Impact of the von Willebrand factor-ADAMTS13 axis on the risk of future venous thromboembolism

Short title: VWF-ADAMTS13 axis and Venous thromboembolism

Magnus S. Edvardsen,[1] Ellen-Sofie Hansen,[1] Thor Ueland,[1,2] Pål Aukrust,[2,3] Sigrid K. Brækkan,[1,4] Vânia M. Morelli,[1,4] and John-Bjarne Hansen[1,4]

[1]Thrombosis Research Center (TREC), Department of Clinical Medicine, UiT - The Arctic University of Norway, Tromsø, Norway; [2]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway; [3]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, Norway; [4]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Corresponding author: Vânia M. Morelli, MD PhD

Thrombosis Research Center (TREC), Department of Clinical Medicine, UiT – The Arctic University of Norway, N-9037 Tromsø, Norway

E-mail: vania.m.morelli@uit.no; Phone/Fax number: +47 77625105

Text word count: 4120 (introduction, methods, results and discussion)

Abstract word count: 244

Figure/table count: 6 (4 tables and 2 figures)

Reference count: 57

Essentials

- von Willebrand factor (VWF) and ADAMTS13 form a pivotal axis that regulates hemostasis
- The role of VWF-ADAMTS13 axis in the risk of future venous thromboembolism (VTE) is unknown
- Low ADAMTS13 levels and increased VWF/ADAMTS13 ratio are associated with increased risk of VTE
- VWF-ADAMTS13 axis may be involved in the pathogenesis of VTE

Summary

Background: von Willebrand factor (VWF) and its cleaving protease, ADAMTS13, form a pivotal axis that regulates hemostasis. However, the role of the VWF-ADAMTS13 axis in the risk of future venous thromboembolism (VTE) is unknown.

Objectives: To investigate whether plasma ADAMTS13 levels and an imbalance with VWF levels, assessed as VWF/ADAMTS13 ratio, are associated with risk of future VTE.

Patients/Methods: A population-based nested case-control study, comprising 383 incident VTE cases and 780 age- and sex-matched controls, was derived from the Tromsø study cohort (1994-2007). Antigen levels of ADAMTS13 and VWF were measured in plasma samples obtained at cohort baseline. Odds ratios (OR) with 95% confidence intervals (CI) were estimated according to quartiles cutoffs of ADAMTS13 and VWF/ADAMTS13 ratio determined in controls.

Results: In age- and sex-adjusted analysis, ADAMTS13 levels were inversely associated with VTE risk, with an OR of 1.40 (95% CI 0.99-1.99) for the lowest versus highest quartile. The VWF/ADAMTS13 ratio was linearly associated with VTE risk (*p* for trend=0.001), with an OR of 1.70 (95% CI 1.19-2.43) for the highest versus lowest quartile, and the association was particularly pronounced for unprovoked VTE (OR 2.81, 95% CI 1.65-4.81). The ORs were only slightly attenuated after additional adjustments for body mass index and C-reactive protein.

Conclusions: Lowered ADAMTS13 levels and an imbalance between ADAMTS13 and VWF levels, reflected by an increased VWF/ADAMTS13 ratio, were associated with increased risk of future VTE. Our findings suggest that the VWF-ADAMTS13 axis is involved in the pathogenesis of VTE.

Keywords: ADAMTS13 Protein, human, venous thrombosis, venous thromboembolism, von Willebrand factor.

Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease and a major cause of morbidity and mortality worldwide.[1,2] Despite intensified efforts to improve awareness and prevention of VTE, time-trend studies have shown a slight increase in the incidence of the disease over the last decades.[3,4] Major knowledge gaps still exist concerning risk factors and mechanisms that drive venous thrombus formation. Understanding the underpinnings of the pathophysiological mechanisms of VTE may reveal novel biomarkers that could help identify individuals at increased VTE risk and provide potential targets for disease prevention and treatment.

Growing evidence suggests that von Willebrand factor (VWF) is involved in the pathogenesis of VTE.[5-7] VWF is a large multimeric glycoprotein that plays critical roles in hemostasis by interacting with platelets to promote platelet plug formation,[8] and by serving as a plasma carrier for coagulation factor VIII.[9] Deficiency of VWF is the cause of von Willebrand disease, characterized by a bleeding tendency,[10] while elevated VWF levels are associated with increased risk of arterial cardiovascular disease (CVD)[11,12] and VTE.[13,14]

The multimeric size and subsequent hemostatic function of VWF is regulated by a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS13), through the cleavage of platelet-hyperadhesive ultra-large VWF (ULVWF) multimers.[15,16] Severe deficiency of ADAMTS13, either congenital or acquired, can result in an excess of ULVWF and cause thrombotic thrombocytopenic purpura (TTP), a rare and potentially fatal disease characterized by VWF-mediated platelet-rich thrombi in the microcirculation.[16,17] Notably, it has been shown that the contribution of ADAMTS13 to thrombogenesis expands beyond the microcirculation, as even slightly to moderately reduced ADAMTS13 levels were found to be associated with increased risk of arterial CVD (e.g. myocardial infarction and ischemic stroke).[18-20] For VTE, the existing data on ADAMTS13 is

limited to cancer patients,[21-23] or to relatively small case-control studies,[24-27] which have an inherent susceptibility to reverse causation due to the lack of temporality between exposure and outcome in the case-control design. Of note, because ADAMTS13 and VWF are functionally linked to each other, forming a pivotal axis that regulates hemostasis, the assessment of an imbalance of their plasma levels may provide further insights into VTE pathogenesis. A useful metric to evaluate such imbalance is the VWF/ADAMTS13 ratio, where an increased ratio may reflect a prothrombotic state. Indeed, an increased VWF/ADAMTS13 ratio in patients with arterial CVD has been consistently associated with cardiovascular complications, including recurrent thrombosis and death.[28-31] However, it remains unknown whether plasma ADAMTS13 and an imbalance with VWF affect the risk of a future VTE.

We hypothesized that reduced ADAMTS13 levels and high VWF/ADAMTS13 ratio would be associated with increased VTE risk. Therefore, we set out to investigate the association of plasma ADAMTS13 levels and VWF/ADAMTS13 ratio with risk of future incident VTE in a population-based nested case-control study.

Methods

Study population and design

The Tromsø Study is a cohort consisting of repeated health surveys of the inhabitants in Tromsø, Norway.[32] The present study was derived from the fourth survey of the Tromsø Study (Tromsø 4), conducted in 1994-95. All inhabitants aged 25 years or older were invited, and 77% (27,158) took part. Participants were followed until incident VTE, death, migration, or end of follow-up (September 1st, 2007). A total of 462 study participants suffered a VTE event during follow-up. To conceive the nested case-control study, for each VTE case, two age- and sex-matched controls (n=924), who were alive at the index date of the corresponding

VTE case, were selected randomly from the parent cohort, as previously described.[14] Seventy-nine cases and 144 controls were excluded from the analyses because plasma samples were not available (64 cases and 115 controls) or were of insufficient quality due to hemolysis (15 cases and 29 controls), leaving 383 cases and 780 controls in the final analytic sample (Figure1). It is important to address that in the nested case-control design, the temporal sequence between exposure and outcome is preserved, as blood samples to measure ADAMTS13 and VWF were collected at cohort baseline. The regional committee for medical and health research ethics approved the study, and informed written consent was obtained from all participants.

VTE registry

As previously described,[33] we identified all first lifetime VTE events by searching the discharge diagnosis registry, radiology procedure registry and autopsy registry from the University Hospital of North Norway (UNN), which is the only hospital in the study area. Events were adjudicated and recorded when 1) a VTE diagnosis was stated in the medical record, 2) signs and symptoms consistent with DVT and/or PE were present, 3) objective radiological confirmation was attained, and 4) treatment was implemented (unless contraindications were specified). For cases obtained from the autopsy registry, an event was recorded when PE was indicated as the cause of death, or a condition significantly contributing to death. All VTE events were further classified as DVT or PE and provoked or unprovoked. If DVT and PE were present simultaneously, the event was classified as a PE. An event was considered provoked if it was closely preceded by one or more of the following provoking factors; trauma, surgery or acute medical conditions (infectious diseases, myocardial infarction or ischemic stroke) within 8 weeks prior to the event, marked immobilization (wheelchair confinement or >3 days bed rest within the last 8 weeks, or long-

distance travel ≥4 hours within the last 14 days), and active cancer. If a treating physician specified another cause for VTE (e.g., venous catheters), the event was also classified as provoked.

Baseline measurements and blood sampling

Questionnaires, physical examination and blood samples were used to acquire baseline information from all participants at inclusion in the parent cohort (1994/95). Weight and height were measured with participants wearing light clothing and no shoes, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Self-administered questionnaires were used to obtain information on history of cancer and arterial CVD (including stroke, angina pectoris or myocardial infarction).

Non-fasting blood samples were collected from an antecubital vein into 5-mL vacutainers (Becton Dickinson, Le Pont-de-Claix, France) containing EDTA as an anticoagulant (40 µL K_3-EDTA, 0.37 mol/L per tube). Platelet-poor plasma (PPP) was prepared by centrifugation at 3000*g* for 10 minutes at room temperature. The supernatant was transferred into cryovials (Greiner Laboratechnik, Nürtringen, Germany) in 1-mL aliquots, and then stored at -80°C until further analysis.

Laboratory analyses

Measurement of ADAMTS13, VWF, and C-reactive protein (CRP) was performed at the Research Institute of Internal Medicine at Oslo University Hospital, Rikshospitalet. Before laboratory analyses, PPP samples were thawed for 5 minutes in a water bath at 37°C and centrifuged at 13500 *g* for 2 minutes to obtain platelet-free plasma (PFP).

Plasma ADAMTS13 antigen (ADAMTS13:Ag) was measured in duplicates by an enzyme immunoassay (EIA) with matched antibodies from R&D Systems (Minneapolis, MN,

USA) in a 384 format using a combination of a SELMA (Jena, Germany) pipetting robot and a Biotek (Winooski, VT, USA) dispenser/washer. Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (Bio-Rad, Hercules, CA, USA). ADAMTS13:Ag intra- and inter-assay coefficients of variation were 9.8% and 8.8%, respectively. The mean value of ADAMTS13:Ag in the control population was set to 100%, and all other values were adjusted accordingly and expressed in percentage. EIAs with antibodies from Dako (Glostrup, Denmark) were applied to measure plasma VWF antigen (VWF:Ag) in duplicates.[14] A polyclonal antibody (A0082) was used for coat, and a horseradish peroxidase-conjugated antibody (P02256) for detection. Parallel-diluted pooled human plasma from 20 healthy individuals was used as standard, and the intra- and inter-assay coefficients of variation were 2.6% and 10.8%, respectively. As for ADATMTS13, the mean value of VWF:Ag in the control population was set to 100%, and all other values were adjusted accordingly and expressed in percentage. C-reactive protein (CRP) was measured with a high sensitive technique ("hsCRP") by EIA, as previously described in detail.[34]

Statistical analyses

STATA version 16.0 (Stata Corporation, College Station, Texas, USA) and R version 4 (The R Foundation for Statistical Computing, Vienna, Austria) were used to perform the statistical analyses. Among controls, ADAMTS13:Ag raw values had a mean of 242 ng/mL (standard deviation [SD]: ± 84 ng/mL) and a median of 228 ng/mL (Percentile 25^{th}: 186 ng/mL - Percentile 75^{th}: 278 ng/mL). After expressing the raw values as a percentage of the control population mean (set to 100%), ADAMTS13:Ag levels were categorized according to the following quartile cutoffs determined in controls: <77%, 77-94%, 94-115%, ≥115%. VWF/ADAMTS13 ratio was calculated for each participant as VWF:Ag divided by ADAMTS13:Ag, and then quartiles cutoffs were determined among controls (<0.42, 0.42-

0.74, 0.74-1.40, ≥1.40). Baseline characteristics stratified on ADAMTS13 and VWF/ADAMTS13 ratio quartiles were analyzed using descriptive statistics, and expressed as percentages (frequency) for categorical variables and as mean (± SD) or median (interquartile range) for continuous variables.

Unconditional logistic regression was performed to estimate odds ratios (OR) with 95% confidence intervals (CI) for VTE according to quartiles of ADAMTS13 levels and VWF/ADAMTS13 ratio. The highest quartile was set as the reference category for ADAMTS13 levels, while the lowest quartile served as the reference for the VWF/ADAMTS13 ratio. The *P* value for linear trend of VTE risk was estimated across quartiles of ADAMTS13 levels and VWF/ADAMTS13 ratio. Associations were adjusted for the matching factors (i.e. age and sex)[35] in Model 1, with addition of BMI and CRP to Model 2 in order to assess potential confounding by obesity-related mechanisms or inflammation. Analyses were performed for overall VTE and VTE subtypes (DVT, PE, provoked, unprovoked) as the outcomes of interest. As ADAMTS13 levels have been shown to be associated with arterial CVD,[18-20] which in turn is found to increase the risk of VTE,[36,37] we performed sensitivity analyses for overall VTE excluding participants with a history of arterial CVD at baseline. In all analyses, plasma ADAMTS13 levels and VWF/ADAMTS13 ratio were also entered into the logistic regression models as continuous variables. To this end, we calculated the mean and SD of ADAMTS13 levels and VWF/ADAMTS13 ratio based on the distribution of the control population. The ORs for VTE were investigated by 1SD decrease for ADAMTS13 levels and by 1SD increase for the VWF/ADAMTS13 ratio. Because the VWF/ADAMTS13 ratio values were not normally distributed, natural logarithm transformation was applied to calculate the SD.

As the follow-up time in the source cohort was long, the results based on baseline ADAMTS13 and VWF measurements could be influenced by regression dilution.[38] To

investigate this, we took into account the time elapsed between blood sampling at cohort baseline in Tromsø 4 (i.e. when samples to measure ADAMTS13 and VWF were drawn) and the occurrence of VTE events. We performed analyses that restricted the maximum time from blood sampling in Tromsø 4 to the VTE events, while keeping all controls in the analyses. The logistic regression analyses on time restrictions were set to require at least 10 VTE events, and ORs were estimated at every time point a new VTE event occurred and were plotted as a function of this maximum time, with adjustment for age, sex, BMI, and CRP.

Results

Baseline characteristics of study participants across quartiles of plasma ADAMTS13:Ag levels are displayed in Table 1. The mean age and the proportion of subjects with self-reported history of arterial CVD increased with decreasing levels of ADAMTS13. Median CRP levels were 1.19 mg/L (interquartile range [IQR] 0.66-2.32) in quartile 4 and increased to 1.46 (IQR 0.85-2.67) in quartile 1. VWF/ADAMTS13 ratio, as expected, increased with decreasing ADAMTS13:Ag levels, while no substantial differences were seen for VWF:Ag levels. When baseline characteristics were analyzed across quartiles of VWF/ADAMTS13 ratio (Supplemental Table 1), the mean age and the proportion of subjects with arterial CVD increased with an increasing VWF/ADAMTS13 ratio. Predictably, plasma levels of VWF:Ag increased whereas ADAMTS13:Ag levels decreased across quartiles of the ratio. The characteristics of patients at the VTE event are shown in Table 2. The mean age at the time of VTE was 68 years, 48.8% were men, and 59% of the events were provoked VTEs and 63.5% were DVTs.

The ORs for overall VTE and subtypes (i.e., provoked and unprovoked VTEs, DVT and PE) across quartiles of plasma ADAMTS13:Ag levels are shown in Table 3. ADAMTS13 levels were inversely associated with VTE risk, with an OR of 1.40 (95% CI 0.99-1.99) for

the lowest versus highest quartile in analysis adjusted for age and sex. No significant trend in risk estimates was seen across quartiles (*P* for trend= 0.12), suggesting a threshold rather than a linear relationship between ADAMTS13 levels and VTE risk. Further adjustment for BMI and CRP had a negligible effect on the risk estimates. For VTE subtypes, the ORs were essentially similar to those observed for overall VTE, with the highest ORs obtained for the lowest versus highest quartile of ADAMTS13, with the exception of provoked VTE. When ADAMTS13 was analyzed as a continuous variable (Supplemental Table 2), 1SD decrease in ADAMTS13:Ag levels was associated with a 16% higher OR of VTE (OR 1.16, 95% CI 1.03-1.30) in the age- and sex-adjusted model, with virtually no change in the risk estimate with additional adjustment for BMI and CRP (OR 1.17, 95% CI 1.04-1.32). In line with the quartile-based analysis, estimates for VTE subtypes did not substantially differ from those of overall VTE.

The ORs for overall VTE and subtypes according to quartiles of plasma VWF/ADAMTS13 ratio are shown in Table 4. A linear association was demonstrated for overall VTE, unprovoked VTE and DVT (*p* for trend ≤0.001). An increasing VWF/ADAMTS13 ratio was associated with increased risk of VTE, with an OR of 1.70 (95% CI 1.19-2.43) for the highest versus lowest quartile in age- and sex-adjusted analysis. The association was most pronounced for DVT (OR 1.77, 95% CI 1.17-2.67) and especially for unprovoked VTE (OR 2.81, 95% CI 1.65-4.81). Further subgroup analysis for unprovoked DVT was conducted as the association between VWF/ADAMTS13 ratio and thrombosis risk was strongest for unprovoked VTE and DVT, and revealed an OR of 4.75 (95% CI 2.27-9.95) for the highest compared to the lowest quartile. Additional adjustment for BMI and CRP had a minor impact on the risk estimates for overall VTE and subtypes. The thrombosis risk by 1SD increase in VWF/ADAMTS13 ratio was in agreement with the quartile-based analysis, with the strongest association also observed for unprovoked VTE (Supplemental Table 3).

Because VWF/ADAMTS13 ratio was consistently associated with VTE risk, we explored whether this association could be driven by either high VWF or low ADAMTS13 plasma levels. As depicted in Supplemental Table 4, the risk estimates from model 2 remained essentially the same after additional adjustment for plasma VWF:Ag (model 3) or ADAMTS13:Ag (model 4), respectively. Sensitivity analyses restricted to participants without self-reported CVD at baseline provided similar risk estimates as the main analyses and are reported in Supplemental Table 5.

To assess the possibility of underestimating the true association due to regression dilution bias, we estimated ORs for overall VTE as a function of time between blood sampling and the events (Figure 2). The ORs for VTE were somewhat higher with shortened time between blood sampling and VTE events, both for ADAMTS13:Ag (OR for the lowest versus highest quartile) and VWF/ADAMTS13 ratio (OR for the highest versus lowest quartile), indicating a certain degree of regression dilution.

Discussion

In this population-based nested case-control study, we found that decreased plasma ADAMTS13:Ag levels were associated with increased risk of future VTE in age- and sex-adjusted analysis. In addition, an increasing VWF/ADAMTS13 ratio displayed a linear association with VTE risk, where subjects in the highest quartile of VWF/ADAMTS13 ratio had a 70% higher OR of VTE than those in the lowest quartile, and the association was strongest for unprovoked VTE. The risk estimates were only slightly attenuated with further adjustment for BMI and inflammation, assessed by CRP. In addition, the risk estimates for VTE by ADAMTS13 levels and VWF/ADAMTS13 ratios were only moderately attenuated by increasing time between blood sampling and the VTE events (regression dilution bias). Our findings support that ADAMTS13, and the VWF/ADAMTS13 ratio in particular, may

serve as biomarkers for risk of future VTE and are involved in the pathogenesis of VTE.

Existing data on the association between ADAMTS13 and VTE has been restricted to case-control studies involving highly selected study populations.[24-27] In accordance with most[24,25], but not all[27] previous case-control studies, Pagliari et al.[26] recently found that decreased ADAMTS13 activity was associated with increased risk of unprovoked DVT in a study comprising 365 patients and 292 controls. Similarly, cohort studies exploring the relationship between ADAMTS13 and VTE in cancer patients[21-23] showed that decreased ADAMTS13 levels were associated with VTE risk in most,[21,23] but not all studies.[22]

To the best of our knowledge, this is the first study to assess the association between plasma ADAMTS13 levels and VTE with a prospective time sequence between the exposure and outcome (i.e., blood samples for assessment of ADAMTS13 were drawn before the VTEs occurred). We found that low ADAMTS13 levels were associated with a 1.4-fold increased risk of VTE. The observed relationship between ADAMTS13 levels and VTE risk pointed toward a threshold effect, as an increase in risk estimates became particularly apparent when comparing the lowest versus highest quartile of ADAMTS13. This finding is in agreement with previous studies on the association of ADAMTS13 with arterial CVD and VTE, in which a threshold effect rather than a dose-response relationship was also suggested.[19,26] The association between ADAMTS13 levels and VTE risk was especially noticeable when modelling the metalloprotease as a continuous variable, with the assessment of the thrombosis risk by 1SD decrease in ADAMTS13 levels. Importantly, in the present study, the effect of ADAMTS13 levels on VTE risk remained virtually the same after adjustment for high-sensitivity CRP, implying that confounding by inflammation would be an unlikely explanation for the potential link between ADAMTS13 and VTE. It is worth noting that rare genetic variants in *ADAMTS13* were associated with slightly reduced activity of ADAMTS13 and with increased DVT risk, implying that ADAMTS13 might be causally related to

VTE.[39,40] However, a recent Mendelian randomization study did not find an association between genetically predicted ADAMTS13 activity levels and VTE risk,[41] suggesting that further research from a causal perspective is warranted.

Growing evidence advocates for a pivotal role of the VWF-ADAMTS13 axis not only in hemostasis but also in thrombosis. In the present study, an imbalance between plasma levels of VWF and ADAMTS13, as reflected by an increased VWF/ADAMTS13 ratio, was dose-dependently associated with increased risk of future VTE events. In patients with coronary artery disease and ischemic stroke, a high VWF/ADAMTS13 ratio was consistently associated with cardiovascular complications, including recurrent thrombosis and death.[28-31] Others assessed the combined effect of high VWF levels and low ADAMTS13 levels on thrombosis risk and found that the two risk factors displayed a synergistic effect on risk of both arterial and venous thrombosis.[26,42] Thus, our findings expand the existing knowledge on the association of an imbalance between plasma VWF and ADAMTS13 levels with arterial thromboembolic conditions to also embrace VTE. Moreover, although the ORs for VTE by increased VWF/ADAMTS13 ratio were somewhat higher with shortened time between blood sampling and VTE events (Figure 2), the component of regression dilution due to intra-individual fluctuation of VWF/ADAMTS13 ratio over time was moderate at most. The stability of VWF/ADAMTS13 ratio underscores its potential to serve as a reliable short- and long-term biomarker of VTE risk.

The consistent finding of increased VTE risk by increasing VWF/ADAMTS13 ratios suggests that the imbalance between VWF and ADAMTS13 plays an important role in the pathogenesis of VTE. It is reasonable to speculate that in the presence of increased VWF levels due to genetic or acquired factors,[43-45] the proteolytic activity of ADAMTS13 towards VWF would be insufficient. The imbalance between the two proteins could also be a consequence of slightly to moderately decreased ADAMTS13 levels due to acquired

conditions (e.g. advancing age, pregnancy, and comorbidities, such as cancer, liver disease and inflammatory disease)[46-48] or genetic factors.[39,40,49,50] Interestingly, common genetic variants were reported to account for up to 20% of the variability in ADAMTS13 antigen levels.[50] Of note, our results suggest that the association between the VWF/ADAMTS13 ratio and VTE was not explained by either high levels of VWF or low levels of ADAMTS13, as adjustment for these variables had a minor impact on the association. This implies that an imbalance between circulating levels of the two proteins contributes to the VTE risk. Irrespective of the primary cause for the imbalance in VWF and ADAMTS13 levels, the regulation of the size of VWF multimers by ADAMTS13 would be disrupted and could result in an excess of ULVWF multimers, which are more prone to bind platelets.[16] The interaction between VWF and platelets is well-known in the pathophysiology of arterial thrombosis[45] but growing evidence from mouse models suggests that this interaction is also implicated in venous thrombus formation.[6,7] The interplay between ADAMTS13 and VWF is a complex and dynamic process in which shear stress has a key role.[16] Therefore, studies are needed to unravel how the VWF-ADAMTS13 axis contributes to venous thrombosis formation, especially because ADAMTS13 has the potential to be a target for therapeutic intervention. Indeed, previous experimental studies in mouse models showed that infusion of recombinant human ADAMTS13 resulted in a reduction of both arterial[51-53] and venous[54] thrombi.

The strengths of the present study include the nested case-control design, allowing prospective assessment and insight into the temporal sequence of the associations. The VTE cases and age- and sex-matched controls were recruited from the same unselected source population, minimizing the likelihood of selection bias. Still, the study has some limitations that require attention. In the quartile-based analysis on the association of ADAMTS13 and VWF/ADAMTS13 ratio with thrombosis risk, the number of VTE events was low in some subgroups, which resulted in limited statistical power, and consequently the need for a

cautious interpretation of the findings, particularly for ADAMTS13. Nonetheless, when both ADAMST13 and VWF/ADAMTS13 ratio were entered into the regression models as continuous variables, the precision of the risk estimates was improved, as reflected by their 95% CIs. Although the number of plasma samples not available or of inadequate quality for the assessment of ADAMTS13 antigen level was somewhat high, missing data on ADAMTS13 was not related to the VTE status, occurring in 17% of the VTE cases and 16% of the controls. Additionally, baseline characteristics of the study participants with and without measurement of ADAMTS 13 were similar (data not shown). Thus, the missing data on ADAMTS13 was presumably completely at random. Plasma samples were stored for more than 20 years between baseline sampling and measurement of ADAMTS13 and VWF, introducing a possibility for discrepancy between true and measured levels. In the present study, ADAMTS13 mean (242 ng/mL) and median (228 ng/mL) antigen levels in controls corresponded to about one third of the levels generally reported for this metalloprotease in a healthy population.[24,25,42,55-57] However, because blood samples were stored in the same way and for the same duration for cases and controls, any potential misclassification would be non-differential with regards to VTE status, which could have led to an underestimation of the true associations.[38] Furthermore, previous studies have predominantly targeted ADAMTS13 activity, as it expresses both quantitative and qualitative alterations of the metalloprotease, while in the present study only ADAMTS13 antigen levels were assessed as we only had stored EDTA plasma samples available for this cohort. In some physiological and pathological conditions (e.g. neonatal period, pregnancies of later maternal age, or cardiac surgery), plasma levels of ADAMTS13 activity and antigen have been shown to not always be well correlated.[47] Nevertheless, our study population was comprised of adult individuals, of whom the majority had no major comorbidities at the time of baseline blood sampling, with existing data indicating a substantial correlation between ADAMTS13 antigen and activity

levels both in healthy individuals and those with thrombotic disease.[28,47] Finally, VWF:Ag assay allows quantitative assessment of VWF but does not evaluate its functional activity. VWF activity assays, which also reflect the protein multimeric size, such as VWF ristocetin-cofactor (VWF:RCo) activity, might provide more accurate and biologically meaningful results on the role of the imbalance between VWF and ADAMTS13 in the risk of VTE. However, as already pointed out, since only stored EDTA plasma samples were available for this study, unfortunately, the VWF activity could not be assessed.

In conclusion, decreased plasma ADAMTS13 levels and an imbalance between VWF and ADAMTS13 levels, reflected by an increased VWF/ADAMTS13 ratio, were associated with increased risk of future VTE. Our findings suggest that the VWF-ADAMTS13 axis might be involved in VTE pathogenesis and serve as a biomarker for risk of future VTE. Further research is needed to unravel the mechanisms by which the VWF-ADAMTS13 axis may contribute to venous thrombus formation and to explore its potential as a therapeutic target for VTE.

Authorship Contributions

M.S. Edvardsen analyzed data, interpreted the results, and drafted the manuscript. E-S. Hansen interpreted the results and revised the manuscript. P. Aukrust and T. Ueland performed the laboratory analysis, interpreted the results, and revised the manuscript. S.K. Brækkan designed the study, organized data collection, interpreted the results, and revised the manuscript. V.M. Morelli designed the study, interpreted the results, contributed to the manuscript draft, and revised the manuscript. J-B. Hansen designed the study, organized data collection, interpreted the results, contributed to the manuscript draft and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Acknowledgments

The Thrombosis Research Center has received an independent grant from Stiftelsen Kristian Gerhard Jebsen (2014-2020).

Conflict of Interest Disclosures

There are no conflicts of interest reported by any of the authors.

References

1. Næss IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrøm J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost*. 2007;5(4):692-699. doi:10.1111/j.1538-7836.2007.02450.x
2. Raskob G, Angchaisuksiri P, Blanco A, et al. Thrombosis: A Major Contributor to Global Disease Burden. *Semin Thromb Hemost*. 2014;40(07):724-735. doi:10.1055/s-0034-1390325
3. Arshad N, Isaksen T, Hansen J-B, Brækkan SK. Time trends in incidence rates of venous thromboembolism in a large cohort recruited from the general population. *Eur J Epidemiol*. 2017;32(4):299-305. doi:10.1007/s10654-017-0238-y
4. Münster AM, Rasmussen TB, Falstie-Jensen AM, et al. A changing landscape: Temporal trends in incidence and characteristics of patients hospitalized with venous thromboembolism 2006–2015. *Thromb Res*. 2019;176:46-53. doi:10.1016/j.thromres.2019.02.009
5. Chauhan AK, Kisucka J, Lamb CB, Bergmeier W, Wagner DD. von Willebrand factor and factor VIII are independently required to form stable occlusive thrombi in injured veins. *Blood*. Mar 15 2007;109(6):2424-9. doi:10.1182/blood-2006-06-028241
6. Brill A, Fuchs TA, Chauhan AK, et al. von Willebrand factor-mediated platelet adhesion is critical for deep vein thrombosis in mouse models. *Blood*. Jan 27 2011;117(4):1400-7. doi:10.1182/blood-2010-05-287623
7. Michels A, Dwyer CN, Mewburn J, et al. von Willebrand Factor Is a Critical Mediator of Deep Vein Thrombosis in a Mouse Model of Diet-Induced Obesity. *Arterioscler Thromb Vasc Biol*. Dec 2020;40(12):2860-2874. doi:10.1161/atvbaha.120.314690
8. Bryckaert M, Rosa J-P, Denis CV, Lenting PJ. Of von Willebrand factor and platelets. *Cell Mol Life Sci*. 2015;72(2):307-326. doi:10.1007/s00018-014-1743-8
9. Federici A. The factor VIII/von Willebrand factor complex: basic and clinical issues. *Haematologica*. 2003;88(6):EREP02-EREP02.
10. Leebeek FWG, Eikenboom JCJ. Von Willebrand's Disease. *N Engl J Med*. 2016;375(21):2067-2080. doi:10.1056/nejmra1601561
11. Vischer UM. von Willebrand factor, endothelial dysfunction, and cardiovascular disease. *J Thromb Haemost*. Jun 2006;4(6):1186-93. doi:10.1111/j.1538-7836.2006.01949.x
12. De Meyer SF, Stoll G, Wagner DD, Kleinschnitz C. von Willebrand Factor: an emerging target in stroke therapy. *Stroke*. 2012;43(2):599-606. doi:10.1161/strokeaha.111.628867
13. Tsai AW, Cushman M, Rosamond WD, et al. Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). *Am J Med*. Dec 1 2002;113(8):636-42.
14. Edvardsen MS, Hindberg K, Hansen E-S, et al. Plasma levels of von Willebrand factor and future risk of incident venous thromboembolism. *Blood Adv*. 2021;5(1):224-232. doi:10.1182/bloodadvances.2020003135

15. Zheng XL. Structure-function and regulation of ADAMTS-13 protease. *J Thromb Haemost*. 2013;11:11-23. doi:10.1111/jth.12221
16. South K, Lane DA. ADAMTS-13 and von Willebrand factor: a dynamic duo. *J Thromb Haemost*. Jan 2018;16(1):6-18. doi:10.1111/jth.13898
17. Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. *Blood*. 2017;129(21):2836-2846. doi:10.1182/blood-2016-10-709857
18. Sonneveld MAH, De Maat MPM, Leebeek FWG. Von Willebrand factor and ADAMTS13 in arterial thrombosis: a systematic review and meta-analysis. *Blood Rev*. 2014;28(4):167-178. doi:10.1016/j.blre.2014.04.003
19. Maino A, Siegerink B, Lotta LA, et al. Plasma ADAMTS-13 levels and the risk of myocardial infarction: an individual patient data meta-analysis. *J Thromb Haemost*. 2015;13(8):1396-1404. doi:10.1111/jth.13032
20. Sonneveld MAH, Kavousi M, Ikram MA, et al. Low ADAMTS-13 activity and the risk of coronary heart disease - a prospective cohort study: the Rotterdam Study. *J Thromb Haemost*. 2016;doi:10.1111/jth.13479
21. Pépin M, Kleinjan A, Hajage D, et al. ADAMTS-13 and von Willebrand factor predict venous thromboembolism in patients with cancer. *J Thromb Haemost*. Feb 2016;14(2):306-15. doi:10.1111/jth.13205
22. Obermeier HL, Riedl J, Ay C, et al. The role of ADAMTS-13 and von Willebrand factor in cancer patients: Results from the Vienna Cancer and Thrombosis Study. *Res Pract Thromb Haemost*. Jul 2019;3(3):503-514. doi:10.1002/rth2.12197
23. Setiawan B, Permatadewi CO, de Samakto B, et al. Von Willebrand factor:antigen and ADAMTS-13 level, but not soluble P-selectin, are risk factors for the first asymptomatic deep vein thrombosis in cancer patients undergoing chemotherapy. *Thromb J*. Nov 11 2020;18(1):33. doi:10.1186/s12959-020-00247-6
24. Karakaya B, Tombak A, Serin MS, Tiftik N. Change in plasma a disintegrin and metalloprotease with thrombospondin type-1 repeats-13 and von Willebrand factor levels in venous thromboembolic patients. *Hematology*. Jun 2016;21(5):295-9. doi:10.1080/10245332.2015.1125079
25. Llobet D, Tirado I, Vilalta N, et al. Low ADAMTS13 levels are associated with venous thrombosis risk in women. *Thromb Res*. Sep 2017;157:38-40. doi:10.1016/j.thromres.2017.07.001
26. Pagliari MT, Boscarino M, Cairo A, et al. ADAMTS13 activity, high VWF and FVIII levels in the pathogenesis of deep vein thrombosis. *Thromb Res*. Jan 2021;197:132-137. doi:10.1016/j.thromres.2020.10.037
27. Mazetto BM, Orsi FL, Barnabé A, De Paula EV, Flores-Nascimento MC, Annichino-Bizzacchi JM. Increased ADAMTS13 activity in patients with venous thromboembolism. *Thromb Res*. Dec 2012;130(6):889-93. doi:10.1016/j.thromres.2012.09.009
28. Kaikita K, Soejima K, Matsukawa M, Nakagaki T, Ogawa H. Reduced von Willebrand factor-cleaving protease (ADAMTS13) activity in acute myocardial infarction. *J Thromb Haemost*. 2006;4(11):2490-2493. doi:10.1111/j.1538-7836.2006.02161.x
29. Matsukawa M, Kaikita K, Soejima K, et al. Serial Changes in von Willebrand Factor-Cleaving Protease (ADAMTS13) and Prognosis After Acute Myocardial Infarction. *Am J Cardiol*. 2007;100(5):758-763. doi:10.1016/j.amjcard.2007.03.095
30. Warlo EMK, Pettersen AR, Arnesen H, Seljeflot I. vWF/ADAMTS13 is associated with on-aspirin residual platelet reactivity and clinical outcome in patients with stable coronary artery disease. *Thromb J*. 2017;15:28. doi:10.1186/s12959-017-0151-3
31. Taylor A, Vendramin C, Singh D, Brown MM, Scully M. von Willebrand factor/ADAMTS13 ratio at presentation of acute ischemic brain injury is predictive of outcome. *Blood Adv*. Jan 28 2020;4(2):398-407. doi:10.1182/bloodadvances.2019000979
32. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso Study. *Int J Epidemiol*. Aug 2012;41(4):961-7. doi:10.1093/ije/dyr049

33. Braekkan SK, Borch KH, Mathiesen EB, Njolstad I, Wilsgaard T, Hansen JB. Body height and risk of venous thromboembolism: The Tromso Study. *Am J Epidemiol*. May 15 2010;171(10):1109-15. doi:10.1093/aje/kwq066
34. Liang RA, Høiland II, Ueland T, et al. Plasma levels of mannose-binding lectin and future risk of venous thromboembolism. *J Thromb Haemost*. 2019;17(10):1661-1669. doi:10.1111/jth.14539
35. Pearce N. Analysis of matched case-control studies. *BMJ*. 2016:i969. doi:10.1136/bmj.i969
36. Rinde LB, Lind C, Småbrekke B, et al. Impact of incident myocardial infarction on the risk of venous thromboembolism: the Tromsø Study. *J Thromb Haemost*. 2016;14(6):1183-1191. doi:10.1111/jth.13329
37. Rinde LB, Smabrekke B, Mathiesen EB, et al. Ischemic Stroke and Risk of Venous Thromboembolism in the General Population: The Tromso Study. *J Am Heart Assoc*. Nov 7 2016;5(11)doi:10.1161/jaha.116.004311
38. Clarke R, Shipley M, Lewington S, et al. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. *Am J Epidemiol*. Aug 15 1999;150(4):341-53. doi:10.1093/oxfordjournals.aje.a010013
39. Lotta LA, Tuana G, Yu J, et al. Next-generation sequencing study finds an excess of rare, coding single-nucleotide variants of ADAMTS13 in patients with deep vein thrombosis. *J Thromb Haemost*. Jul 2013;11(7):1228-39. doi:10.1111/jth.12291
40. Pagliari MT, Cairo A, Boscarino M, et al. Role of ADAMTS13, VWF and F8 genes in deep vein thrombosis. *PLoS One*. 2021;16(10):e0258675. doi:10.1371/journal.pone.0258675
41. Ye Z, Zheng J. Verification of the Role of ADAMTS13 in the Cardiovascular Disease Using Two-Sample Mendelian Randomization. *Front Genet*. 2021;12:660989. doi:10.3389/fgene.2021.660989
42. Andersson HM, Siegerink B, Luken BM, et al. High VWF, low ADAMTS13, and oral contraceptives increase the risk of ischemic stroke and myocardial infarction in young women. *Blood*. Feb 9 2012;119(6):1555-60. doi:10.1182/blood-2011-09-380618
43. Desch KC, Ozel AB, Siemieniak D, et al. Linkage analysis identifies a locus for plasma von Willebrand factor undetected by genome-wide association. *Proc Natl Acad Sci U S A*. Jan 8 2013;110(2):588-93. doi:10.1073/pnas.1219885110
44. de Lange M, de Geus EJ, Kluft C, et al. Genetic influences on fibrinogen, tissue plasminogen activator-antigen and von Willebrand factor in males and females. *Thromb Haemost*. Mar 2006;95(3):414-9. doi:10.1160/th05-09-0596
45. Franchini M, Lippi G. Von Willebrand factor and thrombosis. *Ann Hematol*. Jul 2006;85(7):415-23. doi:10.1007/s00277-006-0085-5
46. Kokame K, Sakata T, Kokubo Y, Miyata T. von Willebrand factor-to-ADAMTS13 ratio increases with age in a Japanese population. *J Thromb Haemost*. Jul 2011;9(7):1426-8. doi:10.1111/j.1538-7836.2011.04333.x
47. Feys HB, Canciani MT, Peyvandi F, Deckmyn H, Vanhoorelbeke K, Mannucci PM. ADAMTS13 activity to antigen ratio in physiological and pathological conditions associated with an increased risk of thrombosis. *Br J Haematol*. 2007;138(4):534-540. doi:10.1111/j.1365-2141.2007.06688.x
48. Mannucci PM, Karimi M, Mosalaei A, Canciani MT, Peyvandi F. Patients with localized and disseminated tumors have reduced but measurable levels of ADAMTS-13 (von Willebrand factor cleaving protease). *Haematologica*. Apr 2003;88(4):454-8.
49. de Vries PS, Boender J, Sonneveld MA, et al. Genetic variants in the ADAMTS13 and SUPT3H genes are associated with ADAMTS13 activity. *Blood*. Jun 18 2015;125(25):3949-55. doi:10.1182/blood-2015-02-629865
50. Ma Q, Jacobi PM, Emmer BT, et al. Genetic variants in ADAMTS13 as well as smoking are major determinants of plasma ADAMTS13 levels. *Blood Adv*. Jun 27 2017;1(15):1037-1046. doi:10.1182/bloodadvances.2017005629
51. Zhao B-Q, Chauhan AK, Canault M, et al. von Willebrand factor–cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. *Blood*. 2009;114(15):3329-3334. doi:10.1182/blood-2009-03-213264

52. De Meyer SF, Savchenko AS, Haas MS, et al. Protective anti-inflammatory effect of ADAMTS13 on myocardial ischemia/reperfusion injury in mice. *Blood*. Dec 20 2012;120(26):5217-23. doi:10.1182/blood-2012-06-439935
53. Doi M, Matsui H, Takeda H, et al. ADAMTS13 safeguards the myocardium in a mouse model of acute myocardial infarction. *Thromb Haemost*. Dec 2012;108(6):1236-8. doi:10.1160/th12-09-0674
54. Tashima Y, Banno F, Akiyama M, Miyata T. Influence of ADAMTS13 deficiency on venous thrombosis in mice. *Thromb Haemost*. Jul 2015;114(1):206-7. doi:10.1160/th14-08-0656
55. Rieger M, Ferrari S, Kremer Hovinga JA, et al. Relation between ADAMTS13 activity and ADAMTS13 antigen levels in healthy donors and patients with thrombotic microangiopathies (TMA). *Thromb Haemost*. Feb 2006;95(2):212-20. doi:10.1160/th05-08-0550
56. Hugenholtz GC, Adelmeijer J, Meijers JC, Porte RJ, Stravitz RT, Lisman T. An unbalance between von Willebrand factor and ADAMTS13 in acute liver failure: implications for hemostasis and clinical outcome. *Hepatology*. Aug 2013;58(2):752-61. doi:10.1002/hep.26372
57. Ladeira VS, Barbosa AR, Oliveira MM, et al. ADAMTS-13-VWF axis in sickle cell disease patients. *Ann Hematol*. Feb 2021;100(2):375-382. doi:10.1007/s00277-020-04385-9

Tables

Table 1. Distribution of baseline characteristics of study participants according to quartiles of plasma levels of ADAMTS13

	Plasma ADAMTS13:Ag (%)			
	Quartile 1 < 77	Quartile 2 77 – 94	Quartile 3 94 – 115	Quartile 4 ≥ 115
n	307	283	295	278
Age, years	62.9 ± 14.2	61.2 ± 14.0	59.4 ± 13.1	57.5 ± 13.5
Sex, male	48.5 (149)	43.8 (124)	50.2 (148)	47.1 (131)
BMI, kg/m^2	26.6 ± 4.5	25.8 ± 3.8	26.5 ± 4.2	26.6 ± 4.5
CRP, mg/L	1.46 (0.85-2.67)	1.30 (0.67-3.20)	1.38 (0.73-2.75)	1.19 (0.66-2.32)
Cancer †	3.9 (12)	5.3 (15)	4.4 (13)	4.3 (12)
CVD †	19.9 (61)	17.3 (49)	13.9 (41)	11.2 (31)
VWF:Ag, %	90 (52-164)	70 (41-128)	75 (44-131)	83 (54-131)
VWF:Ag / ADAMTS13:Ag ratio	1.42 (0.79-2.57)	0.84 (0.48-1.47)	0.73 (0.41-1.28)	0.55 (0.39-0.99)

ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; VWF, von Willebrand factor.
Continuous variables are shown as mean (± standard deviation) or median (interquartile range).
Categorical variables are shown as percentages with numbers in brackets.
† Self-reported history of cancer or arterial CVD (myocardial infarction, angina or stroke) at baseline.

Table 2. Characteristics of the venous thromboembolism (VTE) events (n=383)

Characteristics	
Age at VTE, years	68 ± 14
Sex, male	48.8 (187)
Deep vein thrombosis	63.5 (243)
Pulmonary embolism	36.6 (140)
Unprovoked VTE	41.0 (157)
Provoked VTE	59.0 (226)

Age is presented as mean ± standard deviation.
Categorical variables are shown as percentages with numbers in brackets.

Table 3. Odds ratios (OR) with 95% confidence intervals (CI) for overall venous thromboembolism (VTE) and subtypes across quartiles (Q) of plasma levels of ADAMTS13

Quartiles of ADAMTS13:Ag	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Overall VTE				
Q1 (< 77%)	194	113	1.40 (0.99-1.99)	1.39 (0.98-1.99)
Q2 (77-94%)	195	88	1.09 (0.76-1.57)	1.13 (0.78-1.63)
Q3 (94-115%)	194	101	1.26 (0.88-1.79)	1.23 (0.86-1.76)
Q4 (≥ 115%)	197	81	1 (reference)	1 (reference)
P for trend			*0.12*	*0.11*
Provoked VTE				
Q1 (< 77%)	194	61	1.34 (0.86-2.08)	1.31 (0.84-2.04)
Q2 (77-94%)	195	61	1.35 (0.87-2.08)	1.37 (0.88-2.13)
Q3 (94-115%)	194	59	1.32 (0.85-2.04)	1.27 (0.82-1.98)
Q4 (≥ 115%)	197	45	1 (reference)	1 (reference)
P for trend			*0.2*	*0.2*
Unprovoked VTE				
Q1 (< 77%)	194	52	1.48 (0.92-2.38)	1.49 (0.92-2.42)
Q2 (77-94%)	195	27	0.76 (0.44-1.31)	0.79 (0.46-1.36)
Q3 (94-115%)	194	42	1.18 (0.72-1.93)	1.14 (0.70-1.88)
Q4 (≥ 115%)	197	36	1 (reference)	1 (reference)
P for trend			*0.2*	*0.2*
Deep vein thrombosis				
Q1 (< 77%)	194	68	1.29 (0.85-1.95)	1.29 (0.85-1.96)
Q2 (77-94%)	195	60	1.13 (0.74-1.72)	1.15 (0.75-1.76)
Q3 (94-115%)	194	61	1.15 (0.76-1.75)	1.12 (0.74-1.71)
Q4 (≥ 115%)	197	54	1 (reference)	1 (reference)
P for trend			*0.3*	*0.2*
Pulmonary embolism				
Q1 (< 77%)	194	45	1.63 (0.97-2.75)	1.59 (0.94-2.70)
Q2 (77-94%)	195	28	1.02 (0.58-1.80)	1.06 (0.59-1.88)
Q3 (94-115%)	194	40	1.48 (0.87-2.51)	1.41 (0.83-2.41)
Q4 (≥ 115%)	197	27	1 (reference)	1 (reference)
P for trend			*0.2*	*0.2*

ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13.
Model 1: Adjusted for age and sex.
Model 2: Model 1 + body mass index and C-reactive protein.

Table 4. Odds ratios (OR) with 95% confidence intervals (CI) for overall venous thromboembolism (VTE) and subtypes across quartiles (Q) of plasma VWF/ADAMTS13 ratio

VWF:Ag / ADAMTS13:Ag ratio	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Overall VTE				
Q1 (<0.42)	194	74	1 (reference)	1 (reference)
Q2 (0.42 - 0.74)	195	77	1.04 (0.71-1.51)	1.00 (0.68-1.47)
Q3 (0.74 - 1.40)	194	106	1.45 (1.01-2.08)	1.41 (0.97-2.03)
Q4 (≥ 1.40)	197	126	1.70 (1.19-2.43)	1.61 (1.12-2.31)
P for trend			*0.001*	*0.002*
Provoked VTE				
Q1 (<0.42)	194	51	1 (reference)	1 (reference)
Q2 (0.42 - 0.74)	195	48	0.92 (0.59-1.44)	0.90 (0.58-1.41)
Q3 (0.74 - 1.40)	194	61	1.16 (0.76-1.79)	1.13 (0.74-1.75)
Q4 (≥ 1.40)	197	66	1.23 (0.80-1.89)	1.18 (0.76-1.81)
P for trend			*0.2*	*0.3*
Unprovoked VTE				
Q1 (<0.42)	194	23	1 (reference)	1 (reference)
Q2 (0.42 - 0.74)	195	29	1.29 (0.72-2.32)	1.28 (0.71-2.31)
Q3 (0.74 - 1.40)	194	45	2.09 (1.21-3.63)	2.09 (1.20-3.64)
Q4 (≥ 1.40)	197	60	2.81 (1.65-4.81)	2.68 (1.56-4.61)
P for trend			*<0.001*	*<0.001*
Deep vein thrombosis				
Q1 (<0.42)	194	50	1 (reference)	1 (reference)
Q2 (0.42 - 0.74)	195	43	0.87 (0.55-1.37)	0.86 (0.54-1.36)
Q3 (0.74 - 1.40)	194	64	1.32 (0.86-2.03)	1.31 (0.85-2.00)
Q4 (≥ 1.40)	197	86	1.77 (1.17-2.67)	1.67 (1.10-2.53)
P for trend			*0.001*	*0.003*
Pulmonary embolism				
Q1 (<0.42)	194	24	1 (reference)	1 (reference)
Q2 (0.42 - 0.74)	195	34	1.38 (0.78-2.41)	1.33 (0.75-2.35)
Q3 (0.74 - 1.40)	194	42	1.69 (0.97-2.94)	1.64 (0.94-2.87)
Q4 (≥ 1.40)	197	40	1.58 (0.90-2.76)	1.54 (0.87-2.70)
P for trend			*0.09*	*0.11*

VWF, von Willebrand factor; ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13.
Model 1: Adjusted for age and sex.
Model 2: Model 1 + body mass index and C-reactive protein.

Figure legends

Figure 1. Flowchart of the study population. The chart illustrates the nested case-control design. Subjects (aged ≥25 years) were derived from Tromsø 4 cohort. Cases and controls were matched on age and sex. VTE, venous thromboembolism.

Figure 2. Plots of estimated odds ratios (ORs) for overall venous thromboembolism (VTE) as a function of time between blood sampling in Tromsø 4 (T4, 1994–95) and VTE events. Participants with plasma levels of ADAMTS13:Ag in the lowest quartile (Q1) were compared with those with ADAMTS13:Ag in the highest quartile (Q4, reference) (**panel 2A**). Participants with plasma VWF:Ag/ADAMTS13:Ag ratio in the highest quartile (Q4) were compared with those with VWF:Ag/ADAMTS13:Ag ratio in the lowest quartile (Q1, reference) (**panel 2B**). ORs were adjusted for age, sex, body mass index and high-sensitivity C-reactive protein. The large blue circles indicate ORs with *P* values <0.05. The number of VTE events are shown above the plots. ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13; VWF, von Willebrand factor.