Combined effect of high factor VIII levels and high mean platelet volume on the risk of future incident venous thromboembolism

Short title: Factor VIII, mean platelet volume and venous thromboembolism

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ESSENTIALS

- How high FVIII combined with large platelets affects venous thromboembolism (VTE) risk is unknown
- This combined effect was investigated in a population-based nested case-control study
- High FVIII plasma levels were robustly associated with increased risk of future VTE
- High FVIII levels combined with large platelets resulted in a supra-additive effect on VTE risk

ABSTRACT

Background: High factor VIII (FVIII) levels and large platelets, as reflected by a high mean platelet volume (MPV), are separately associated with increased risk of venous thromboembolism (VTE). Whether the combination of high FVIII and large platelets has supra-additive effect on VTE risk is unknown.

Objective: We aimed to investigate the joint effect of high FVIII levels and large platelets, as reflected by high MPV, on the risk of future incident VTE.

Methods: A population-based nested case-control study with 365 incident VTE cases and 710 controls was derived from the Tromsø Study. FVIII antigen levels and MPV were measured in blood samples drawn at baseline. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated across FVIII tertiles (<85%, 85-108%, ≥108%) and within predefined MPV strata (<8.5, 8.5-9.5, 9.5 fL).

Results: The VTE risk increased linearly across FVIII tertiles (P_{trend}<0.001) in models adjusted for age, sex, body mass index and C-reactive protein. In the combined analysis, participants with FVIII levels in the highest tertile and MPV≥9.5 fL (i.e., joint exposure) had an OR for VTE of 2.71 (95% CI 1.44-5.11) compared with those with FVIII in the lowest tertile and MPV<8.5 fL (reference). In the joint exposure group, 52% (95% CI 17-88%) of VTEs were attributable to the biological interaction between FVIII and MPV.

Conclusion: Our results suggest that large platelets, as reflected by high MPV, might play a role in the mechanism by which high FVIII increases the risk of incident VTE.

KEYWORDS: factor VIII, mean platelet volume, interaction, deep vein thrombosis, venous thromboembolism

INTRODUCTION

Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease associated with serious short- and long-term complications, including post-thrombotic syndrome, post-PE syndrome, recurrence and death [1-3]. Effective prevention and subsequent reduction of VTE burden in the general population are dependent on expanded clarification of risk factors and molecular disease mechanisms. However, up to 50% of incident VTE events occur in the absence of any recognized predisposing factor [1, 4], implying that there are still major knowledge gaps on the pathophysiological mechanisms of the disease.

During the past decades, a substantial body of evidence has implicated that coagulation factor VIII (FVIII) is a key component in the pathogenesis of VTE [5-7]. FVIII is a plasma sialoglycoprotein that plays an essential role in hemostasis. Upon cleavage by thrombin, activated FVIII dissociates from its carrier protein von Willebrand factor (VWF) and acts as a cofactor for activated FIX on the surface of negatively-charged phospholipid membranes provided mainly by platelets, leading to accelerated thrombin generation [8]. The association between FVIII and VTE in epidemiological studies was first recognized by Koster *et al*. in 1995, who reported that high FVIII levels were associated with increased VTE risk in a dose-response manner [9]. In the following years, an increasing number of studies, mostly with a case-control design, has supported an association between high FVIII levels and increased risk of a first lifetime VTE [10-14]. Still, only a few studies have prospectively evaluated this relationship in the general population [15-17]. The role of FVIII in venous thrombosis is further reinforced by animal models, which have shown that elevated FVIII levels enhance thrombus formation and stabilization [5-7].

Although the association between FVIII levels and VTE is well-established, the mechanisms underlying this association are not fully understood, especially regarding to what extent other components of the hemostatic system modify the VTE risk in the presence of high FVIII. One of the possible components are platelets. Large platelets, as reflected by a high mean platelet volume (MPV), are associated with increased risk of incident VTE [18]. *In vitro*, large platelets display increased reactivity and are more prone to expose phosphatidylserine on their membrane [19-21]. As the exposure of phosphatidylserine upon platelet activation is a fundamental step for activated FVIII and FIX to form the tenase complex that facilitates thrombin generation [8], we hypothesized that the combination of high FVIII levels and large platelets could have a supra-additive effect on VTE risk due to biological interaction. To examine this hypothesis, we first assessed the association between FVIII plasma levels and risk of future incident VTE in a nested case-control study derived from the general population, and secondly investigated the combined effect of high FVIII levels and large platelets, as reflected by a high MPV, on the risk of VTE.

METHODS

Study population and study design

The Tromsø Study is a population-based cohort, with repeated health surveys of inhabitants in Tromsø, Norway [22]. All inhabitants aged ≥25 years living in the Tromsø municipality were invited to participate in the fourth survey (Tromsø 4, 1994-1995). A total of 27,158 individuals participated (77% of those invited) and were followed from the date of inclusion until an incident VTE, migration, death, or end of follow-up (September 1, 2007), whichever came first. All first lifetime VTE events occurring during follow-up were identified using the hospital discharge diagnosis, autopsy and radiology procedure registries from the University

Hospital of North Norway (UNN), which is the only hospital in the Tromsø region. Trained personnel confirmed and recorded each VTE event by extensive review of medical records. A VTE was confirmed if there were signs and symptoms of PE or DVT combined with objective confirmation by radiological procedures, which resulted in treatment initiation [23]. A VTE event was further classified as provoked or unprovoked based on provoking factors closely preceding the VTE diagnosis. A provoked VTE was defined as an event occurring in the presence of one or more of the following provoking factors: trauma, surgery or acute medical conditions (acute ischemic stroke, acute myocardial infarction, or acute infection) within 8 weeks before the event, active cancer at the time of VTE diagnosis, immobilization (bed rest for longer than 3 days, confinement to a wheelchair within the past 8 weeks, or long distance travel of 4 hours or longer within the past 14 days), or other factors specifically described as provoking by a physician (e.g. intravascular catheter).

We created a nested-case control study derived from the Tromsø 4 cohort for the assessment of biological variables from stored blood samples that were obtained at cohort inclusion, as previously described [24, 25]. Briefly, during the follow-up period (1994-2007), 462 individuals experienced an incident VTE. For each case, two age- and sex-matched controls were randomly sampled from the parent cohort, who were alive at the index date of the VTE event (n=924). From this population, 97 cases and 214 controls were excluded because plasma samples were not available or were of insufficient quality for the analyses. Therefore, 365 VTE cases and 710 controls were included in the final analysis (Fig. 1). The regional committee for medical and health research ethics approved the study, and all participants provided written informed consent.

Baseline measurements and blood sampling

Height (to the nearest cm) and weight (to the nearest 0.5 kg) were measured in participants wearing light clothing and no shoes [23]. Body mass index (BMI) was calculated as weight divided by the square of height in meters (kg/m^2). A self-administered questionnaire was used to collect a detailed history of previous cancer and arterial cardiovascular disease (CVD) events (i.e., stroke, angina pectoris, transient ischemic attack, and myocardial infarction).

Procedures for blood collection and storage of blood products have been previously described elsewhere [24, 25]. In brief, at baseline inclusion in 1994-1995 (Tromsø 4), non-fasting blood was collected from an antecubital vein into 5-mL vacutainers (Becton Dickinson, Le Pont de Claix, France) containing EDTA (K_3-EDTA 40 µL, 0.37 mol/L per tube) as an anticoagulant. Platelet-poor plasma was prepared by centrifugation at 3000*g* for 10 minutes at room temperature, after which the supernatant was transferred into cryovials (Greiner Bio-One, Nürtingen, Germany) in 1-mL aliquots and stored at -80°C until further analysis.

Laboratory analyses

Platelet parameters (platelet count and MPV) were analyzed within 12 hours of blood sampling, using an automated blood cell counter (Coulter Counter; coulter Electronics, Luton, UK) [18]. Measurement of FVIII and C-reactive protein (CRP) were performed at the Research Institute of Internal Medicine at Oslo University Hospital, Rikshospitalet. Platelet-poor plasma samples were thawed at 37°C in a water bath for 5 minutes and prepared for analyses by centrifugation at 13500g for 2 minutes in order to obtain platelet-free plasma [25].

Plasma levels of FVIII antigen were measured by enzyme immuno assay (EIA) with matched antibodies from Affinity Biologicals (Ancaster, Ontario, Canada). EIA was performed

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). FVIII intra- and inter-assay coefficients of variation were <10%. The mean value of FVIII in the control population was set to 100%, and all other values were adjusted accordingly and expressed in percentages. High-sensitivity CRP was measured by EIA, as previously described [26].

Statistical analyses

Association between plasma factor VIII levels and risk of future VTE

Statistical analyses were carried out using Stata version 16 (StataCorp LLC, Texas, USA) and R version 4.0.5 (The R Foundation for Statistical Computing, Vienna, Austria). Participants were categorized according to tertiles of plasma FVIII antigen levels, with cut-offs determined in the control population (<85%, 85-108%, ≥108%). Baseline characteristics across FVIII tertiles were expressed as proportions for categorical variables, and as mean (± standard deviation [SD]) or median (25^{th}-75^{th} percentiles) for continuous variables using descriptive statistics.

Unconditional logistic regression was used to calculate odds ratios (ORs) for VTE with 95% confidence intervals (CIs) according to tertiles of FVIII, and the lowest tertile served as the reference. We performed analyses for overall VTE and for subgroups according to VTE location (DVT or PE), and presence of provoking factors (provoked or unprovoked). The P value for linear trend of VTE risk was estimated across increasing tertiles of FVIII levels. The association between FVIII levels and VTE was adjusted for age and sex in a first model to take into account the matching variables in the analyses [27]. BMI and inflammation, as reflected by high-sensitivity CRP, can influence both FVIII levels [28, 29] and VTE risk [30, 31], thereby

acting as potential confounders in the association between FVIII and VTE. We therefore added BMI and high-sensitivity CRP to a second model. We chose not to adjust plasma FVIII for VWF because the levels of these factors are known to be closely related, as they circulate in a tight non-covalent complex and can be considered as one entity [32]. Further, in this scenario, potential errors in the measured levels could have a substantial impact on the adjustment, limiting the ability to draw reliable conclusions [33]. Because cancer and arterial CVD have been reported to be associated with both FVIII levels [34, 35] and VTE risk [36-38] we performed sensitivity analyses for overall VTE after excluding participants with self-reported history of cancer or arterial CVD at cohort baseline. In all analyses, plasma FVIII was also entered into the logistic regression models as a continuous variable. For this purpose, we calculated the mean and SD of FVIII levels based on the distribution of the control population. The ORs for VTE were assessed by 1SD increase in FVIII levels.

Results based only on baseline measurement of FVIII could be affected by regression dilution bias due to the long follow-up time in the parent cohort [39]. To address this, we took into account the time elapsed between blood sampling at baseline (i.e., when samples to measure FVIII were drawn) and the occurrence of VTE events. We performed analyses that restricted the maximum follow-up time for the VTE cases, 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 adjusted for age, sex, BMI, and CRP (model 2) were generated at every time point a new VTE occurred and plotted as a function of this maximum time.

Combined effect between plasma FVIII levels and MPV on the risk of future VTE

To investigate the combined effect between plasma FVIII antigen levels and MPV, categories of MPV were conceived based on cut-offs at 8.5 and 9.5 fL, according to our previous study

on platelets and VTE risk [18]. The 3-level variables of FVIII (<85%, 85-108%, ≥108%) and MPV (<8.5, 8.5-9.5, 9.5 fL) were combined to yield a 9-level variable; subjects with both low FVIII levels (<85%) and low MPV (<8.5 fL) served as the reference group. Unconditional logistic regression was used to calculate ORs for overall VTE according to combined categories of FVIII and MPV, using the aforementioned adjustment models but with the addition of platelet count to the second model. The presence of interaction on an additive scale between FVIII and MPV was assessed by calculating the following measures of biological interaction: the relative excess risk due to interaction (RERI), and the attributable proportion (AP) due to interaction with corresponding 95% CIs [40]. The RERI can be explained as the part of the total effect on the outcome that is attributable to the interaction, and the AP as the proportion of cases in the joint exposure group that is due to the interaction between the two exposures. RERI and AP > 0 indicate positive interaction or more than additivity, meaning that the effect of the joint exposure to the two risk factors is greater than the sum of the separate effects [41].

RESULTS

The distribution of baseline characteristics across tertiles of plasma FVIII antigen levels in the study population is shown in Table 1. The mean age and BMI, and the median levels of CRP increased across FVIII tertiles, while the mean MPV did not substantially differ according to FVIII tertiles. The characteristics of the VTE patients are shown in Table 2. The mean age at the time of VTE was 67 years, 48.5% were men, 63.3% of the events were DVTs and 59.5% were provoked VTEs.

Association between plasma factor VIII levels and risk of future VTE

The ORs for overall VTE and subgroups (i.e., DVT, PE, and provoked and unprovoked VTE) according to tertiles of plasma FVIII antigen levels are shown in Table 3. The ORs for VTE increased linearly across FVIII tertiles in the age- and sex-adjusted model (P_{trend}<0.001). Participants with FVIII levels in the highest tertile had a 2.1-fold higher OR for VTE (OR 2.13, 95% CI 1.53-2.98) compared with those with FVIII in the lowest tertile. A dose-response relationship between FVIII levels and thrombosis risk was also observed for the VTE subgroups, except for PE. Of note, FVIII levels were more strongly associated with risk of DVT (OR for highest vs. lowest tertile: 2.63, 95% CI 1.77-3.92) than with risk of PE (OR for highest vs. lowest tertile: 1.46, 95% CI 0.89-2.39). For overall and subgroup analyses, further adjustment for BMI and CRP had a minor impact on risk estimates. The thrombosis risk by 1SD increase in FVIII levels was in line with the tertile-based analysis, with the strongest association being for DVT (Table 3). In the sensitivity analyses, exclusion of participants with self-reported history of arterial CVD (Supplemental Table 1) or cancer (Supplemental Table 2) at baseline yielded results similar to those obtained in the main analysis.

To investigate the possibility of underestimating the true association due to regression dilution bias, we considered the time elapsed between the blood sampling at baseline and the occurrence of VTE events, estimating ORs for VTE among participants with high (highest tertile) versus low (lowest tertile) FVIII levels. As shown in Fig. 2, the ORs for overall VTE by high levels of FVIII were higher with shortened time between blood sampling and VTE events, with risk estimates being especially high during the first 4 years after blood sampling. However, along the entire study period (≥ than 12 years for many individuals), FVIII levels remained significantly associated with VTE.

Combined effect between plasma FVIII levels and MPV on the risk of future VTE

The ORs for VTE according to plasma levels of FVIII within each stratum of MPV are described in Table 4. In each MPV stratum, there was a dose-response relationship between FVIII levels and VTE risk. It is worth noting that the highest estimates were observed in the highest MPV stratum (≥9.5 fL), where subjects with FVIII levels in the highest tertile had an OR for VTE of 3.42 (95% CI 1.60-7.30) compared with those with FVIII in the lowest tertile in age- and sex- adjusted analysis, with only a slight change in the OR after further adjustment for BMI, CRP and platelet count (OR 3.74, 95% CI 1.69-8.29).

As shown in Table 4, participants in the combined high-high category (i.e., FVIII in the highest tertile and MPV ≥9.5 fL) had an OR for VTE of 2.77 (95% CI 1.52-5.03) compared with those in the combined low-low category (i.e., FVIII in the lowest tertile and MPV <8.5 fL) in analysis adjusted for age and sex. Additional adjustment for BMI, CRP and platelet count had a minor impact on the risk estimate (OR 2.71, 95% CI 1.44-5.11). The combined exposure to high FVIII and high MPV had a supra-additive effect on VTE risk, with a RERI of 1.42 (95% CI 0.01-2.85) in the fully adjusted analyses. The estimation of AP revealed that 52% (95% CI 17%-88%) of the VTE events in the joint exposure group could be attributed to the biological interaction between FVIII and MPV. The combined effect of high factor VIII levels and high MPV on VTE risk did not seem to be driven by platelet count, as adjustment for platelet count did not affect the ORs. Moreover, the combination of high FVIII and high platelet count (≥300 x 10^9/L) had no supra-additive effect on VTE risk, with RERI and AP estimates around zero (Supplemental Table 3).

DISCUSSION

In this population-based nested case-control study, FVIII levels were linearly associated with risk of future incident VTE in analyses adjusted for age and sex, and the association was

particularly strong for DVT. The risk estimates were only slightly attenuated after further adjustment for BMI and CRP. Although the ORs for VTE were higher with shortened time between blood sampling and the thrombotic events, the association between high FVIII levels and VTE remained significant even several years after blood sampling. Furthermore, high FVIII levels combined with high MPV displayed a supra-additive effect on VTE risk. In the joint exposure group, 52% of the VTE events could be attributed to the biological interaction between high FVIII and high MPV. Our results indicate that plasma FVIII levels are robustly associated with risk of future VTE in the general population and suggest that large platelets, as reflected by a high MPV, might play a role in the mechanism by which high FVIII increases thrombosis risk.

Although there are several case-control studies addressing the association between FVIII and VTE [9-14, 42], only a few reports have prospectively evaluated this relationship in the general population [15-17]. In the Longitudinal Investigation of Thromboembolism Etiology study, a cohort with 19237 participants, of whom 159 experienced a VTE event during a median follow-up time of 7.8 years, authors observed a linear relationship between FVIII levels and VTE risk in models adjusted for age, sex, race, BMI, diabetes, factor VII and VWF [15]. Results from a case-cohort study derived from the Reasons for Geographic and Racial Differences in Stroke study [16] and from a cohort (Multi-Ethnic Study of Atherosclerosis) [17] also showed that high FVIII plasma levels were associated with increased risk of incident VTE in analyses adjusted for several demographics and comorbidities.

In the present study, we confirmed the previous findings on the prospective association between high FVIII levels and risk of incident VTE in the general population [15-17]. Moreover, our results are consistent with the linear relationship between FVIII levels

and VTE risk observed in several reports [9, 13, 17]. Because FVIII levels were dose-dependently associated with VTE risk in the present study, it was not surprising that FVIII levels were associated with increased risk of VTE even within the normal range, e.g., at percentiles 33.3th (85%) and 66.6th (108%) of the distribution of the controls. In the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, a large population-based case-control study, participants with FVIII antigen levels in the 25-50th percentile of the control distribution (versus those with FVIII < 25th percentile) were already at a significant increased risk of VTE [13]. In the MEGA study, the VTE risk increased linearly up to the extreme levels of FVIII (i.e., > 99th percentile). Unfortunately, due to limited statistical power, the assessment of the association between extreme levels of FVIII and VTE risk was not feasible in our study. As FVIII is an acute phase protein [43], it is crucial to take inflammation into account when studying the association between FVIII and VTE. Here, we demonstrated that the association was not explained by inflammation, as adjustment for high-sensitivity CRP had minor impact on risk estimates. Another relevant feature of our study is the fact that although the ORs for VTE by increased FVIII levels were higher with shortened time between blood sampling and VTE events (Figure 2), the strength of the association between plasma FVIII and VTE risk remained substantial even several years after blood sampling. Importantly, results from a Mendelian randomization study advocated a causal relationship between FVIII levels and VTE [44]. Taken together, these findings highlight that FVIII not only behaves as a robust short- and long-term biomarker of VTE risk in the general population but may also be causally related to VTE.

Interestingly, the association between plasma FVIII levels and VTE was mostly driven by the relationship with DVT, which is in agreement with results from a case-control study [13], but not reported in previous prospective studies [15-17]. In a mouse model of venous

thrombosis [5], authors observed increased embolization in VWF-deficient mice as compared with wild-type mice. This was due to reduced FVIII levels, since infusion of recombinant FVIII in VWF-deficient mice resulted in thrombus stabilization, with significantly less embolization. The critical role that activated FVIII plays for an efficient propagation of the coagulation system and thrombin generation [8] is a plausible mechanism by which FVIII promotes thrombus stabilization, thus predominantly affecting DVT risk. It is worth noting that factor V Leiden (FVL) is also associated with a higher risk of DVT than of PE, a phenomenon known as the FVL paradox [45]. According to experimental data in mice, FVL carriers develop larger and more stable thrombi that are less likely to embolize compared with wild-type mice [46]. Because FVIII increases activated protein C (APC) resistance [47], the same phenotype associated with FVL, FVIII may also contribute to thrombus stabilization through APC resistance.

To our knowledge, we are the first to investigate the combined effect of FVIII and MPV on VTE risk. Exposure to both high FVIII levels and high MPV resulted in a supra-additive effect on VTE risk, with 52% of the VTEs in the combined exposure group being attributed to the biological interaction between the two exposures. Our findings suggest that the risk of VTE conferred by high FVIII levels synergistically increases in the presence of large platelets, as determined by a high MPV, which have been shown to be more hemostatically active than small platelets [19-21]. Indeed, upon stimulation, large platelets are more prone to expose negatively-charged phospholipids (phosphatidylserine in particular) on their membranes compared with small platelets [20]. Hence, large platelets may provide a more efficient surface for the assembly of activated FVIII and FIX, accelerating FX activation and subsequent thrombin generation [8, 48].

As FVIII circulates in a tight non-covalent complex with VWF [32], it might be argued that the interaction between high FVIII and high MPV could have been mainly driven by plasma VWF. We previously reported that high plasma VWF levels had a synergistic effect on VTE risk, not only in the presence of high MPV, but also in the presence of high platelet count [49]. If the synergistic effect between FVIII and MPV on VTE risk were due to VWF, the combination of FVIII and platelet count would be expected to have a similar impact on thrombosis risk. However, there was no biological interaction between high FVIII and high platelet count (RERI and AP estimates were close to zero), making VWF an unlikely explanation for the synergism that we found between FVIII and MPV in relation to VTE risk. In light of these findings, one might speculate that the interaction between high FVIII and high MPV could be due to an increased exposure of negatively-charged phospholipids on the surface of large platelets that would accelerate thrombin generation in the presence of high FVIII levels, ultimately resulting in a prothrombotic state and increased risk of VTE. However, it is important to address that because plasma levels of FVIII and VWF are closely related [32], any epidemiological approach would be insufficient to reliably disentangle the effect of FVIII from VWF (and vice-versa) on thrombosis risk. Experimental models of venous thrombosis conceived to fully disentangle the effect of these coagulation factors would be warranted to reveal to what extent high FVIII in combination with large platelets would affect thrombogenesis independent of VWF. Finally, platelet size, as reflected by MPV, is reported to have a strong genetic component [50, 51] and be relatively stable within an individual over time [52, 53]. Whether the identification of subjects with high FVIII levels and high MPV could be useful for risk stratification and targeted VTE prevention, particularly in high risk situations for developing a VTE, is a further open question worth pursuing.

The main strengths of our study include the nested case-control study design, where the VTE cases and controls were selected from the same source population (i.e., the Tromsø study cohort), thus mitigating the likelihood of selection bias. Because of the prospective design, where samples used to measure plasma FVIII levels and platelet parameters were collected at cohort baseline, we could make assumptions on the temporal sequence between exposure and outcome. Some limitations of this study merit attention. Although the number of plasma samples not available or of inadequate quality for the assessment of FVIII antigen levels was somewhat high, missing data on FVIII did not seem to be related to the VTE status, occurring in 21% of the VTE cases and 23% of the controls. Additionally, relevant baseline characteristics were similar for study participants with and without measurement of FVIII (data not shown). Thus, the missing data on FVIII was presumably completely at random. Blood samples were drawn in 1994-1995 and stored for more than 20 years before analyses, and this could potentially have affected FVIII levels. However, because blood samples were stored in the same way and for the same duration in cases and controls, any potential misclassification would be non-differential with regards to VTE status, thereby introducing a possibility for underestimation of the true associations. Finally, prior studies have predominantly assessed FVIII activity, while in the present study FVIII antigen levels were investigated, since we only had stored EDTA plasma samples available in the parent cohort. It is important to note that FVIII activity and FVIII antigen levels have shown similar impact on VTE risk in previous studies [13, 54].

In conclusion, increasing levels of plasma FVIII were linearly associated with increased risk of future incident VTE, and the association was particularly strong for DVT. We found a supra-additive effect of high FVIII levels and high MPV on the risk of VTE. Our findings suggest that large platelets, as reflected by a high MPV, interact biologically with high FVIII

levels, and play a role in the pathophysiological mechanism by which FVIII increases VTE risk. Future studies are needed to confirm our findings and unravel the mechanisms that underlie the interaction between large platelets and FVIII.

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AUTHORSHIP CONTRIBUTIONS

E-S. Hansen analyzed data, interpreted the results, and drafted the manuscript. M.S. Edvardsen interpreted the results and revised the manuscript. P. Aukrust and T. Ueland performed the laboratory analysis, interpreted the results, and revised the manuscript. J-B. Hansen and 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. All authors reviewed and approved the final version of the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

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

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TABLES

Table 1. Distribution of baseline characteristics of the study population across tertiles of plasma FVIII antigen levels

	FVIII levels		
	Tertile 1 <85%	Tertile 2 85-108%	Tertile 3 ≥108%
n	320	358	397
Age, years	54.9±13.5	61.6±13.3	64.1±13.0
Sex, men	50.9 (163)	46.9 (168)	44.8 (178)
BMI, kg/m^2	25.7±3.9	26.2±4.1	27.1±4.6
hsCRP, mg/L	1.08 (0.63-2.20)	1.33 (0.74-2.54)	1.76 (0.90-3.51)
MPV, fL	8.8±0.99	8.9±0.99	8.8±0.96
Platelet, *10^9 L^{-1}	252±53	245±55	240±54
Cancer†	2.2 (7)	4.8 (17)	5.8 (23)
CVD†	13.4 (43)	17.6 (63)	17.4 (69)

Continuous variables are shown as mean (± standard deviation) or median (25th percentile -75th percentile). Categorical variables are shown as percentages with numbers in brackets.
BMI, body mass index; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein.
†Self-reported history of cancer or arterial cardiovascular disease (myocardial infarction, angina, stroke) at baseline.

Table 2. Characteristics of the venous thromboembolism events (n=365)

Characteristics	Value
Age at VTE (years)	67.4±13.8
Sex (males)	48.5 (177)
Deep vein thrombosis	63.3 (231)
Pulmonary embolism	36.7 (134)
Unprovoked	40.5 (148)
Provoked VTE	59.5 (217)
Surgery/trauma	21.9 (80)
Cancer	23.6 (86)
Immobilization	19.2 (70)
Acute medical condition	15.9 (58)
Other factors	4.4 (16)

Age is shown as mean ± standard deviation, and categorical variables as percentages with numbers in brackets.

Table 3. Odds ratios (OR) with 95% confidence interval (CI) for overall venous thromboembolism (VTE) and subgroups according to plasma FVIII antigen levels

	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Overall VTE				
T1 (<85%)	238	82	1 (reference)	1 (reference)
T2 (85-108%)	236	122	1.58 (1.13-2.22)	1.57 (1.11-2.21)
T3 (≥108%)	236	161	2.13 (1.53-2.98)	1.97 (1.40-2.76)
P for trend			*<0.001*	*<0.001*
Per 1SD increase			1.32 (1.16-1.50)	1.28 (1.12-1.45)
DVT				
T1 (<85%)	238	48	1 (reference)	1 (reference)
T2 (85-108%)	236	72	1.64 (1.09-2.49)	1.62 (1.07-2.46)
T3 (≥108%)	236	111	2.63 (1.77-3.92)	2.45 (1.63-3.67)
P for trend			*<0.001*	*<0.001*
Per 1SD increase			1.41 (1.22-1.63)	1.37 (1.19-1.59)
PE				
T1 (<85%)	238	34	1 (reference)	1 (reference)
T2 (85-108%)	236	50	1.46 (0.90-2.37)	1.44 (0.88-2.35)
T3 (≥108%)	236	50	1.46 (0.89-2.39)	1.31 (0.79-2.16)
P for trend			*0.150*	*0.334*
Per 1SD increase			1.11 (0.92-1.33)	1.05 (0.87-1.27)
Provoked				
T1 (<85%)	238	50	1 (reference)	1 (reference)
T2 (85-108%)	236	69	1.42 (0.94-2.15)	1.41 (0.93-2.13)
T3 (≥108%)	236	98	2.04 (1.37-3.05)	1.90 (1.27-2.86)
P for trend			*<0.001*	*0.002*
Per 1SD increase			1.25 (1.07-1.45)	1.21 (1.04-1.41)
Unprovoked				
T1 (<85%)	238	32	1 (reference)	1 (reference)
T2 (85-108%)	236	53	1.85 (1.14-3.01)	1.82 (1.12-2.97)
T3 (≥108%)	236	63	2.29 (1.42-3.72)	2.03 (1.24-3.31)
P for trend			*0.001*	*0.006*
Per 1SD increase			1.37 (1.17-1.62)	1.32 (1.12-1.55)

FVIII, factor VIII; SD, standard deviation; T, tertile.
Model 1, adjusted for age and sex.
Model 2, adjusted for age, sex, body mass index and high-sensitivity C-reactive protein.
The mean and standard deviation (32%) of plasma FVIII antigen levels were determined in the control population.

Table 4. Odds (OR) ratios with 95% confidence intervals (CI) for overall venous thromboembolism (VTE) across tertiles of plasma FVIII antigen levels and strata of MPV

				Within stratum OR (95% CI)		Combined effects OR (95% CI)	
MPV (fL)	FVIII (%)	Controls (n=710)	Cases (n=365)	Model 1	Model 2	Model 1	Model 2
<8.5	T1	92	33	1 (reference)	1 (reference)	1 (reference)	1 (reference)
	T2	81	44	1.73 (0.99-3.01)	1.68 (0.96-2.95)	1.61 (0.93-2.79)	1.57 (0.90-2.72)
	T3	98	55	1.85 (1.08-3.17)	1.70 (0.98-2.96)	1.69 (1.00-2.87)	1.56 (0.91-2.65)
	P for trend			*0.032*	*0.071*		
8.5-9.5	T1	90	35	1 (reference)	1 (reference)	1.11 (0.63-1.94)	1.10 (0.62-1.94)
	T2	104	47	1.20 (0.70-2.03)	1.25 (0.73-2.14)	1.35 (0.79-2.30)	1.40 (0.81-2.41)
	T3	93	66	1.91 (1.13-3.21)	1.79 (1.05-3.05)	2.18 (1.29-3.66)	2.06 (1.21-3.50)
	P for trend			*0.012*	*0.028*		
≥9.5	T1	56	14	1 (reference)	1 (reference)	0.71 (0.35-1.44)	0.73 (0.35-1.50)
	T2	51	31	2.32 (1.09-4.94)	2.41 (1.10-5.26)	1.84 (1.00-3.36)	1.79 (0.95-3.37)
	T3	45	40	3.42 (1.60-7.30)	3.74 (1.69-8.29)	2.77 (1.52-5.03)	2.71 (1.44-5.11)
	P for trend			*0.002*	*0.001*		
RERI (95% CI)						1.37 (-0.03, 2.76)	1.42 (0.01, 2.85)
AP (95% CI)						0.49 (0.13, 0.85)	0.52 (0.17, 0.88)

AP, attributable proportion; FVIII, factor VIII; MPV; mean platelet volume; RERI, relative excess risk due to interaction; T, tertile.

T1 corresponds to FVIII <85%, T2 to FVIII 85-108%, and T3 to FVIII ≥108%.

Model 1, adjusted for age and sex.

Model 2, adjusted for age, sex, body mass index, high-sensitivity C-reactive protein and platelet count.

FIGURE LEGENDS

Figure 1. Flowchart of the study population. The flowchart illustrates the nested case-control study derived from the fourth survey of the Tromsø Study (1994-1995). Venous thromboembolism (VTE)

Figure 2. Plots of estimated odds ratios (ORs) for overall venous thromboembolism (VTE) as a function of time from blood sampling in T4 (Tromsø 4, 1994-1995) to VTE events. Participants with plasma FVIII levels in the highest tertile (T3) were compared with those with FVIII levels in the lowest tertile (T1, reference category). Analyses were adjusted for age, sex, body mass index and high-sensitivity C-reactive protein. Blue solid circles indicate that risk estimates were statistically significant at a *P*-value <0.05. The number of VTE events are depicted above the plot.