Combined effects of plasma von Willebrand factor and platelet measures on the risk of incident venous thromboembolism

Short title: VTE risk by VWF and platelets

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Text word count: 3651 (introduction, methods, results and discussion)

Abstract word count: 250

Figure/table count: 7 (4 tables and 3 figures); **Reference count:** 56

Scientific category: Thrombosis and Hemostasis

Key points

- Both platelet reactivity and platelet count interact biologically with high VWF plasma levels, resulting in an increased risk of VTE

Abstract

Plasma von Willebrand factor (VWF) and platelet reactivity are both risk factors for venous thromboembolism (VTE), and VWF can promote hemostasis by interaction with platelets. In this study, we explored the combined effects of plasma VWF and platelet measures on the risk of incident VTE. A population-based nested case-control study with 403 cases and 816 controls was derived from the Tromsø Study. VWF, platelet count and mean platelet volume (MPV) were measured in blood samples drawn at baseline. Odds ratios (ORs) with 95% confidence intervals (CIs) for VTE were estimated across VWF tertiles, within predefined MPV (<8.5, 8.5-9.5, ≥9.5 fL) and platelet count (<230, 230-299, $\geq 300 \cdot 10^9$ L^{-1}) strata. Here, participants with VWF levels in the highest tertile and MPV ≥9.5 fL had an OR of 1.98 (95% CI 1.17-3.36) for VTE compared with those in the lowest VWF tertile and with MPV <8.5 fL in the age- and sex-adjusted model. In the joint exposure group, 48% (95% CI 15% to 96%) of VTEs were attributable to the biological interaction between VWF and MPV. Similarly, individuals with VWF in the highest tertile and platelet count $\geq 300 \cdot 10^9$ L^{-1} had an OR of 2.91 (95% CI 1.49-5.67) compared with those with VWF in the lowest tertile and platelet count <230, and 39% (95% CI -2% to 97%) of VTEs in the joint exposure group were explained by the interaction. Our results suggest that both platelet reactivity and platelet count interact biologically with high plasma VWF, resulting in an increased risk of incident VTE.

Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease associated with severe complications.[1,2] The incidence of VTE is increasing, contributing to a substantial disease burden globally.[3,4] Even though the pathophysiology of VTE has gradually been unraveled over the last decades, still up to half of all events arise in absence of any known attributable factors.[5,6] Therefore, it is essential to identify risk markers and expand our insight into the mechanisms involved in the disease development.

Von Willebrand Factor (VWF) is a multimeric glycoprotein involved in hemostasis. It is synthesized by endothelial cells and megakaryocytes, and stored and released from Weibel-Palade bodies and platelet α-granules upon stimulation.[7-9] VWF has two distinct roles in hemostasis, namely (i) to serve as the carrier and protector of coagulation factor VIII (FVIII) and (ii) to promote adhesion and aggregation of platelets through interaction with their glycoproteins.[10] We and others have demonstrated that elevated plasma levels of VWF is associated with risk of VTE.[11-13] Moreover, a recent Mendelian randomization (MR) analysis indicated a causal role of VWF in the development of VTE, but this causality could not be dissected from FVIII due to the tight correlation.[14] It is a common notion that the increased VTE risk by elevated VWF levels is mediated primarily through the parallel increase in FVIII levels.[12,14,15] However, studies in mice have suggested that VWF-mediated platelet adhesion has a critical role in VTE formation independent of FVIII.[16,17]

Platelets constitute a crucial part of hemostasis, and have been connected with thrombogenesis through several mechanisms.[18,19] A high platelet count is a marker of VTE risk in cancer patients, but not in cancer-free subjects.[20,21] Mean platelet volume (MPV), an indirect marker of platelet reactivity,[22,23] is associated with future risk of VTE, and unprovoked events in particular.[24] However, studies using other measures of platelet function have provided conflicting results,[25] and the association between platelet function and VTE

remains insufficiently understood.

Expanded knowledge on the role of the VWF-platelet interaction in the pathogenesis of VTE may facilitate development of novel therapeutic targets and improve strategies for prevention. In order to focus on the FVIII-independent role of VWF, our aim was to explore the combined effects of categories of VWF levels and platelet measures on the risk of VTE. To address this question, we carried out a nested case-control study derived from a population-based cohort, with the hypothesis that VWF levels and platelet measures would have more than additive effects on VTE risk.

Methods

Study design

The Tromsø Study is a single-center, population-based cohort with repeated health surveys of the inhabitants of Tromsø municipality in Norway.[26] The fourth survey (Tromsø 4) was conducted in 1994-95. All inhabitants aged ≥ 25 years were invited, with 27,158 individuals (77% of the eligible) eventually taking part in the survey. All participants were followed from the date of inclusion until an incident VTE, death, migration or end of follow-up (September 1, 2007).

During follow-up, 462 participants developed an incident VTE event. For each case, two age- and sex-matched controls, who were alive at the index date of the case, were randomly sampled from the source cohort (Figure 1). The age-matching was based on the same year of birth. Due to insufficient quality of plasma samples, 59 VTE cases and 108 controls were excluded, leaving 403 cases and 816 controls in the final analytic sample. Written informed consent was given by all participants, and study approval was obtained from the regional committee for medical and health research ethics.

Validation of events

To identify all first lifetime VTE events, we searched the hospital discharge diagnosis registry, the radiology procedure registry and the autopsy registry from the University Hospital of North Norway (UNN), which is the only hospital in the study region. The medical record of every potential VTE case was reviewed by trained personnel, and the event was only recorded when clinical signs and symptoms were followed by objective radiological confirmation, resulting in a VTE diagnosis requiring treatment (unless contraindications were specified).

All events were classified as a DVT or PE, with simultaneous evidence of both conditions classified as a PE. Further classification into provoked and unprovoked events was additionally performed. If the patient had one or more provoking factor closely preceding the event, it was categorized as provoked. The provoking factors included were: trauma or surgery within 8 weeks prior to the event; an acute medical condition (myocardial infarction, ischemic stroke or infectious disease); active cancer; and immobilization (> 3 days bed rest, wheelchair confinement, or long-distance travel of ≥ 4 hours within the last 14 days). If the treating physician specified another factor to have provoked the event (e.g., venous catheters), this was also recognized.

Baseline measurements and blood sampling

Baseline information was collected by physical examinations, self-administered questionnaires and blood samples. Height (to the nearest cm) and weight (to the nearest 0.5 kg) was measured with participants in light clothing and no shoes, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Trained personnel performed three consecutive blood pressure measurements on all participants, using an automatic device (Dinamap Vital Signs Monitor). Participants rested in

a sitting position for 2 minutes before and between the measurements, and the two last measurements were used to calculate mean systolic and diastolic blood pressure. Self-administered questionnaires were used to obtain information regarding smoking habits and estrogen use, and history of cancer and cardiovascular disease (myocardial infarction, stroke or angina pectoris).

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

Measurement of platelet count, MPV and VWF

Platelet count and MPV were analyzed within 12 hours of blood sampling, using an automated blood cell counter (CoulterCounter®, Coulter Electronics, Luton, UK).

Measurement of VWF was performed at the Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet. Stored plasma samples were first thawed in a water bath at 37°C for 5 minutes, and then centrifuged at 13500*g* for 2 minutes to obtain platelet free plasma (PFP). Plasma VWF levels were measured by enzyme immunoassays with antibodies (A0082, P02256) obtained from Dako (Glostrup, Denmark), using a polyclonal antibody for coat (A0082) and a horseradish peroxidase-conjugated polyclonal antibody for detection (P02256). Parallel diluted pooled human plasma from 20 healthy individuals was used as standard, and the measurements were expressed as percentage of the control population mean (100%). The intra- and inter-assay coefficients of variation were 2.6% and 10.8%, respectively.

Statistical analyses

STATA version 16.0 (Stata Corporation, College Station, Texas, USA) and R version 3.6.3 (The R Foundation for Statistical Computing, Vienna, Austria) were used to carry out the statistical analyses.

Categories of VWF (low, medium, high) were defined according to tertiles of plasma VWF levels in the control group. Categories of platelet variables were made with cutoffs at 8.5 and 9.5 fL for MPV, and 230 and $300 \cdot 10^9$ L^{-1} for platelet count, according to our previous study on platelets and VTE risk.[24] The three-level variables of VWF and MPV were combined to yield a nine-level variable, and subjects with concomitant low VWF levels and low MPV served as the reference group. A combined category of VWF and platelet count was created using the same approach.

Baseline characteristics across tertiles of VWF levels were expressed as mean (± standard deviation [SD]) or median (25^{th} – 75^{th} percentile) for continuous variables, and as percentages (quantity) for categorical variables. Unconditional logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to combined categories of VWF and platelet measures. The ORs were adjusted for age and sex in Model 1 to take into account the matching factors in the analyses,[27] with further adjustment for BMI, C-reactive protein (CRP), smoking, hypertension, estrogen use, self-reported history of cancer at baseline and platelet count/MPV in Model 2.

The relative excess risk due to interaction (RERI)[28] was used to evaluate biological interaction: $RERI = (OR_{AB} - 1) - (OR_A + OR_B - 2)$, where the exposures $_A$ and $_B$ represent high plasma VWF and high platelet measures, respectively. A RERI>0 indicates that the combined effects of the two exposures exceeds the sum of the individual effects, thereby suggesting biological interaction. The attributable proportion (AP), i.e. the proportion of

events in the joint exposure group that could be attributed to the biological interaction, was estimated as RERI divided by OR_{AB}.[28] The RERI and AP were presented with 95% CIs.[28]

Results

The baseline characteristics of study participants across tertiles of plasma VWF levels are shown in Table 1. The mean age and proportion with hypertension increased, whereas platelet count decreased slightly across tertiles of VWF. In the lowest tertile, history of cardiovascular disease was less frequent, while smoking was more common. Mean MPV, mean BMI, median CRP, proportion of men/women, estrogen use and history of cancer did not differ across tertiles of VWF. Median time from baseline to VTE event was 7.5 years. The characteristics of the 403 VTE events are presented in Table 2. The mean age of the patients at the time of their first VTE was 67 years, and 48% were male. DVT (62%) was more common than PE (38%), and 58% of all events were classified as provoked.

The ORs for VTE by VWF levels within each MPV stratum and by the combined categories of VWF and MPV are displayed in Table 3 and Figure 2A. In participants with low MPV (<8.5 fL), those with high VWF levels had an age- and sex-adjusted OR of 1.31 (95% CI 0.80 – 2.17) for VTE compared with those with a low VWF level. In participants with high MPV (≥9.5 fL), high VWF levels yielded an OR of 2.18 (95% CI 1.15 – 4.16) for VTE compared with those with low VWF. Compared with those with low MPV and low VWF levels, participants with high MPV and high VWF levels had an OR of 1.98 (95% CI 1.17 – 3.36) in analyses adjusted for age and sex. The risk estimates were only modestly affected by multivariable adjustment (Table 3 and Figure 2A). Interaction analyses yielded a RERI of 1.00 (95% CI -0.04 to 2.03), and by calculating the AP, we found that 48% (95% CI 15% to 96%) of the cases in the joint exposure group could be attributed to the biological interaction between VWF and MPV.

Subgroup analyses showed that the combined effect of VWF and MPV was particularly strong for unprovoked VTE events, where participants with high VWF levels and MPV had an age- and sex-adjusted OR of 6.63 (95% CI 2.89 – 15.17) compared with those with low MPV and low VWF levels. Multivariable adjusted analyses yielded similar risk estimates (Figure 2B). The RERI was 5.18 (95% CI -2.29 to 9.93), and the AP implied that 59% (95% CI 6% to 96%) of the unprovoked VTEs in the joint exposure group were attributable to this interaction. Stratified analyses further pointed towards a stronger association for DVT (Figure 2C) than for PE, where joint exposure of high VWF levels and high MPV yielded age- and sex-adjusted ORs of 2.23 (95% CI 1.21 – 4.11) for DVT and 1.61 (95% CI 0.75 – 3.46) for PE. The RERI was 0.62 (95% CI -0.50 to 1.83), and the AP suggested that 33% (95% CI -16% to 99%) of the DVT cases in the joint exposure group could be attributed to the interaction between VWF and MPV.

The ORs for VTE across combined categories of plasma VWF level and platelet count are shown in Table 4 and Figure 3A. Table 4 also depicts the ORs for VTE according to VWF levels within each stratum of platelet count. In the low platelet count stratum, a high VWF level yielded an age- and sex-adjusted OR of 1.52 (95% CI 0.95 – 2.44) compared with those with a low VWF. The corresponding OR in the high platelet count stratum was 2.77 (95% CI 1.25 – 6.14). The combination of high platelet count and plasma VWF level in the highest tertile yielded an OR of 2.91 (95% CI 1.49 – 5.67) for VTE compared with those with low platelet count and VWF level in the age- and sex-adjusted model. The ORs were hardly affected by further adjustment for BMI, CRP, smoking, hypertension, estrogen use, cancer at baseline and MPV (Table 4 and Figure 3A). Interaction analyses provided a RERI of 0.94 (95% CI -0.59 to 2.52), indicating that the combined effects of high VWF and high platelet count on VTE risk were more than additive, and the AP implied that 39% (95% CI -2% to 97%) of the events in the joint exposure group could be attributed to the interaction.

Subgroup analyses of the interaction between VWF and platelet count provided similar results as for MPV, with strongest associations for unprovoked events and DVT. The combination of a high platelet count and high VWF yielded age-and sex-adjusted ORs of 6.54 (95% CI 2.47 – 17.32) for unprovoked VTE and 3.01 (95% CI 1.45 – 6.23) for DVT, compared with those with low levels in both exposures. Multivariable adjustment did not affect the estimates substantially (Figure 3B and Figure 3C). The RERI for unprovoked VTE was 2.73 (95% CI -0.93 to 4.36), and the AP indicated that 40% (95% CI 10% to 100%) of unprovoked VTEs in those with combined high platelet count and high VWF could be attributed to the interaction. For DVT, the RERI was 1.36 (95% CI -0.68 to 3.54), and the AP implied that 56% (95% CI 8% to 100%) of all DVTs in the joint exposure group were attributable to the interaction.

Discussion

In the present population-based nested case-control study, we found that the combination of elevated plasma VWF levels and high MPV or a high platelet count had a more than additive effect on the VTE risk. In those with high MPV and high VWF, 48% of the VTE events in the joint exposure group could be attributed to the biological interaction. Similarly, 39% of the VTE events in those with high platelet count and high VWF could be attributed to the interaction. The combinations of elevated VWF levels and high platelet measures were most strongly associated with unprovoked VTE events. Our findings suggest that the presence of both high VWF levels and a high platelet count or reactivity is required to yield an increased risk of VTE, implying a co-dependency between VWF and platelet measures in the biology of the VTE risk.

MPV is a marker of platelet reactivity and functional capacity,[29] as large platelets are observed to have a higher turnover of thromboxane A2 and release larger amounts of

signaling substances upon activation.[22,30] Platelet size is primarily determined by the state of the parent megakaryocyte at the time of discharge.[31,32] In situations with enhanced platelet consumption, such as hypoxia, smoking, high BMI and immune thrombocytopenic purpura, the megakaryocytes have higher cytoplasmic volumes and release larger platelets.[22,33,34] Large and more reactive (and hemostatically active) platelets are shown to be a risk factor for both arterial[35,36] and venous[24] thrombosis.

Plasma VWF levels are shown to be associated with future risk of VTE.[11,13] VWF is acknowledged as an important protagonist of arterial thrombosis under high-flow conditions,[37] whereas the role of VWF in the causal pathway of VTE is still not fully understood. Since the discovery of the association between VWF and DVT in 1995, it has been perceived that the association is primarily explained by concurrent high levels of FVIII.[12] However, in the Longitudinal Investigation of Thromboembolism Etiology (LITE) study, VWF and FVIII were reported to be independently associated with the risk of VTE.[11] A recent MR analysis was not able to address the independent role of VWF, as no loci were found to regulate VWF independent of FVIII.[14] In addition to serving as the carrier of FVIII, VWF promotes hemostasis by interacting with platelet glycoprotein Ib-IX-V and αIIbβ3 integrin, resulting in adhesion of platelets to the endothelium and other platelets, respectively.[38] It is therefore likely to assume that VWF also is involved in FVIII-independent mechanisms in the pathogenesis of VTE.

To our knowledge, this is the first study to explore the combined effects of VWF with platelet count and platelet reactivity on VTE risk. The observation that VWF and platelet measures displayed synergism on an additive scale on the risk of VTE suggests that a biological interaction may contribute to the pathophysiology of VTE. Our results add knowledge regarding the presence and impact of this interaction in the general population. Notably, for overall VTE, VWF and platelet measures only yielded increased VTE risk when

both components were elevated. Specifically, the two variables did not merely interact to add excess risk,[39] but appeared to be dependent on each other to mediate increased risk. Thus, co-dependency, rather than synergism, may be a more fitting term for the interaction.

Similar to previous studies investigating the separate associations of VWF and MPV in relation to VTE risk,[24] we found that the combined effect was strongest for unprovoked events. This implies a contribution by VWF-platelet interaction on unprovoked events in particular.[40,41] Furthermore, the combined exposure of high VWF levels and high MPV or high platelet count resulted in a particularly strong risk increase for DVT, compared with PE. This finding is analogous with our previous study on VWF and risk of VTE,[13] and suggests that thrombi formed in the presence of high plasma levels of VWF and either many or highly reactive platelets may be more tightly anchored to the endothelium, and thus be less likely to embolize.

Evidence from mouse studies suggests an essential role of VWF-platelet interaction in the pathogenesis of VTE. First, Chauhan and colleagues found that VWF-deficient mice had impaired thrombus growth in ferric chloride-injured veins.[16] Second, Brill and colleagues reported that the VWF-platelet interaction was necessary for development of venous thrombosis in a flow-restricted model.[17] Traditionally, the increased thrombin potential by elevated FVIII levels has been thought to explain the observed VWF-associated VTE risk.[12,14] However, infusion of recombinant FVIII in VWF-deficient mice only resulted in increased thrombus stability, and did not increase thrombus formation.[16,17] Recently, Michels and colleagues found that VWF is critical in obesity-associated DVT in mice, and demonstrated that targeting the VWF-platelet interaction with antibodies or nanobodies protected against thrombogenicity.[42] Furthermore, observational studies have shown that VWF and FVIII are independent risk factors for VTE.[11,43] Taken together, growing evidence suggests that enhanced platelet reactivity contributes to the increased VTE risk observed by elevated

plasma VWF levels. On this basis, the VWF-platelet interaction may represent a promising target for VTE prevention and treatment, as for arterial thrombosis.[42,44] Several studies have been carried out on possible therapeutic approaches for VWF inhibition, but the focus has thus far been on arterial thrombosis.[45]

Platelet count is inversely correlated with MPV and bleeding time[22] but is not regarded as a useful risk factor for hemostatic dysfunction as no association is established with arterial or venous thrombotic events,[24,46,47] and both low and high platelet count is associated with increased mortality and risk of bleeding events in the general population.[48-52] However, the risk of VTE was substantially increased in subjects with high platelet count and concomitant high VWF, suggesting that the VWF-platelet interaction is important for thrombogenesis also in the presence of a high platelet count.

The strengths of this study include the population-based nested case-control design with a large population of cases and controls sampled from the same parent cohort with close follow-up. Due to the prospective design, we were able to gain insight into the temporal sequences of the associations. The study also has some limitations that require attention. Guidelines recommend collecting blood into tubes containing citrate as anticoagulant to measure VWF levels,[53] but in the present study, only plasma obtained from samples collected into EDTA was available. Of note, previous studies comprising healthy volunteers suggested a strong positive correlation between VWF antigen levels measured in EDTA and citrate plasma.[54,55] Even though participants from this population-based nested case-control study were not necessarily healthy at baseline, it is unlikely that they would have clinically relevant coagulation disorders. Still, because blood samples were collected into EDTA in both cases and controls, any discrepancy between true and measured levels of VWF would be non-differential in relation to VTE status. In addition, blood samples were stored for more than 20 years between baseline sampling and measurement of VWF and subjected to one additional

freeze thaw cycle before assessment of VWF, which could have introduced a discrepancy between true and measured levels. However, the samples were stored in the same way, for the same duration and subjected to the same number of freeze thaw cycles in cases and controls, and potential alterations would be non-differential with regards to VTE status, thereby introducing a possibility for regression dilution bias and a weakening of the results compared to the true associations. Interaction analyses have inherent statistical limitations since they require dividing the study population into smaller groups.[56] Thus, our results on measures of biological interaction and their 95% CIs should be interpreted with caution, especially for the VTE subgroups. The majority of participants in this study were Caucasians, and we therefore encourage caution when extrapolating these findings to individuals of other ethnicities.

In conclusion, we found a synergistic effect of elevated plasma VWF levels and high MPV on the VTE risk, which was most prominent for unprovoked events. High platelet count also yielded increased risk of VTE when VWF levels were high. Our findings suggest that the presence of both high VWF levels and a high platelet count or reactivity is required to yield an increased risk of VTE. Further research is necessary to disentangle the mechanisms of interaction between VWF and platelet number and reactivity, and explore potential targets for VTE prevention and treatment.

Authorship Contributions

Contribution: M.S.E. contributed to statistical analysis, interpreted data and drafted the manuscript. K.H., S.K.B and L.H.E. contributed to statistical analysis, interpreted data and revised the manuscript. E-S.H. and V.M.M. interpreted data and revised the manuscript. T.U. and P.A. performed the laboratory analysis, interpreted data and revised the manuscript. J-B.H. conceived and designed the study, interpreted data and revised the manuscript.

Conflict of Interest Disclosures

The authors declare no competing financial interests.

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Tables

Table 1 Distribution of baseline characteristics according to tertiles of plasma levels of von Willebrand Factor (VWF).

	Plasma VWF, %		
	Tertile 1 < 90.4	Tertile 2 90.4 – 100.9	Tertile 3 ≥ 100.9
n	387	410	422
Platelet count, 10^9 L^{-1}	251 ± 54	245 ± 53	238 ± 55
MPV, fL	8.8 ± 0.9	8.8 ± 0.9	8.9 ± 1.1
Age, years	57.0 ± 14.3	60.0 ± 13.7	63.4 ± 12.9
Sex, male	47.8 (185)	47.3 (194)	45.7 (193)
Body mass index, kg/m^2	25.8 ± 3.9	26.6 ± 4.3	26.8 ± 4.6
CRP, mg/L	1.18 (0.65-2.23)	1.14 (0.63-1.89)	1.31 (0.65-2.33)
Hypertension ∫	49.2 (190)	56.5 (231)	60.7 (256)
Cancer †	5.0 (16)	6.6 (21)	5.4 (17)
CVD †	12.9 (50)	17.3 (71)	18.0 (76)
Estrogen use ‡	4.7 (18)	5.1 (21)	4.3 (18)
Smoking ‡	41.1 (159)	25.1 (103)	26.1 (110)

MPV, mean platelet volume; CRP, C-reactive protein; CVD, cardiovascular disease.
Continuous variables are shown as mean ± standard deviation or median (25^{th} – 75^{th} percentile).
Categorical variables are shown as percentages (quantity).
∫ Defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg.
† Self-reported history of cancer, or myocardial infarction, angina or stroke at baseline.
‡ Self-reported daily use of oral contraceptives or hormonal replacement therapy (estrogen use), or smoking of cigarettes or cigars (smoking).

Table 2 Characteristics of the venous thromboembolism (VTE) events (n=403).

Characteristics	
Age at VTE, years	67.4 ± 13.7
Sex, male	48.1 (194)
Deep vein thrombosis	62.3 (251)
Pulmonary embolism	37.7 (152)
Unprovoked VTE	41.9 (169)
Provoked VTE	58.1 (234)
Surgery/trauma	22.3 (90)
Acute medical condition	15.6 (63)
Active cancer	21.8 (88)
Immobilization	18.1 (73)
Other*	4.0 (16)

Age is presented as mean ± standard deviation.
Categorical variables are presented as percentages (quantity).
* Other factor specified by treating physician to have provoked the event (e.g. intravascular catheter).

Table 3 Odds ratios (OR) with 95% confidence intervals (CI) for venous thromboembolism (VTE) across tertiles (T) of plasma von Willebrand Factor (VWF) and categories of mean platelet volume (MPV).

				Within stratum		Combined effects	
MPV, fL	**VWF, %**	**Controls n = 816**	**Cases n = 403**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**
<8.5		314	148				
	T1	112	46	Ref	Ref	Ref	Ref
	T2	106	54	1.27 (0.79-2.05)	1.42 (0.81-2.48)	1.25 (0.78-2.01)	1.32 (0.77-2.27)
	T3	96	48	1.31 (0.80-2.17)	1.40 (0.77-2.54)	1.24 (0.76-2.03)	1.25 (0.70-2.24)
	P for trend			*0.3*	*0.3*		
8.5 - 9.5		324	163				
	T1	99	47	Ref	Ref	1.16 (0.71-1.90)	1.16 (0.67-2.02)
	T2	107	57	1.12 (0.70-1.81)	1.33 (0.77-2.27	1.31 (0.82-2.11)	1.48 (0.85-2.58)
	T3	118	59	1.05 (0.65-1.69)	0.97 (0.57-1.65)	1.24 (0.77-1.98)	1.11 (0.64-1.91)
	P for trend			*0.9*	*0.9*		
≥9.5		178	92				
	T1	62	21	Ref	Ref	0.84 (0.46-1.53)	0.85 (0.42-1.72)
	T2	60	26	1.24 (0.63-2.45)	1.08 (0.49-2.41)	1.07 (0.60-1.90)	0.95 (0.48-1.90)
	T3	56	45	2.18 (1.15-4.16)	2.16 (1.01-4.60)	1.98 (1.17-3.36)	2.10 (1.11-3.98)
	P for trend			*0.014*	*0.041*		

Model 1: Adjusted for age and sex.

Model 2: Adjusted for age, sex, body mass index, C-reactive protein, smoking, hypertension, estrogen use, self-reported history of cancer at baseline and platelet count.

Table 4 Odds ratios (OR) with 95% confidence intervals (CI) for venous thromboembolism (VTE) across tertiles (T) of plasma von Willebrand Factor (VWF) and categories of platelet count.

				Within stratum		Combined effects	
Platelet count, 10^9 L^{-1}	**VWF, %**	**Controls n = 816**	**Cases n = 403**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**
<230		349	176				
	T1	112	41	Ref	Ref	Ref	Ref
	T2	112	64	1.56 (0.97-2.51)	1.44 (0.84-2.45)	1.57 (0.98-2.52)	1.52 (0.90-2.59)
	T3	125	71	1.52 (0.95-2.44)	1.13 (0.66-1.95)	1.56 (0.98-2.49)	1.28 (0.76-2.17)
	P for trend			*0.1*	*0.7*		
230-299		349	157				
	T1	111	49	Ref	Ref	1.22 (0.74-1.99)	1.04 (0.60-1.82)
	T2	117	52	1.01 (0.63-1.61)	1.06 (0.62-1.83)	1.22 (0.75-1.99)	1.09 (0.63-1.90)
	T3	121	56	1.03 (0.64-1.64)	1.17 (0.69-2.00)	1.29 (0.80-2.08)	1.23 (0.71-2.10)
	P for trend			*0.9*	*0.6*		
≥300		118	70				
	T1	50	24	Ref	Ref	1.30 (0.71-2.39)	1.18 (0.59-2.33)
	T2	44	21	1.09 (0.53-2.27)	1.30 (0.54-3.13)	1.32 (0.70-2.48)	1.34 (0.64-2.83)
	T3	24	25	2.77 (1.25-6.14)	2.52 (0.95-6.69)	2.91 (1.49-5.67)	2.40 (1.08-5-34)
	P for trend			*0.017*	*0.073*		

Model 1: Adjusted for age and sex.
Model 2: Adjusted for age, sex, body mass index, C-reactive protein, smoking, hypertension, estrogen use, self-reported history of cancer at baseline and mean platelet volume.

Figure legends

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

Figure 2. Forest plot of multivariable analyses, with odds ratios (ORs) and 95% confidence intervals (CIs) for overall venous thromboembolism (VTE) (2A), unprovoked VTE (2B) and deep vein thrombosis (2C) by categories of von Willebrand Factor (VWF) and mean platelet volume (MPV). The group with VWF level in the lowest tertile (T) and low MPV (<8.5 fL) is set as reference. ORs adjusted for age, sex, body mass index, C-reactive protein, smoking, hypertension, estrogen use, self-reported history of cancer at baseline, and platelet count.

Figure 3. Forest plot of multivariable analyses, with odds ratios (ORs) and 95% confidence intervals (CIs) for overall venous thromboembolism (VTE) (3A), unprovoked VTE (3B) and deep vein thrombosis (3C) by categories of von Willebrand Factor (VWF) and platelet count. The group with VWF level in the lowest tertile (T) and low platelet count ($<230 \cdot 10^9$ L^{-1}) is set as reference. ORs adjusted for age, sex, body mass index, C-reactive protein, smoking, hypertension, estrogen use, self-reported history of cancer at baseline, and mean platelet volume.

Figure 1

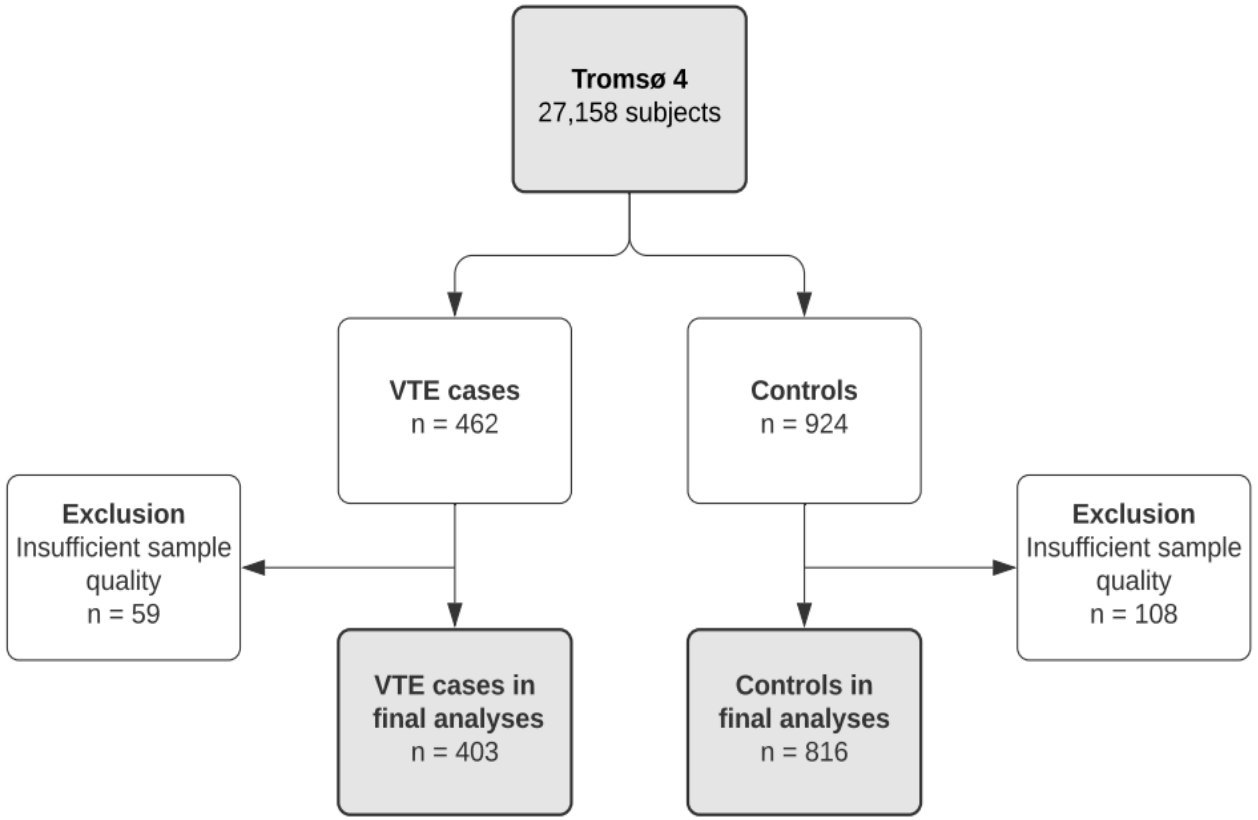

Tromsø 4
27,158 subjects
VTE cases
n = 462
Controls
n = 924
Exclusion
Insufficient sample quality
n = 59
Exclusion
Insufficient sample quality
n = 108
VTE cases in final analyses
n = 403
Controls in final analyses
n = 816

Figure 2

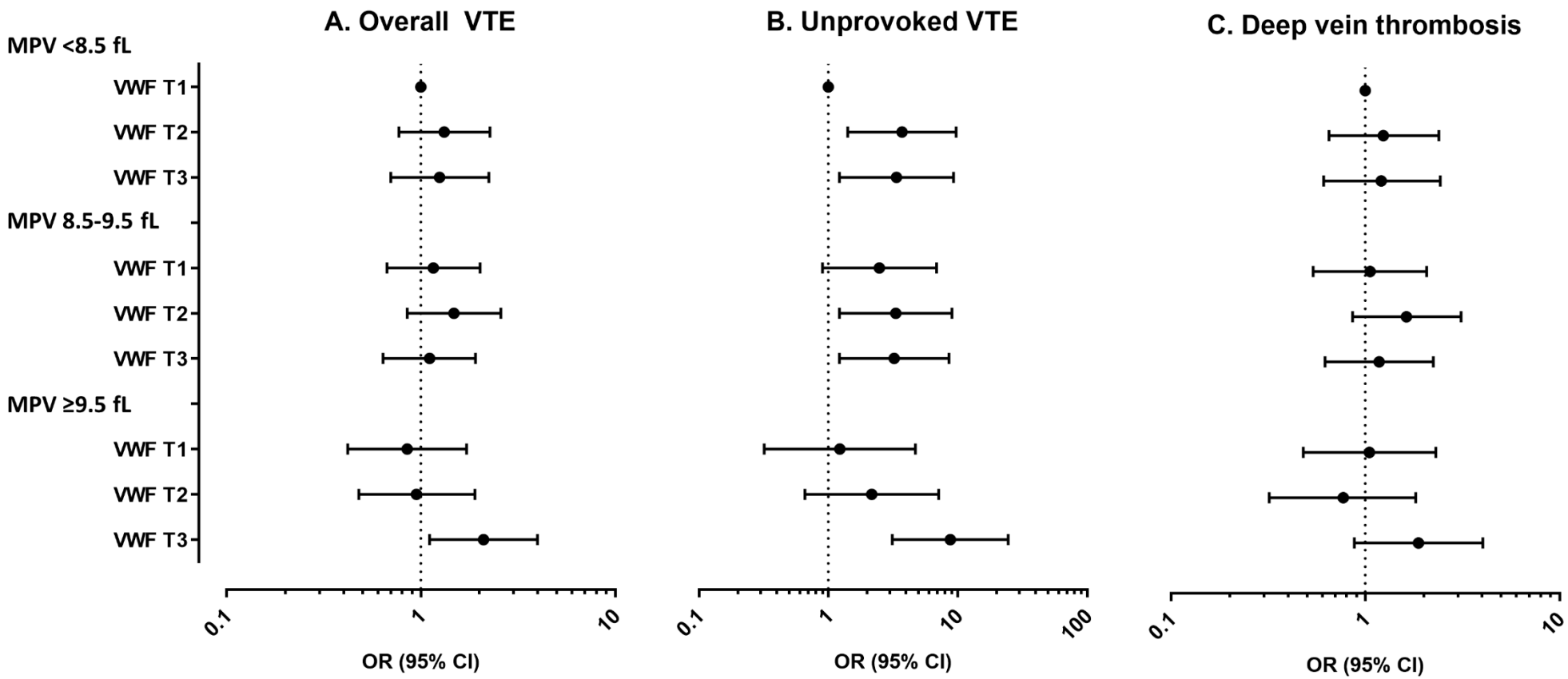
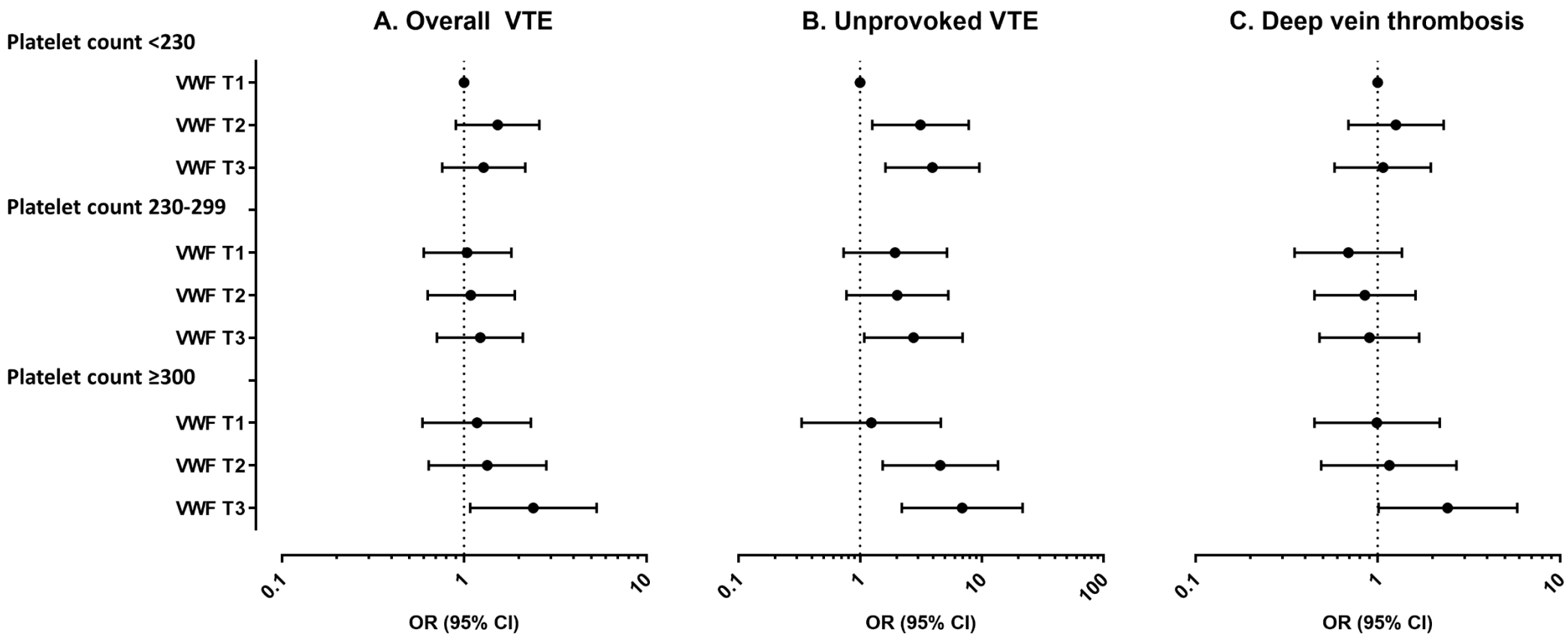

A. Overall VTE
B. Unprovoked VTE
C. Deep vein thrombosis
MPV <8.5 fL
VWF T1
VWF T2
VWF T3
MPV 8.5-9.5 fL
VWF T1
VWF T2
VWF T3
MPV ≥9.5 fL
VWF T1
VWF T2
VWF T3
0.1
1
10
100
OR (95% CI)

Figure 3


A. Overall VTE
B. Unprovoked VTE
C. Deep vein thrombosis
Platelet count <230
VWF T1
VWF T2
VWF T3
Platelet count 230-299
VWF T1
VWF T2
VWF T3
Platelet count ≥300
VWF T1
VWF T2
VWF T3
0.1
1
10
100
OR (95% CI)