Plasma Levels of von Willebrand Factor in the Etiologic Subtypes of Ischemic Stroke

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Summary

Background: Compared to coronary artery disease, there are few studies on von Willebrand factor (VWF) in ischemic stroke (IS). Moreover, there is little information on VWF in the etiologic subtypes of IS.

Objectives: The aim of this study was to investigate VWF in IS and in the etiologic subtypes of IS.

Patients/methods: The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) is a case-control study comprising 600 patients and 600 matched controls. Etiologic IS subtype was defined according to the TOAST criteria. Blood sampling was performed in the acute phase and after three months.

Results: The levels of VWF were increased in overall IS, at both time-points. The 3-month VWF levels were increased in the subtypes of large-vessel disease (LVD), cardioembolic (CE) stroke and cryptogenic stroke, but not in the subtype of small-vessel disease (SVD), as compared with the controls. The acute phase VWF levels were significantly increased in all four subtypes. In the multivariate regression analysis, with vascular risk factors as covariates, the 3-month VWF levels were associated with CE stroke and cryptogenic stroke, and the acute phase VWF levels with all subtypes. There were significant subtype-specific differences in VWF, with the highest levels in LVD and CE stroke.

Conclusions: The present results show that VWF levels are increased in patients with IS. Furthermore, the VWF levels differ between etiologic IS subtypes and thus, it is important to consider etiologic subtypes in future studies of VWF in patients with IS.

Introduction

Thrombus formation is a key mechanistic event in ischemic stroke (IS), which explains why acute thrombolytic treatment is effective in patients with IS. von Willebrand factor (VWF) plays crucial roles in platelet adhesion and aggregation, which are the initial steps in thrombus formation [1]. VWF is also a carrier protein for coagulation factor VIII, it is mainly produced by endothelial cells, and the plasma concentrations of VWF have a wide normal range [2].

Previous studies have reported increases in the levels of VWF in patients with coronary heart disease (CHD) and high VWF levels have also been suggested as a predictor of CHD [3, 4]. There are fewer studies on VWF in IS, although increased levels of VWF have been reported in some case-control studies [5-11]. Furthermore, high plasma levels of VWF have been identified as a predictor of stroke in three prospective studies of subjects with and without atrial fibrillation [12-14]. However, other case-control and prospective studies have not found an association between VWF and IS [15-18]. IS is a more heterogeneous disease than CHD, and there are no systematic studies on VWF in relation to the etiologic subtypes of IS. Therefore, the apparent discrepancies between the previous studies on IS may in part be explained by a failure to make clear distinctions between the etiologic subtypes of IS. Moreover, small sample sizes and/or different time-points for blood sampling in relation to the index event may also have contributed to the discrepancies.

In the light of this, we aimed to determine (1) whether acute and/or 3-month follow-up levels of VWF are increased in patients with IS compared with healthy controls, (2) whether VWF levels are increased in all four major etiologic subtypes of IS compared to controls, and (3) whether there are differences in VWF levels between the

etiologic subtypes of IS. This was carried out by using data collected in the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), which is a large case-control study that involves careful characterization of the etiologic IS subtypes and standardized blood sampling, both in the acute phase and at the 3-month follow-up [19].

Subjects and Methods

Study Population

The study population comprised the participants in SAHLSIS, the design of which has been reported elsewhere [19]. Briefly, 600 patients who presented with first-ever (n=486) or recurrent (n=114) acute ischemic stroke before reaching the age of 70 years were recruited consecutively at four Stroke Units in western Sweden. Inclusion started in August 1998 and was continued until 600 patients had been recruited in December 2003. For each subject, one healthy community control, matched for age (±1 year), sex, and geographic area of residence, was randomly selected from participants in a population-based health survey or the Swedish Population Register. All participants provided written informed consent prior to enrolment. For those participants who were unable to communicate, consent was obtained from the next-of-kin. This study was approved by the Ethics Committee of the University of Gothenburg.

Risk Factor Definition and Stroke Subtyping

Information on the subjects' vascular risk factors was collected, as described in detail elsewhere [19]. All patients were examined by a physician trained in stroke medicine during the acute stage and at follow-up after three months, and underwent neuroimaging with computed tomography. Additional diagnostic work-up was performed as clinically indicated which included magnetic resonance imaging in 62% [19]. Ischemic stroke was defined as an episode of focal neurological deficits with acute onset and lasting >24 hours or until death, with no apparent non-vascular cause, and no signs of primary hemorrhage on brain-imaging. The patients were classified into etiologic subtypes according to the criteria of the Trial of Org 10172 in Acute Stroke Treatment (TOAST), as follows; large-vessel disease (LVD); small-vessel disease (SVD); cardioembolic (CE) stroke; cryptogenic stroke; other determined cause of stroke; and undetermined stroke. Cryptogenic stroke was defined for cases in which no cause was identified despite extensive investigation. The undetermined stroke group included patients for whom more than one cause was identified or for whom the evaluation was cursory. Adjudication of ischemic stroke diagnosis and etiologic subtype was centralized and performed by two neurologists (K.J., C.B.). Patients were also classified based on clinical presentation, as assessed using the Oxfordshire Community Stroke Project (OCSP) system, into the categories of: total anterior circulation infarct (TACI); partial anterior circulation infarcts (PACI); lacunar infarct (LACI); and posterior circulation infarct (POCI).

Blood Sampling and VWF Measurement

For each patient, blood sampling was performed in the acute phase within 10 days (median 4 days) of the stroke event. An additional sample was drawn at follow-up approximately three months after the event (median 101 days, range 85-125 days). For the control subjects,

blood sampling was performed once. Venous blood was collected in tubes that contained 10% by volume of 0.13 mol/L sodium citrate. Blood sampling was performed between 8:30 AM and 10:30 AM after overnight fasting. Plasma was isolated within 2 hours by centrifugation 2000 × *g* at 4°C for 20 minutes and stored at -80°C for 5-10 years prior to assay. The levels of VWF antigen were measured using an ELISA (Asserachrom VWF:Ag; Diagnostica Stago, Asnières, France), and calibrated against reference standard plasma (determined against a secondary standard of the International Standard established in 2003, National Institute for Biological Standards and Controls, Potters Bar, Hertfordshire, UK, NIBSC code 02/150). The acute phase and 3-month samples from each patient were analyzed together with the sample from the matching control on the same microtiter plate. The intra- and inter-assay coefficients of variation were 5.3% and 13.3%, respectively. The serum levels of high-sensitivity C-reactive protein (hsCRP) were analyzed in a solid-phase chemiluminescent immunometric assay, the plasma levels of tissue type plasminogen activator (t-PA) antigen were analyzed by ELISA, and plasma fibrinogen was measured with an automated clot rate assay, as described previously [20-22].

Statistical Analyses

VWF levels were logarithmically transformed. Differences in characteristics between cases and controls were examined with the χ^2 test for proportions and with Student's t-test or Mann-Whitney U test for continuous variables. Associations between VWF and overall IS were investigated using conditional univariate and multivariate logistic regression analyses, with adjustments for hypertension, smoking status, diabetes mellitus, and hyperlipidemia (model 1). For the TOAST subtypes, cases were compared with the whole control population and thus an unconditional logistic regression analysis was used, also including age, sex and geographic area as covariates. In order to investigate whether the associations were independent of inflammatory markers, a second multivariate model was used that also included the rapid acute phase reactant hsCRP and the slow reactant fibrinogen; both variables were logarithmically transformed (model 2). In addition, we investigated whether the associations were independent of another marker of endothelial damage, i.e. t-PA antigen (model 3). All the reported odds ratios (ORs) were scaled to estimate the ORs associated with an increase of 1 standard deviation (SD) in the log VWF concentration. Differences in VWF levels between IS subtypes and between IS subtypes and controls, were analyzed with ANCOVA using Bonferroni correction and adjusting for significant covariates among age, sex, hypertension, smoking status, diabetes mellitus, and hyperlipidemia. All the statistical analyses were performed using SPSS for Windows ver. 16.0. The statistical significance level was 0.05 and *P*-values were two-tailed.

Missing data

The number of individuals for whom the values for risk factors and other covariates were missing, have been reported previously [19-22]. In the logistic regression, missing values were replaced by dummy variables for the covariates being categorical variables. For the VWF measurements, blood samples were missing for 7 controls and 28 patients in the acute phase of IS. With regard to the 3-month follow-up, the samples for 49

patients were missing due to: technical difficulties (n=18); intervening death (n=7); and unwillingness to take part in the follow-up examination or to provide blood samples (n=24).

Results

Plasma VWF Levels in Patients with Ischemic Stroke

Baseline characteristics of the study subjects, hsCRP, fibrinogen, and t-PA antigen levels in the IS subtypes in SAHLSIS have been described elsewhere [19-22]. These data are summarized in Table 1. There were 51 patients with other determined cause of stroke, and 92 patients had undetermined stroke. There were no significant differences with regard to median time of blood sampling in relation to stroke onset, among the TOAST subtypes. Furthermore, there was no correlation between acute phase VWF levels and the time of the first blood draw (P=0.42), or between 3-month VWF levels and the time of the follow-up blood draw (P=0.56).

The level of VWF was significantly increased in the overall IS group both in the acute phase and at the 3-month follow-up, as compared to the controls (Fig. 1). In patients, VWF was significantly higher in the acute phase compared with the follow-up measurement (geometric mean acute phase VWF of 235 IU/dL (95% confidence interval (CI) 227-243) vs. geometric mean follow-up VWF levels of 214 IU/dL (95% CI 207-221), respectively). The exclusion of those patients who suffered a recurrent stroke within 3 months of inclusion (n=31) did not alter these results (data not shown).

Univariate regression analysis revealed significant associations between overall IS and VWF, both in the acute phase and at the 3-month follow-up (Table 2). For both time-points, the associations remained after adjustment for vascular risk factors (Table 2). After the incorporation of hsCRP and fibrinogen levels into the model, the strengths of the associations were slightly diminished and remained significant (Table 2). This was also true when the inflammatory markers were replaced by t-PA antigen (Table 2).

To investigate whether preexisting vascular disease contributed to the association between overall IS and VWF, an additional regression analysis was performed in which patients with a history of stroke, coronary artery disease or peripheral artery disease (n=174) were excluded. In this analysis, the associations remained with multivariate ORs for IS of 2.06 (95% CI 1.63-2.62) and 1.46 (95% CI 1.20-1.79) for the acute phase and 3-month VWF levels, respectively. In addition, there were no significant differences in VWF levels between patients with or without a history of vascular disease at any of the two time-points (geometric mean acute phase VWF of 243 IU/dL (95% CI 229-257) vs. 231 IU/dL (95% CI 220-242), and mean 3-month VWF of 223 IU/dL (95% CI 211-235) vs. 210 IU/dL (95% CI 202-219), respectively). Furthermore, there were no significant differences in VWF levels between patients with or without statins at follow-up (P=0.35). This was also true for anti-hypertensive drugs at follow-up (P=0.31). The only subtype with a significant proportion of patients on anticoagulant therapy was CE stroke, and in this subtype there was no significant difference in VWF levels between patients with (n=61) or without anticoagulant therapy (P=0.96).

Plasma VWF Levels in the Etiologic Subtypes of IS

The levels of VWF were investigated in the four major TOAST subtypes. Differences in VWF levels between IS subtypes as well as between IS subtypes and controls were adjusted for age, which was the only significant covariate in the ANCOVA. The acute phase VWF levels were significantly higher in all subtypes, as compared with controls (Fig. 1). At the 3-month follow-up, the IS subtypes of LVD, CE stroke and cryptogenic stroke displayed significantly higher VWF levels than the controls (Fig. 1). In the acute phase, both the LVD and CE stroke groups displayed significantly higher VWF levels than the SVD group (geometric mean VWF of 253 IU/dL (95% CI 230-279) and 263 IU/dL (95% CI 242-286), vs. geometric mean VWF of 213 IU/dL (95% CI 197-229), respectively). At the 3-month follow-up, the CE stroke group displayed significantly higher VWF than the SVD group (geometric mean VWF of 240 IU/dL (95% CI 224-256) vs. geometric mean VWF of 201 IU/dL (95% CI 189-215), respectively).

For all subtypes, the reductions in VWF levels between the acute phase and 3-month follow-up measures were significant (mean ΔVWF of 38.1, 9.9, 23.5, and 15.7 IU/dL for LVD, SVD, CE stroke, and cryptogenic stroke, respectively, $P<0.05$ throughout). Although the reduction was most pronounced for the LVD group, there were no significant differences either in the absolute reduction ($P>0.61$ throughout) or in the relative reduction ($P>0.68$ throughout) between the subtypes.

In the univariate binary logistic regression, VWF levels showed significant associations with all subtypes at both time-points (Table 2). After adjustment for traditional vascular risk factors, all the associations for acute phase VWF levels were retained, and independent associations with the 3-month follow-up VWF were observed for CE and cryptogenic stroke (Table 2). When the hsCRP and fibrinogen levels were included together with the vascular risk factors as covariates, the associations for acute phase VWF were diminished, for LVD and CE stroke, whereas the associations for 3-month follow-up VWF remained essentially the same (Table 2). Similar findings were made when the inflammatory markers were replaced by t-PA antigen (Table 2).

Plasma VWF Levels in Relation to OCSP subtypes

Patients with a clinical presentation indicating an extensive infarction (i.e. TACI, n=62), displayed significantly higher VWF levels than the other OCSP subtypes (PACI, n=176; LACI, n=206; and POCI, n=150) at both time-points ($P<0.01$ for both measurements). Therefore, an additional analysis for TOAST subtypes was performed from which the patients with TACI were excluded. This analysis showed a similar pattern for VWF across the TOAST subtypes, as when patients with TACI were included (data not shown).

Discussion

Our findings demonstrate increased VWF levels in overall IS, both in the acute phase and at the 3-month follow-up, as compared to controls. In addition, we found significant differences between the etiologic subtypes at both time-points, with the highest levels in LVD and CE stroke.

The result for acute VWF in overall IS is consistent with the results of most previous studies involving much fewer patients [5-10, 18]. Data on VWF in the chronic phase of IS are more scarce. Although we found that the VWF levels were significantly lower at the 3-month follow-up than in the acute phase, the VWF levels were still significantly increased in patients with IS at follow-up, as compared to controls. This is in agreement with the study by Catto *et al.* [7], which involved 169 patients with ischemic or hemorrhagic stroke. In contrast, significantly increased VWF levels at the 3-month follow-up were not detected in three other studies [5, 6, 17]. However, these studies only included a small number of patients at follow-up (26-64 patients) [5, 6, 17].

From a mechanistic point of view the chronic VWF levels are more interesting than the acute ones, and the present study is the first case-control study to examine VWF levels at follow-up in the different IS subtypes. The levels were increased in LVD, CE stroke and cryptogenic stroke as compared to the controls. Interestingly, the associations between follow-up VWF and both CE and cryptogenic stroke were independent of vascular risk factors, but also of both inflammatory markers and t-PA antigen. This indicates that the prothrombotic effects of VWF may play a role independent of atherosclerosis/ inflammation or endothelial damage in these subtypes. In support of this interpretation, there are data speaking in favour of that VWF plays a role in arterial thrombosis, but not in the atherosclerotic process *per se* [2]. For instance, one study has reported that intima-media thickness of the atherosclerotic plaques in the carotid and femoral arteries do not differ between patients with severe von Willebrand disease and healthy controls [2]. This may also explain why we did not find an independent association between 3-month VWF and LVD. We have previously reported that hsCRP shows an independent association to LVD exclusively [19, 20]. Thus, taken together our results suggest that while the inflammatory pathway is of importance in LVD, prothrombotic mechanisms play an independent role in CE and cryptogenic stroke.

The finding for cryptogenic stroke is particularly interesting in view of that this group has the smallest proportion of patients with traditional vascular risk factors and pre-existing vascular disease. Furthermore, we have recently reported an association between a genetic variant in the ADAMTS13 gene and this subtype [23]. ADAMTS13 cleaves VWF multimers, thereby regulating the biological activity of VWF in plasma. Although the functional importance of the ADAMTS13 gene variants remain to be determined [23], one may speculate that both increased VWF levels and ADAMTS13 contribute to a prothrombotic phenotype in cryptogenic stroke.

In contrast to the other subtypes, plasma VWF at 3-month follow-up was not significantly increased in SVD, as compared to controls. We also found increased VWF levels in the CE stroke group compared to the SVD group. Because destructive lesions of vessel walls in lacunes have been suggested as a plausible mechanism for SVD [24], and because VWF is often referred to as a marker of endothelial dysfunction [3], this finding was somewhat unexpected. The lacunar hypothesis has, however, been challenged and it is suggestive that the mechanisms causing SVD are far more complex. Furthermore, the concept of endothelial dysfunction is not well

defined [3], and as far as we are aware there are no data showing an association between plasma VWF and endothelial dysfunction as determined by invasive measures. It is also of note that the present results are in accordance with results from the prospective ARIC study, in which patients with CE stroke showed increased VWF levels, as compared to patients with lacunar stroke [25]. Thus, although further studies clearly are warranted, in contrast to CE and cryptogenic stroke, plasma VWF does not seem to play an important role in SVD.

With respect to the acute phase, there is only one previous study in which acute phase VWF levels were compared between TOAST subtypes: in that study, which included only 120 patients, CE stroke displayed higher VWF levels than the other subtypes, although this difference was not significant [9]. In the present study, the CE stroke and LVD groups had the highest acute phase VWF levels. If infarct size *per se* plays a role, this may clearly explain this finding. However, when patients with a clinical presentation indicating a large infarct, i.e. TACI, were excluded from the analysis, the subtype-specific difference in acute phase VWF levels remained significant. This may indicate that the difference in VWF level between the subtypes is not merely related to infarct size. In this context it is of course of note that OCSP subtype is a very crude measure of infarct size. Another possible explanation may be that the LVD and CE stroke groups display the greatest acute phase response and/or have the highest degree of endothelial damage. This is supported by the fact that although the associations remained significant in these two subtypes after the incorporation of either inflammatory markers or t-PA antigen, the ORs were diminished, particularly in CE stroke.

Interestingly, there is one recent study in which both VWF antigen and VWF propeptide were investigated in two case-control samples of patients with IS or transient ischemic attacks (n=204 and n=342) [11]. The results suggest that increased secretion of VWF from the endothelium is an important mechanism underlying the association between total VWF levels and IS, but a lower clearance of VWF may also contribute. Future studies on VWF propeptide levels in IS subtypes may thus contribute to our knowledge on mechanisms underlying subtype-specific differences in VWF in IS.

The present study has the advantages of large sample size and a comprehensive classification of the etiologic IS subtypes. Furthermore, blood sampling was standardized and performed at two time-points, whereof one was in the chronic phase. Only a few previous studies [5-7, 11, 17] have examined VWF levels at more than one well-defined time-point. There are also some limitations that should be considered. First, this study is based on hospitalized cases. Nevertheless, the stroke admission rate in Sweden is high, with >87% of the cases aged <75 years being admitted to hospital [26]. Second, the case-control design presents a limitation to interpreting the results for the plasma levels. In addition to the effects of the event itself, the effects of medication is difficult to account for, especially with regard to acute phase levels. Therefore, our results need to be confirmed in additional large prospective studies of etiologic IS subtypes. With the routine clinical work-up used to date, this is difficult to achieve in prospective studies. For instance, in one recent study, only approximately 40% of the patients

could be classified according to the TOAST criteria [27], as compared to 85% of the patients in the present study. As the routine clinical work-up for IS is improving, future prospective studies have greater potential for detailed classifications of the etiologic subtypes. Third, it should be noted that the population in the present study is relatively young, which means that it is not possible to extrapolate these results to older populations. In contrast, an advantage is that comorbidities, which may confound the results, occur less frequently in a younger population than in an older population.

In conclusion, the present study shows that VWF levels are increased in overall IS both in the acute phase and three months after the event, and that the levels decline from the acute phase to follow-up. There are significant differences in VWF levels between the four major etiologic subtypes of IS, and these differences vary between the two sample points. Thus, it is important to take etiologic IS subtypes into account in future studies of VWF in patients with IS, and also to standardize sampling time in case-control studies. Furthermore, VWF levels three months after the event show an association with both CE stroke and cryptogenic stroke, which is independent of traditional risk factors and inflammatory markers. This finding points to the pathophysiologic importance of prothrombotic mechanisms for these two subtypes.

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Disclosure of Conflict of Interest

The authors state that they have no conflict of interest.

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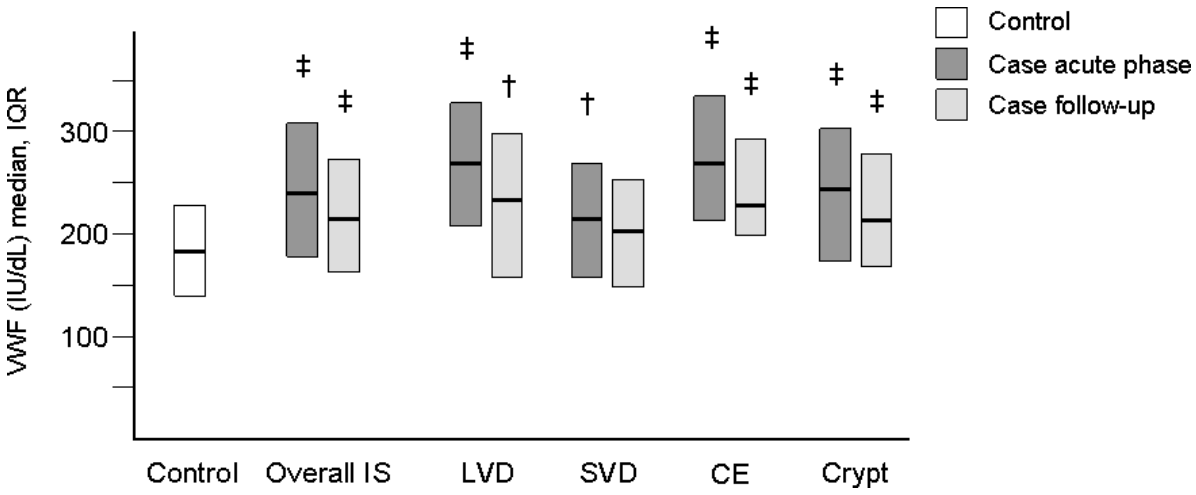

Fig. 1. Median levels and interquartile ranges (IQR) of plasma VWF in the acute phase and at the 3-month follow-up for overall IS and the TOAST subtypes. IS indicates ischemic stroke; LVD, large-vessel disease; SVD, small-vessel disease; CE, cardioembolic stroke; Crypt, cryptogenic stroke. Differences in logarithmically transformed VWF levels between overall IS and controls were examined with Student's t-test, and between TOAST subtypes and controls with ANCOVA adjusting for age and using Bonferroni correction. †P<0.01; ‡P<0.001.

Table 1. Baseline characteristics and plasma levels of hsCRP, fibrinogen and t-PA antigen in controls, overall ischemic stroke and the TOAST subtypes.

	Control (n=600)	IS (n=600)	LVD (n=73)	SVD (n=124)	CE (n=98)	Crypt (n=162)
Mean age, y (SD)	56 (10)	56 (10)	59 (8)	58 (7)	57 (10)	53 (12)
Male sex, n (%)	385 (64)	385 (64)	54 (74)	77 (62)	66 (67)	95 (59)
Hypertension*, n (%)	224 (37)	354 (59)	44 (60)	89 (72)	50 (51)	87 (54)
Diabetes, n (%)	33 (6)	114 (19)	25 (34)	26 (21)	19 (19)	23 (14)
Current smoking, n (%)	109 (18)	233 (39)	39 (53)	54 (44)	34 (35)	60 (37)
Hyperlipidemia†, n (%)	403 (67)	413 (76)	53 (82)	77 (71)	73 (82)	107 (71)
History of CAD and/or PAD, n (%)	**...**	109 (18)	21 (29)	10 (8)	40 (41)	16 (10)
History of stroke, n (%)	**…**	114 (19)	21 (29)	25 (20)	22 (22)	18 (11)
Statins at follow-up, n (%)	31 (5)	198 (33)	36 (49)	40 (32)	32 (33)	55 (34)
Anti-hypertensive drugs at follow-up, n (%)	89 (15)	292 (49)	35 (48)	71 (57)	65 (66)	61 (38)
Anticoagulant drugs at follow-up, n (%)	**…**	113 (19)	8 (11)	2 (2)	61 (62)	18 (11)
hsCRP, mg/L						
Control/acute phase, median (IQR)	1.6 (0.9-3.4)	3.5 (1.5-9.4)	4.7 (1.8-13.9)	3.1 (1.5-5.8)	7.1 (2.4-17.8)	2.3 (1.1-6.3)
3-month follow-up, median (IQR)	**…**	2.4 (1.1-5.5)	3.5 (1.4-10.0)	2.7 (1.2-4.8)	2.7 (1.1-5.5)	2.2 (1.1-5.7)
Fibrinogen, g/L						
Control/acute phase, median (IQR)	2.9 (2.6-3.3)	3.7 (3.1-4.4)	4.1 (3.3-5.1)	3.6 (2.9-4.2)	3.9 (3.4-4.6)	3.4 (2.9-4.1)
3-month follow-up, median (IQR)	**…**	3.3 (2.9-3.8)	3.6 (3.1-4.7)	3.3 (2.9-3.7)	3.4 (2.9-4.1)	3.3 (2.8-3.7)
t-PA antigen, µg/L						
Control/acute phase, median (IQR)	9.8 (7.2-12.7)	11.9 (8.7-15.7)	12.6 (9.7-18.0)	11.8 (8.4-14.7)	14.1 (10.2-18.7)	11.1 (8.1-13.8)
3-month follow-up, median (IQR)	**…**	11.6 (8.8-14.9)	12.5 (9.7-16.3)	11.2 (8.9-13.2)	13.2 (9.5-16.3)	10.1 (10.1-13.6)

IS indicates overall ischemic stroke; LVD, large-vessel disease; SVD, small-vessel disease; CE, cardioembolic stroke; Crypt, cryptogenic stroke; CAD, coronary artery disease; PAD, peripheral artery disease; hsCRP, high-sensitivity C-reactive protein; t-PA, tissue type plasminogen activator. *Hypertension was defined as pharmacological treatment, SBP ≥160 mmHg and/or DBP ≥90 mm Hg. †Hyperlipidemia was defined as pharmacological treatment, total fasting serum cholesterol level >5.0 mmol/L, and/or LDL >3.0 mmol/L.

Table 2. ORs and 95% CI for overall ischemic stroke and TOAST subtypes per 1 SD increase in the log plasma VWF concentration in the acute phase and at the 3-month follow-up, as compared to the controls.

	Unadjusted OR (95% CI)	**Adjusted, model 1*** OR (95% CI)	**Adjusted, model 2†** OR (95% CI)	**Adjusted, model 3‡** OR (95% CI)
Acute phase VWF				
Overall IS, n=572	2.00 (1.74-2.29)	2.01 (1.73-2.34)	1.73 (1.40-2.14)	1.87 (1.54-2.27)
LVD, n=69	3.02 (2.17-4.21)	2.14 (1.45-3.15)	1.71 (1.12-2.62)	1.85 (1.25-2.73)
SVD, n=123	1.56 (1.25-1.95)	1.37 (1.05-1.77)	1.20 (0.92-1.58)	1.35 (1.04-1.75)
CE, n=94	3.24 (2.40-4.37)	3.39 (2.39-4.80)	2.34 (1.62-3.36)	2.85 (2.01-4.06)
Crypt, n= 152	1.83 (1.48-2.25)	1.94 (1.54-2.44)	1.71 (1.35-2.18)	1.85 (1.47-2.34)
Follow-up VWF				
Overall IS, n=551	1.55 (1.36-1.75)	1.49 (1.29-1.71)	1.39 (1.16-1.66)	1.36 (1.15-1.62)
LVD, n=65	1.66 (1.25-2.19)	1.31 (0.96-1.79)	1.17 (0.84-1.63)	1.25 (0.91-1.71)
SVD, n=115	1.35 (1.08-1.67)	1.12 (0.87-1.44)	1.08 (0.83-1.40)	1.11 (0.86-1.42)
CE, n=87	2.34 (1.77-3.09)	2.33 (1.71-3.17)	2.16 (1.57-2.98)	2.12 (1.55-2.90)
Crypt, n= 153	1.47 (1.21-1.77)	1.46 (1.19-1.78)	1.37 (1.10-1.70)	1.44 (1.17-1.76)

Abbreviations as in Table 1. Conditional and unconditional regression analysis was used for overall IS and for the subtypes, respectively. *Model 1; the covariates were smoking, diabetes, hypertension, and hyperlipidemia for overall IS, as well as age, sex and geographic area for subtypes. †Model 2; the covariates were the same as in model 1, with the addition of hsCRP and fibrinogen. ‡Model 3; the covariates were the same as in model 1, with the addition of t-PA antigen.