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ORIGINAL ARTICLE

Plasma levels of P-selectin and future risk of incident venous thromboembolism

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Abstract

Background: P-selectin levels are elevated following acute deep vein thrombosis and reported to predict recurrent venous thromboembolism (VTE) and cancer-associated VTE. Yet, it is unknown whether plasma P-selectin levels are associated with incident VTE.

Objectives: We aimed to investigate the association between plasma P-selectin levels and risk of future incident VTE.

Methods: We performed a nested case-control study in 415 patients with VTE and 843 age- and sex-matched controls derived from the general population (Tromsø IV Study). Plasma P-selectin levels were measured using enzyme-linked immunosorbent assay. Logistic regression models were used to estimate odds ratios (ORs) for VTE across quartiles of plasma P-selectin level. Sex-stratified analysis was also performed.

Results: Plasma P-selectin levels were higher in men (41.4 ng/mL) than in women (38.7 ng/mL, $p = .0046$). We found no association between plasma P-selectin levels and risk of VTE in the overall analyses. However, sex-stratified analyses revealed that women with P-selectin levels in the highest quartile (>44.3 ng/mL) had higher risk of VTE (OR, 1.63; 95% CI, 1.01-2.64) than women with P-selectin levels in the lowest quartile (≤29.9 ng/mL). In contrast, higher levels of P-selectin were apparently associated with lower risk of VTE in men (OR for highest vs lowest quartile of P-selectin, 0.69; 95% CI, 0.42-1.15). The observed associations were stronger when the time between blood sampling and VTE was shorter.

Conclusion: Elevated levels of plasma P-selectin were associated with increased risk of VTE in women but not in men, suggesting a differential impact of sex on the association between P-selectin and VTE risk.

KEYWORDS
deep vein thrombosis, P-selectin, pulmonary embolism, venous thromboembolism, VTE

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1 | INTRODUCTION

Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a frequently occurring, life-threatening condition that affects approximately 1.1 million Europeans each year [1]. VTE presents a challenge to health care systems and public health due to frequent hospitalizations, severe long-term complications, and high rates of recurrence and mortality [2]. Despite identification of new VTE risk factors and more widespread use of thromboprophylaxis, the incidence of VTE has increased over the past decades [3,4]. It is, therefore, imperative to identify novel biomarkers and reveal disease mechanisms to improve VTE risk assessment as well as provide targeted prevention and treatment.

P-selectin is a single-chain glycoprotein with a molecular weight of 140 kDa, making it the largest member of the selectin family of cell adhesion molecules [5,6]. P-selectin is stored in the α-granules of platelets and the Weibel-Palade bodies in endothelial cells and is rapidly expressed on the surface of these cells upon activation [7–10]. Activation of platelets and endothelial cells also triggers the release of soluble P-selectin (sP-selectin) into plasma [11,12]. P-selectin interacts with P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes, facilitating leukocytes' adhesion and rolling on endothelial cells as well as interaction between activated platelets and leukocytes [13–15]. P-selectin levels are correlated with platelet-derived extracellular vesicle (EV) count, and these EVs have been shown to induce thrombin generation [16]. In addition, P-selectin increases tissue factor expression on monocytes [17] and facilitates the formation of large and stable platelet aggregates [18].

Transmembrane P-selectin is present in dimers or oligomers, whereas sP-selectin, which results from proteolytic cleavage of transmembrane P-selectin, circulates in plasma in its monomeric form and is unable to interact with functional ligands [19,20]. High levels of sP-selectin in plasma are suggested to reflect cell activation and the degree of functional P-selectin expressed on the surface of platelets and endothelial cells [21].

Several studies support a possible involvement of P-selectin in the pathogenesis of VTE. Higher levels of sP-selectin have been reported in DVT cases compared with healthy controls [22–26] and in patients with suspected VTE in whom DVT was confirmed compared with those in whom DVT was ruled out [25–28]. sP-selectin has also been identified as a risk factor for VTE recurrence [29] as well as cancer-associated VTE [30] and cancer-associated VTE recurrence [31]. More recently, sP-selectin was identified as a predictive biomarker for VTE risk among patients with COVID-19 [32]. *In vitro* and animal studies showed that inhibition of P-selectin interaction with its ligand, PSGL-1, contributed to lower thrombus weight, reduced thrombus formation, and less leukocyte accumulation [33,34].

Although small case-control studies have indicated an association between sP-selectin and first lifetime VTE, the timing of blood sampling in these studies (ie, in the acute phase or shortly after the VTE) and the limited control population make them susceptible to biases such as reverse causation and selection bias [22,24,25,28]. Due to the lack of investigation in larger cohorts, it remains unknown whether sP-selectin in plasma can be used to identify individuals at increased risk of future VTE in the general population. Moreover, the levels of plasma sP-selectin are reported to be slightly higher in men than in women [35,36], but no study has investigated the association between sP-selectin and risk of VTE in men and women separately. Therefore, the aim of the present study was to investigate the association between plasma levels of P-selectin and risk of future VTE, overall and in men and women separately, in a nested case-control study derived from a general population cohort.

Essentials

- P-selectin levels are elevated in patients following venous thromboembolism (VTE).
- We investigated the relationship between baseline serum P-selectin and future VTE risk.
- Women with higher baseline levels of P-selectin had significantly higher risk of future VTE.
- This association was stronger with shorter time between sampling and VTE event.

2 | METHODS

2.1 | Study population

The Tromsø Study is a single-center, population-based cohort study with repeated health surveys of the population of Tromsø, Norway. Inhabitants of Tromsø aged ≥25 years were invited to participate in the fourth survey, which was conducted during 1994 to 1995. A total of 27158 subjects (77% of invited individuals) participated in the survey and were followed from the date of inclusion in the study until a confirmed incident VTE event, migration, death, or end of follow-up on 1 September 2007.

Participants with a history of VTE before baseline were excluded from this study. All incident VTE events occurring among study participants during the study period (1994-2007) were identified via the hospital discharge diagnosis registry, autopsy registry, and/or radiology procedure registry from the University Hospital of North Norway, which is the primary diagnostic radiology and treatment provider for VTE in the Tromsø municipality. Trained health care personnel adjudicated and recorded each VTE event through meticulous review of medical records. This process for identifying and adjudicating VTE based on firm criteria (symptomatic DVT or PE objectively confirmed via radiological methods) has been previously described [37]. A VTE that occurred in the presence of 1 of the following factors was designated as provoked: surgery or trauma (within 8 weeks preceding the VTE event), immobilization (bed rest for ≥3 days or wheelchair confinement within 8 weeks preceding the VTE event or long-distance travel for ≥4 hours within the 14 days preceding VTE event), acute medical condition (myocardial infarction, ischemic stroke, or infections), or other factors specifically characterized as provoking by a physician in the patient's medical record (eg, intravascular catheter).

Among those included in the Tromsø 4 Study, 462 individuals experienced a first-in-lifetime VTE event during the follow-up period (1994-2007). For each VTE case, 2 age- and sex-matched controls who were alive at the index date of VTE event were randomly sampled from the source cohort (n = 924). As 47 cases and 81 controls did not have plasma samples of sufficient quality for further analysis, our final nested case-control study consisted of 415 cases and 843 controls (Figure 1). The regional committee for medical and health research ethics approved the study, and all participants provided written consent.

2.2 | Baseline measurements

Height (to the nearest centimeter) and weight (to the nearest 0.5 kg) were measured in participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). A self-administered questionnaire was used to gather a detailed history of participants' previous cardiovascular events (stroke, angina pectoris, transient ischemic attack, and myocardial infarction), cancer, and diabetes as well as participants' dietary habits, physical exercise, smoking, and alcohol consumption.

2.3 | Blood sample collection and storage of blood products

At the point of inclusion in Tromsø 4, nonfasting blood was collected into 5-mL vacutainers (Becton Dickinson) containing EDTA (40 μL of K3-EDTA, 0.37 mol/L per tube) as an anticoagulant. Platelet-poor plasma was prepared via centrifugation at 3000× g for 10 minutes at room temperature. Platelet-poor plasma was then transferred to cryovials (Greiner Laboratechnik) in 1-mL aliquots and stored at −80 °C. At the point of biomarker measurements in plasma, samples were thawed for 5 minutes at 37 °C in a water bath and centrifuged for 2 minutes at 13000× g at room temperature to obtain platelet-free plasma.

2.4 | Measurements of plasma levels of C-reactive protein and P-selectin

sP-selectin and high sensitivity C-reactive protein (hsCRP) were measured in platelet-free plasma using enzyme-linked immunosorbent assay using commercially available agents, according to the manufacturer's instructions (R&D Systems). The enzyme-linked immunosorbent assay was performed in a 384 format using the combination of a SELMA pipetting robot and a BioTek EL406 dispenser/washer. Using a Synergy H1 Hybrid microplate reader (BioTek), absorption was read at 450 nm with a wavelength correction set to 540 nm. The intra- and interindividual coefficients of variation were 3.0% and 8.5% for sP-selectin and 2.6% and 9.1% for hsCRP, respectively.

2.5 | Statistical analysis

Statistical analyses were carried out using Stata version 16 (StataCorp LLC) and R (version 4.1.2). sP-selectin levels were categorized according to quartile cut-offs in the control population (≤31.2, 31.3-

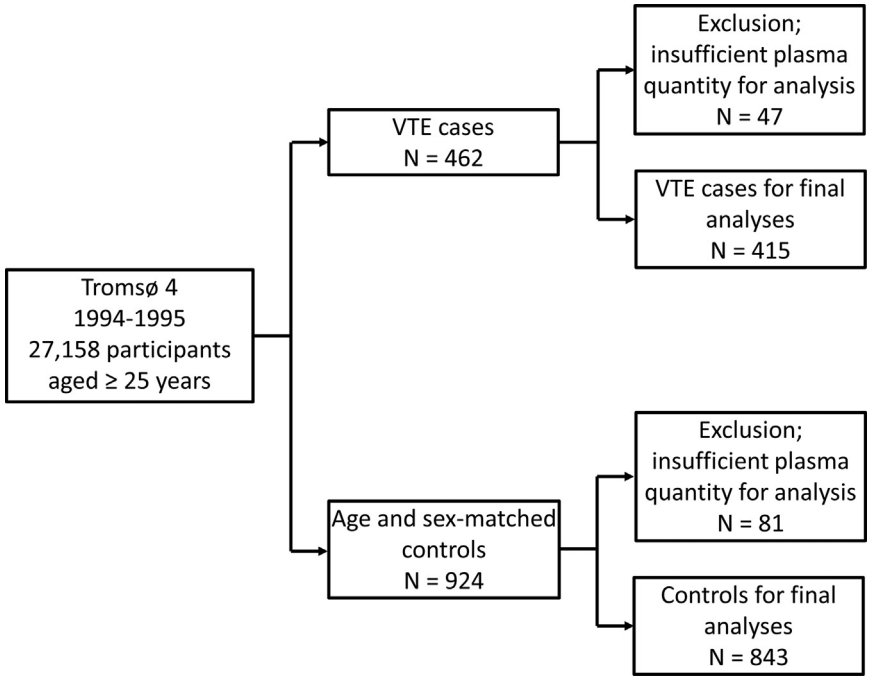

FIGURE 1 Flowchart of study participants from the Tromsø 4 Study and the current nested case-control study of plasma P-selectin in patients with VTE and age- and sex-matched controls. VTE, venous thromboembolism.

38.5, 38.6-45.9, and ≥46 ng/mL overall; ≤29.9, 30.0-37.4, 37.5-44.2, and ≥44.3 ng/mL for women; and ≤32.4, 32.5-39.5, 39.6-47.2, and ≥47.5 ng/mL for men). Means and proportions of baseline characteristics across P-selectin quartiles were calculated using descriptive statistics. Logistic regression models were used to calculate odds ratios (ORs) of VTE with 95% CIs according to sP-selectin quartiles using the lowest sP-selectin quartile as the reference group. Subgroup analyses were also conducted with unprovoked VTE, provoked VTE, DVT, and PE as the outcomes. Four adjustment models were applied in the logistic regression. We adjusted for age and sex in model 1, whereas BMI and hsCRP were sequentially added to models 2 and 3, respectively. Model 4 additionally included platelet and leukocyte counts.

Because these results were based on single baseline measurements with a long follow-up time in the source cohort (>12 years for many individuals), estimates based on baseline measurement of sP-selectin could be influenced by natural fluctuations over time, which would induce regression dilution bias [38]. To investigate this, we performed analyses in which we limited the maximum time from blood sampling in the Tromsø 4 Study to 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 new ORs were generated every time a new VTE occurred (ie, including all VTE events accrued up to this time-point) and plotted as a function of this accrued follow-up time.

3 | RESULTS

The distribution of baseline characteristics of study participants according to quartiles of sP-selectin is shown in Table 1. The mean age (ranging from 59 to 62 years) and BMI (ranging from 26.3 to 26.5) were similar across quartiles, whereas the mean CRP levels increased from 1.38 to 1.91 mg/L. Plasma P-selectin levels were higher in men (41.35 ng/mL) than in women (38.68 ng/mL, p = .0046), and the proportion of men thereby increased across quartiles from 38.6% to 55.2%.

The characteristics of the patients with VTE in this study are shown in Table 2. The mean age at the time of VTE was 67.6 years overall, 65.6 years for men, and 69.5 years for women. In total, 63.1% of the VTE events were DVT (men, 59.3%; women, 66.7%) and 37.8% of the VTE events were PE (men, 39.7%; women, 36.1%). The majority of VTE events were provoked (58.1%), and the most common provoking factor was surgery/trauma (22.4%), followed by active cancer (21.5%), immobilization (18.1%), and acute medical conditions (15.7%) with similar distributions in men and women (Table 2). In the 8-week period preceding the VTE, estrogens were used by 15.3% of the women with VTE.

The overall and sex-specific risk of VTE across quartiles of sP-selectin levels is shown in Table 3. Overall, there was no association between increasing quartiles (Q) of sP-selectin and risk of VTE after multivariable adjustment (OR for Q4 vs Q1, 1.05; 95% CI, 0.73-1.50). Sex-specific analysis revealed that women with sP-selectin levels in the fourth quartile (>44.3 ng/mL) had a significantly higher risk of VTE (OR, 1.87; 95% CI, 1.13-3.07) than those in the reference quartile (<29.9 ng/mL) in the fully adjusted model (adjusted for age, sex, BMI, CRP, platelet count, and white blood cell count). Men in the fourth quartile (>47.5 ng/mL), however, had an apparently lower risk of VTE (OR, 0.73; 95% CI, 0.43-1.24) than those in the reference quartile (<32.4 ng/mL) in the fully adjusted model, although this result was not statistically significant.

The risk of DVT and PE for the overall study sample, as well as in men and women, across quartiles of sP-selectin is shown in Table 4. When analyzing all subjects, there was no clear association between sP-selectin and risk of DVT or PE. In women, those with sP-selectin levels in the fourth quartile had a higher risk of DVT (OR, 1.90; 95% CI, 1.05-3.43) and higher risk of PE (OR, 1.74; 95% CI, 0.87-3.51) than those in the reference quartile in the fully adjusted model. However, as observed for overall VTE, men with sP-selectin levels in the fourth quartile had a lower risk of both DVT (OR, 0.72; 95% CI, 0.39-1.34) and PE (OR, 0.86; 95% CI, 0.39-1.89) than those in the reference quartile in the fully adjusted model. The ORs for unprovoked and provoked VTEs across quartiles of sP-selectin showed similar patterns as the ORs observed for overall VTE in men and women (Supplementary Table). Of note, women with sP-selectin levels in the fourth quartile had a higher risk of provoked VTE (OR, 1.87; 95% CI, 1.02-3.04) than those in the reference quartile in the fully adjusted model.

To consider the likelihood of underestimating ORs due to natural fluctuations of sP-selectin over time, we estimated ORs for overall VTE in the total study population and in men and women by plotting the ORs for subjects in the highest vs lowest quartile of sP-selectin levels as a function of the follow-up time since blood sampling (Figure 2). The higher risk in women and lower risk in men both appeared to be stronger with shorter follow-up time.

4 | DISCUSSION

We investigated the association between plasma sP-selectin levels and future risk of VTE in a large, nested case-control study derived from the general population. Due to differences in plasma sP-selectin levels between men and women, we performed both overall and sex-specific analysis. In our overall analysis and in men, we found no significant association with VTE across quartiles of sP-selectin levels. In contrast, women with sP-selectin levels in Q4 had an 87% higher OR for VTE than women in Q1 after adjustment for age, BMI, hsCRP, platelet count, and white blood cell count. The OR for VTE by plasma sP-selectin in women increased substantially with shortened time between blood sampling and VTE event. Our findings suggest that elevated plasma P-selectin levels are associated with increased risk of future VTE in women but not in men.

The majority of previous studies on sP-selectin in VTE assessed P-selectin levels in the acute phase of DVT, either in patients with suspected DVT in whom the diagnosis was ruled out [25–28] or in healthy controls [24,26] as comparison groups. These studies reported

TABLE 1 Distribution of baseline characteristics according to quartiles of sP-selectin.

Baseline characteristics	Overall sP-selectin (ng/mL)			
	≤31.2	<31.3-38.5	>38.6-45.9	>46.0
	(*n* = 305)	(*n* = 313)	(*n* = 330)	(*n* = 310)
Age, y	58.9 ± 14.3	59.0 ± 14.4	61.3 ± 13.6	61.6 ± 12.7
Sex, % men (*n*)	38.6 (118)	44.1 (138)	49.7 (164)	55.2 (171)
BMI, kg/m^2	26.3 ± 4.3	26.5 ± 4.4	26.4 ± 3.8	26.5 ± 4.5
CRP, mg/L	1.38 ± 1.21	1.52 ± 1.34	1.69 ± 1.48	1.91 ± 1.39
CVD[a], % (*n*)	11.8 (36)	13.7 (43)	19.7 (65)	16.8 (52)
Cancer[b], % (*n*)	4.3 (13)	3.2 (10)	5.8 (19)	5.2 (16)
Platelet count, ×10^9/L	228.5 ± 51.0	241.8 ± 53.3	248.0 ± 53.1	259.8 ± 55.8
White blood cell count, ×10^9/L	6.48 ± 3.0	6.73 ± 1.8	7.17 ± 1.7	7.74 ± 2.0
Baseline characteristics	**Men sP-selectin (ng/mL)**			
	≤32.4	<32.4-39.5	>39.6-47.2	>47.5
	(*n* = 153)	(*n* = 151)	(*n* = 147)	(*n* = 140)
Age, y	56.7 ± 12.8	58.6 ± 12.1	59.2 ± 12.4	58.5 ± 11.6
BMI, kg/m^2	26.0 ± 2.9	26.5 ± 3.7	26.0 ± 3.1	25.7 ± 3.7
CRP, mg/L	1.35 ± 1.15	1.66 ± 1.48	1.71 ± 1.50	1.89 ± 1.43
CVD[a], % (*n*)	16.3 (25)	19.2 (29)	23.1 (34)	17.1 (24)
Cancer[b], % (*n*)	0.7 (1)	0.7 (1)	4.8 (7)	5.0 (7)
Platelet count, ×10^9/L	221.0 ± 49.0	236.5 ± 50.7	239.9 ± 56.0	250.3 ± 54.5
White blood cell count, ×10^9/L	6.40 ± 1.5	6.96 ± 2.0	7.19 ± 1.8	7.94 ± 2.2
Baseline characteristics	**Women sP-selectin (ng/mL)**			
	≤29.9	<30.0-37.4	>37.5-44.2	>44.3
	(*n* = 152)	(*n* = 168)	(*n* = 164)	(*n* = 183)
Age, y	58.8 ± 15.8	61.7 ± 15.3	63.3 ± 14.1	63.6 ± 13.8
BMI, kg/m^2	25.9 ± 4.7	26.8 ± 5.1	26.5 ± 4.6	27.3 ± 5.1
CRP, mg/L	1.39 ± 1.20	1.44 ± 1.29	1.64 ± 1.45	1.84 ± 1.32
CVD[a], % (*n*)	9.2 (14)	13.1 (22)	11.6 (19)	15.8 (29)
Cancer[b], % (*n*)	6.6 (10)	7.1 (12)	6.7 (11)	4.9 (9)
Platelet count, ×10^9/L	230.8 ± 51.9	245.9 ± 51.7	251.5 ± 49.1	274.5 ± 54.8
White blood cell count, ×10^9/L	6.31 ± 1.5	6.85 ± 3.8	7.12 ± 1.6	7.50 ± 1.9

Continuous variables are shown as mean (±SD) or median (25th percentile-75th percentile).

BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; sP-selectin, soluble P-selectin.

[a] Self-reported history of CVD (myocardial infarction, angina, and stroke).

[b] Self-reported history of cancer at baseline.

significantly higher sP-selectin levels in patients with acute DVT [24–28]. However, the sample size of these studies was small (ranging from 48 to 189 participants in total), and the generalizability of the controls with regard to the source population could, therefore, be questioned. Moreover, as sP-selectin was measured in the acute phase, it could not be determined whether sP-selectin levels were a cause of the DVT or merely a marker of the ongoing acute thrombotic process. In a subgroup of the Leiden Thrombophilia Study, including 89 DVT cases and 126 controls, the OR was 2.1 for sP-selectin levels >238 ng/mL when cases were compared with controls and blood samples were collected >6 months after the DVT [22]. Our study is, to the best of our knowledge, the first to investigate the association between plasma levels of sP-selectin and risk of future VTE in a large sample recruited from the general population with sP-selectin measured years before the VTE occurred. The risk estimates were weaker than those reported in the previous acute phase studies [23–28] and only present in women, which support the notion that the elevated sP-selectin levels observed in patients with acute DVT, to a

TABLE 2 Characteristics of VTE events.

Characteristics of VTE events	All (*n* = 415) % (*n*)	Men (*n* = 199) % (*n*)	Women (*n* = 216) % (*n*)
Age at VTE (y)	67.6 ± 13.7	65.6 ± 11.9	69.5 ± 14.9
Deep vein thrombosis	63.1 (262)	59.3 (118)	66.7 (144)
Proximal	79.0 (207)	83.1 (98)	75.7 (109)
Distal	21.0 (55)	16.9 (20)	24.3 (35)
Pulmonary embolism	37.8 (157)	39.7 (79)	36.1 (78)
Unprovoked VTE	41.9 (174)	43.2 (86)	40.7 (88)
Provoked VTE	58.1 (241)	56.8 (113)	59.3 (128)
Surgery/trauma	22.4 (93)	21.1 (42)	23.6 (51)
Active cancer	21.5 (89)	20.6 (41)	22.2 (48)
Acute medical condition	15.7 (65)	17.1 (34)	14.4 (31)
Immobilization	18.1 (75)	17.1 (34)	19.0 (41)
Other factors	4.1 (17)	5.0 (10)	3.2 (7)

VTE, venous thromboembolism.

large extent, are a consequence of the ongoing thrombotic process in these patients.

In agreement with previous studies [35,39], we found that the overall levels of sP-selectin in plasma is higher in men than in women. However, increased sP-selectin levels were associated with higher VTE risk in women but not in men. Our study is the first to investigate the association between sP-selectin and VTE in men and women separately. As our findings of sex differences are unchallenged, further

TABLE 3 ORs with 95% CIs for VTE according to quartiles of sP-selectin.

Quartiles of sP-selectin (ng/mL)	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)
			Overall			
≤31.2	210	95	Ref	Ref	Ref	Ref
31.3-38.5	211	102	1.07 (0.76-1.50)	1.06 (0.75-1.49)	1.04 (0.74-1.47)	0.99 (0.70-1.41)
38.6-45.9	210	120	1.26 (0.90-1.75)	1.27 (0.91-1.78)	1.23 (0.88-1.73)	1.29 (0.92-1.8)
>46	212	98	1.01 (0.72-1.43)	1.02 (0.72-1.44)	0.95 (0.67-1.35)	1.05 (0.73-1.50)
P for trend	843	415	0.94	0.93	0.79	0.81
			Men			
≤32.4	98	55	Ref	Ref	Ref	Ref
32.4-39.5	98	53	0.97 (0.60-1.55)	0.92 (0.57-1.49)	0.86 (0.53-1.39)	0.89 (0.54-1.45)
39.6-47.2	98	49	0.89 (0.56-1.44)	0.90 (0.56-1.46)	0.84 (0.52-1.37)	0.91 (0.55-1.50)
>47.5	98	42	0.77 (0.47-1.25)	0.78 (0.47-1.28)	0.69 (0.42-1.15)	0.73 (0.43-1.24)
P for trend	392	199	0.29	0.32	0.15	0.251
			Women			
≤29.9	112	40	Ref	Ref	Ref	Ref
30.0-37.4	113	55	1.36 (0.84-2.21)	1.35 (0.83-2.21)	1.36 (0.83-2.22)	1.33 (0.80-2.20)
37.5-44.2	112	52	1.30 (0.79-2.12)	1.32 (0.80-2.16)	1.31 (0.80-2.15)	1.44 (0.86-2.40)
>44.3	114	69	1.69 (1.05-2.71)	1.67 (1.04-2.70)	1.63 (1.01-2.64)	1.87 (1.13-3.07)
P for trend	451	216	0.029	0.035	0.046	0.014

Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, and body mass index. Model 3: adjusted for age, sex, body mass index, and C-reactive protein. Model 4: adjusted for age, sex*, body mass index, C-reactive protein, platelet count, and white blood cell count.

OR, odds ratio; Ref, reference; sP-selectin, soluble P-selectin.

TABLE 4 ORs with 95% CIs for DVT and PE according to quartiles of sP-selectin.

Quartiles of sP-selectin (ng/mL)	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)
			DVT—overall			
≤31.2	210	60	Ref	Ref	Ref	Ref
31.3-38.5	211	70	1.16 (0.78-1.72)	1.15 (0.77-1.71)	1.14 (0.77-1.70)	1.13 (0.75-1.69)
38.6-45.9	210	70	1.17 (0.78-1.73)	1.18 (0.79-1.75)	1.15 (0.77-1.72)	1.25 (0.83-1.88)
>46	212	62	1.02 (0.68-1.54)	1.03 (0.68-1.55)	0.99 (0.65-1.50)	1.10 (0.72-1.69)
P for trend	843	262	0.90	0.89	0.96	0.656
			DVT—men			
≤32.4	98	37	Ref	Ref	Ref	Ref
32.4-39.5	98	29	0.79 (0.45-1.39)	0.77 (0.44-1.36)	0.74 (0.42-1.31)	0.79 (0.44-1.41)
39.6-47.2	98	26	0.71 (0.40-1.26)	0.72 (0.40-1.29)	0.68 (0.38-1.23)	0.77 (0.42-1.39)
>47.5	98	26	0.71 (0.40-1.26)	0.73 (0.41-1.30)	0.66 (0.37-1.20)	0.72 (0.39-1.34)
P for trend	392	118	0.24	0.28	0.17	0.298
			DVT—women			
≤29.9	112	25	Ref	Ref	Ref	Ref
30.0-37.4	113	39	1.53 (0.87-2.70)	1.55 (0.87-2.76)	1.55 (0.87-2.76)	1.60 (0.89-2.86)
37.5-44.2	112	37	1.45 (0.82-2.57)	1.47 (0.82-2.63)	1.47 (0.82-2.63)	1.67 (0.91-3.04)
>44.3	114	43	1.65 (0.94-2.89)	1.67 (0.94-2.95)	1.67 (0.94-2.95)	1.90 (1.05-3.43)
P for trend	451	144	0.08	0.08	0.08	0.033
			PE—overall			
≤31.2	210	35	Ref	Ref	Ref	Ref
31.3-38.5	211	32	0.99 (0.65-1.51)	0.98 (0.64-1.49)	0.86 (0.51-1.46)	0.82 (0.48-1.40)
38.6-45.9	210	52	1.17 (0.78-1.74)	1.15 (0.77-1.73)	1.41 (0.87-2.28)	1.41 (0.86-2.30)
>46	212	38	1.11 (0.74-1.67)	1.11 (0.74-1.67)	0.97 (0.58-1.62)	1.03 (0.61-1.75)
P for trend	843	157	0.90	0.87	0.90	0.913
			PE—men			
≤32.4	98	17	Ref	Ref	Ref	Ref
32.4-39.5	98	21	1.22 (0.60-2.45)	1.14 (0.57-2.33)	1.09 (0.53-2.21)	1.10 (0.53-2.28)
39.6-47.2	98	25	1.45 (0.73-2.85)	1.44 (0.73-2.87)	1.36 (0.68-2.70)	1.38 (0.67-2.80)
>47.5	98	16	0.93 (0.44-1.95)	0.95 (0.45-2.00)	0.85 (0.40-1.81)	0.86 (0.39-1.89)
P for trend	392	79	0.85	0.90	0.68	0.715
			PE—women			
≤29.9	112	17	Ref	Ref	Ref	Ref
30.0-37.4	113	19	1.10 (0.54-2.23)	1.05 (0.51-2.15)	1.05 (0.51-2.15)	1.00 (0.48-2.09)
37.5-44.2	112	13	0.76 (0.35-1.64)	0.74 (0.34-1.61)	0.72 (0.34-1.57)	0.74 (0.33-1.67)
>44.3	114	29	1.67 (0.86-3.20)	1.60 (0.82-3.12)	1.53 (0.78-3.00)	1.74 (0.87-3.51)
P for trend	451	78	0.13	0.17	0.22	0.120

Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, and body mass index. Model 3: adjusted for age, sex, body mass index, and C-reactive protein. Model 4: adjusted for age, sex, body mass index, C-reactive protein, platelet count, and white blood cell count. Sex was not included as a covariate in the sex-specific models.

DVT, deep vein thrombosis; OR, odds ratio; PE, pulmonary embolism; Ref, reference; sP-selectin, soluble P-selectin.

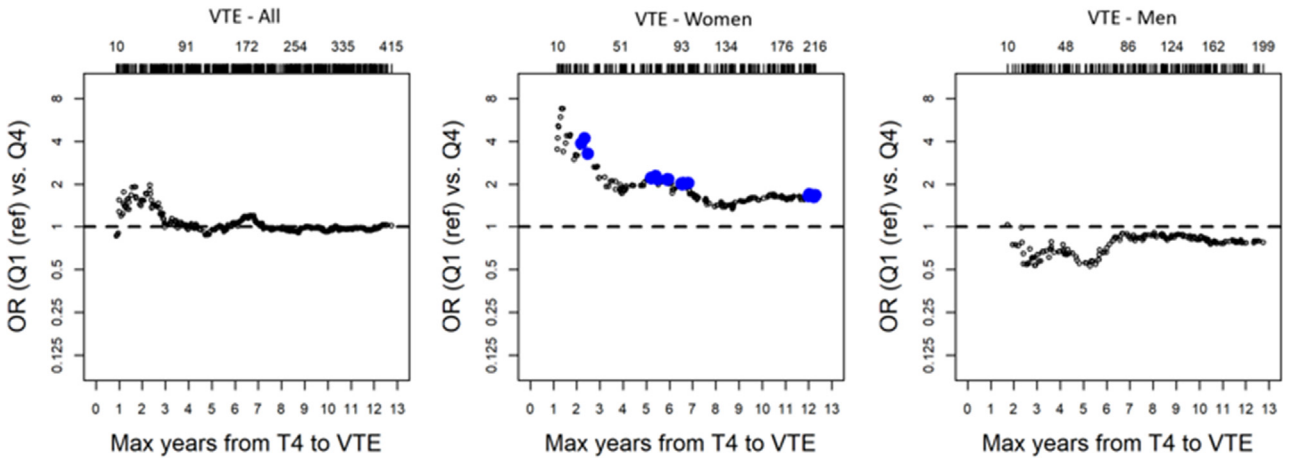

FIGURE 2 Plots of estimated ORs for overall VTE in all subjects, men, and women as a function of time from blood sampling in the fourth survey of the Tromsø Study (1994-1995) and event in analyses adjusted for age, sex, and BMI. Blue circles indicate ORs with p values of <.05. BMI, body mass index; OR, odds ratio; ref, reference; VTE, venous thromboembolism.

studies are needed to confirm our findings and elucidate potential mechanisms of sex differences. The reason for the observed differential impact of sP-selectin on VTE risk among sexes is not known. Inflammation induces expression of P-selectin on platelets and endothelial cells and is correlated with the amount of sP-selectin detected in plasma [7–9]. In the Tromsø Study, we reported that inflammation, assessed using CRP, was associated with risk of VTE in women but not in men [40], and this could potentially explain the sex difference observed for sP-selectin. However, adjustment for CRP only slightly attenuated the association between sP-selectin and VTE risk in women.

The association between sP-selectin levels and future VTE has been previously assessed in patients with cancer and patients with a first VTE. In a cohort of 687 patients with cancer, of who 44 developed VTE during follow-up, sP-selectin levels above the 75th percentile were associated with a 2.6-fold higher risk of VTE [30]. Furthermore, in a cohort of 544 patients with a first unprovoked VTE, sP-selectin levels above the 75th percentile cut-off, measured after cessation of anticoagulation, predicted 4-year risk of recurrence (20.6% vs 10.8% recurrence probability, respectively) [29].

Both cancer and VTE are inflammatory conditions, and higher sP-selectin levels in these selected patient groups may, to a larger extent, be a marker of the underlying prothrombotic state caused by inflammation.

Several animal studies have explored the relationship between sP-selectin and VTE formation. In a mouse model of inferior vena cava ligation, mice with elevated sP-selectin levels displayed a 50% increase in thrombus mass (defined as thrombus weight/inferior vena cava length) compared with wild-type mice [41]. In a study on formation of neutrophil extracellular traps (NETs) in mice, NETosis was induced by thrombin-activated platelets from wild-type mice but not from platelets in P-selectin knockout mice [42]. In addition, mice that were engineered to overproduce soluble P-selectin displayed significantly elevated NET formation [42].

Activated platelets release sP-selectin and EVs into plasma, and platelet-derived EVs express phosphatidylserine and P-selectin on their surface [21,43]. As P-selectin is expressed on the EV membrane, it retains its dimerized form and binds to PSGL-1 with higher avidity than monomeric P-selectin [20]. Dimeric P-selectin can trigger integrin-dependent adhesion of leukocytes to immobilized intercellular adhesion molecule and NET formation and increases the number of neutrophils rolling on P-selectin [20]. Further research is needed to determine the distribution and functional characteristics of monomeric and dimeric forms of sP-selectin in which EVs from activated platelets or endothelial cells may represent nanobodies displaying the dimeric (or multimeric) form of the protein.

The strengths of our study include the recruitment of patients with VTE and controls from the same population cohort, with blood samples collected before the VTEs occurred. Thus, there is a clear temporal sequence between exposure (plasma levels of sP-selectin) and outcome (first-in-lifetime VTE event). The study also has some limitations. The blood samples were collected at the time of inclusion in the Tromsø 4 cohort (1994 or 1995) and stored at −80 °C for up to 22 years. This long storage time could have potentially affected the plasma levels of sP-selectin. However, the plasma sP-selectin levels in our control population were similar to the levels reported in other healthy populations [23,25,29]. Furthermore, as the effect of plasma storage would be similar in both cases and controls, any misclassification of sP-selectin levels would likely be nondifferential and lead to underestimation of the true effect. Plasma sP-selectin levels were measured only at baseline, and intraindividual changes in sP-selectin levels during follow-up could also result in underestimation of the true association [38]. This was supported by the stronger association observed with shorter follow-up time in our study.

In conclusion, the results of our nested case-control study indicate that high levels of plasma sP-selectin were associated with increased risk of future VTE in women but not in men. Further studies are

needed to validate our unchallenged observation and to elucidate underlying mechanism(s).

AUTHOR CONTRIBUTIONS

S.S. analyzed the data, interpreted the results, and drafted the manuscript. T.U. performed laboratory analysis of the samples, interpreted the results, and revised the manuscript. J.B.H., O.S., and S.K.B. designed the study, collected the data, interpreted the results, and revised the manuscript. All authors read and approved the final manuscript.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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