Thromb. Vasc. Biol. 38 (2018) 520–528.
[53] L. Daza, M. Aguirre, M. Jimenez, R. Herrera, J.J. Bollain, Common carotid intima-media thickness and von Willebrand factor serum levels in rheumatoid arthritis female patients without cardiovascular risk factors, Clin. Rheumatol. 26 (2007) 533–537.
[54] C.D. Seaman, K.M. George, M. Ragni, A.R. Folsom, Association of von Willebrand factor deficiency with prevalent cardiovascular disease and asymptomatic carotid atherosclerosis: the Atherosclerosis Risk in Communities Study, Thromb. Res. 144 (2016) 236–238.
[55] J. Ding, Q. Chen, X. Zhuang, Z. Feng, L. Xu, F. Chen, Low paraoxonase 1 arylesterase activity and high von Willebrand factor levels are associated with severe coronary atherosclerosis in patients with non-diabetic stable coronary artery disease, Med. Sci. Monit. 20 (2014) 2421–2429.
[56] F. Bilora, C. Dei Rossi, B. Girolami, A. Casonato, E. Zanon, A. Bertomoro, A. Girolami, Do hemophilia A and von Willebrand disease protect against carotid atherosclerosis? A comparative study between coagulopathics and normal subjects by means of carotid echo-color Doppler scan, Clin. Appl. Thromb. Hemost. 5 (1999) 232–235.
[57] A. Sramek, P. Bucciarelli, A.B. Federici, P.M. Mannucci, V. De Rosa, G. Castaman, M. Morfini, M.G. Mazzucconi, A. Rocino, M. Schiavoni, F.A. Scaraggi, J.H. Reiber, F.R. Rosendaal, Patients with type 3 severe von Willebrand disease are not protected against atherosclerosis: results from a multicenter study in 47 patients, Circulation 109 (2004) 740–744.
[58] F. Bilora, E. Zanon, A. Casonato, A. Bertomoro, F. Petrobelli, M. Cavraro, E. Campagnolo, A. Girolami, Type IIb von Willebrand disease: role of qualitative defects in atherosclerosis and endothelial dysfunction, Clin. Appl. Thromb. Hemost. 13 (2007) 384–390.
[59] F.W. Leebeek, I.M. van der Meer, J.C. Witteman, Genetic variability of von Willebrand factor and atherosclerosis, Circulation 110 (2004) e57.
[60] K.P. van Galen, A. Tuinenburg, E.M. Smeets, R.E. Schutgens, Von Willebrand factor deficiency and atherosclerosis, Blood Rev. 26 (2012) 189–196.
[61] K.D. Kovacevic, J.C. Gilbert, B. Jilma, Pharmacokinetics, pharmacodynamics and safety of aptamers, Adv. Drug Deliv. Rev. 134 (2018) 36–50.
[62] C. Firbas, J.M. Siller-Matula, B. Jilma, Targeting von Willebrand factor and platelet glycoprotein Ib receptor, Expert Rev. Cardiovasc Ther. 8 (2010) 1689–1701.
[63] M. Galbusera, C. Zoja, R. Donadelli, S. Paris, M. Morigi, A. Benigni, M. Figliuzzi, G. Remuzzi, A. Remuzzi, Fluid shear stress modulates von Willebrand factor release from human vascular endothelium, Blood 90 (1997) 1558–1564.
[64] C. Kawecki, P.J. Lenting, C.V. Denis, von Willebrand factor and inflammation, J. Thromb. Haemost. 15 (2017) 1285–1294.
[65] N. Dhanesha, P. Prakash, P. Doddapattar, I. Khanna, M.J. Pollpeter, M.K. Nayak, J.M. Staber, A.K. Chauhan, Endothelial cell-derived von Willebrand factor is the major determinant that mediates von Willebrand factor-dependent acute ischemic stroke by promoting postischemic thrombo-inflammation, Arterioscler. Thromb. Vasc. Biol. 36 (2016) 1829–1837.
[66] A. Bernardo, C. Ball, L. Nolasco, J.F. Moake, J.F. Dong, Effects of inflammatory cytokines on the release and cleavage of the endothelial cell–derived ultralarge von Willebrand factor multimers under flow, Blood 104 (2004) 100–106.
[67] E.H. Olsen, A.S. McCain, E.P. Merricks, T.H. Fischer, I.M. Dillon, R.A. Raymer, D.A. Bellinger, S.A. Fahs, R.R. Montgomery, J.C. Keith Jr., R.G. Schaub, T.C. Nichols, Comparative response of plasma VWF in dogs to up-regulation of VWF mRNA by interleukin-11 versus Weibel-Palade body release by desmopressin (DDAVP), Blood 102 (2003) 436–441.
[68] C.V. Denis, K. Kwack, S. Saffaripour, S. Maganti, P. André, R.G. Schaub, D.D. Wagner, Interleukin 11 significantly increases plasma von Willebrand factor and factor VIII in wild type and von Willebrand disease mouse models, Blood 97 (2001) 465–472.
[69] M.V. Ragni, R.C. Jankowitz, H.L. Chapman, E.P. Merricks, M.T. Kloos, A.M. Dillow, T.C. Nichols, A phase II prospective open-label escalating dose trial of recombinant interleukin-11 in mild von Willebrand disease, Haemophilia 14 (2008) 968–977.
[70] A. Hillarp, M. Stadler, C. Haderer, J. Weinberger, C.M. Kessler, J. Romisch, Improved performance characteristics of the von Willebrand factor ristocetin cofactor activity assay using a novel automated assay protocol, J. Thromb. Haemost. 8 (2010) 2216–2223.
[71] R.C. Gosselin, K. Honeychurch, H.J. Kang, D.M. Dwyre, Effects of storage and thawing conditions on coagulation testing, Int J Lab Hematol 37 (2015) 551–559.
[72] M.A. Sonneveld, M.P. de Maat, F.W. Leebeek, Von Willebrand factor and ADAMTS13 in arterial thrombosis: a systematic review and meta-analysis, Blood Rev. 28 (2014) 167–178.
[73] E. Kastritis, I. Papassotiriou, E. Terpos, M. Roussou, M. Gavriatopoulou, A. Komitopoulou, C. Skevaki, E. Eleutherakis-Papaiakovou, C. Pamboucas, E. Psimenou, E. Manios, S. Giannouli, M. Politou, H. Gakiopoulou, E. Papadopoulou, K. Stamatelopoulos, A. Tasidou, M.A. Dimopoulos, Clinical and prognostic significance of serum levels of von Willebrand factor and ADAMTS-13 antigens in AL amyloidosis, Blood 128 (2016) 405–409.
[74] G. Meucci, F. Pareti, M. Vecchi, S. Saibeni, C. Bressi, R. de Franchis, Serum von Willebrand factor levels in patients with inflammatory bowel disease are related to systemic inflammation, Scand. J. Gastroenterol. 34 (1999) 287–290.
[75] N.M. Bornstein, J.W. Norris, Benign outcome of carotid occlusion, Neurology 39 (1989) 6–8.
[76] M. Hennerici, H.B. Hulsbomer, W. Rautenberg, H. Hefter, Spontaneous history of asymptomatic internal carotid occlusion, Stroke 17 (1986) 718–722.