Complement factors B, D, C3bBbP and risk of future venous thromboembolism

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Highlights

- Venous thromboembolism (VTE) is a frequent and devastating disease with incompletely understood links between coagulation and the complement system.
- We investigated the possible association of specific components of the alternative pathway of complement and future risk of VTE in a nested case-control study.
- Plasma levels of CFB and CFD of the alternative pathway were not associated with future risk of VTE, whereas specific activation, assessed by plasma C3bBbP, was associated with increased risk in the subgroup of provoked VTE.
- In the subgroup of provoked VTE cases, subjects with C3bBbP levels in quartile (Q) 4 had a 1.68-fold higher OR compared with Q1.

Abstract

The complement system appears to be involved in the pathogenesis of venous thromboembolism (VTE).

We investigated the association of complement factors (CF) B, D, and the alternative pathway convertase, C3bBbP, measured at inclusion, with the risk of future VTE in a nested case-control study; 380 VTE patients and 804 age- and sex-matched controls derived from the Tromsø study. Odds ratios (ORs) with 95% confidence intervals (95% CI) for VTE across tertiles of CF concentrations were estimated using logistic regression.

There was no association between CFB or CFD and risk of future VTE. Higher levels of C3bBbP gave an increased risk of provoked VTE; subjects in Q4 had a 1.68-fold higher OR compared with Q1 in the age-, sex- and BMI-adjusted model (OR 1.68; 95% CI 1.08-2.64).

There was no increased risk of future VTE in individuals with higher levels of complement factors B or D of the alternative pathway. Increased levels of the alternative pathway activation product, C3bBbP, showed an association with future risk of provoked VTE.

Keywords

Blood Coagulation, Case-Control Studies, Complement System Proteins, Logistic Models, Venous Thromboembolism

1.1 Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), affects 1-2 per 1000 individuals annually and represents a major public health challenge [1,2]. There are approximately 1.1 million VTE events per year in Europe with more than 500,000 VTE-related fatalities [3]. In contrast to the declining incidence in arterial thrombotic disease [4,5], the incidence of VTE has remained stable or slightly increased over the past decades [2,6,7]. Anticoagulants can prevent VTE prophylactically, but also carry a risk of iatrogenic bleeding [8,9]. Identification of novel biomarkers may not only improve risk stratification and targeted prevention, but also unravel underlying disease mechanisms suitable for therapeutic intervention, and ultimately reduce the burden of VTE.

The complement system is a key part of the innate immune system, and comprises a network of proteins that collectively mediate immunosurveillance and maintain tissue homeostasis [10]. The cascade is initiated by three distinct pathways, namely the classical, lectin and alternative pathway (AP). These pathways converge at complement factor C3, with subsequent activation of C5 and the terminal complement pathway, resulting in formation of the terminal C5b-9 complex (TCC) [11]. Unlike the classical and lectin pathways, which are activated upon recognition of specific proteins, the alternative pathway is either activated secondarily to classical or lectin pathway activation, or is constitutively active at a low level due to spontaneous hydrolysis of C3 ("tick-over") [12,13]. Depending on the context, C3b binds to complement factor B (CFB) forming C3bB, whereupon CFB is cleaved by complement factor D (CFD) and stabilized by properdin, to form the C3bBb (i.e., the AP C3 convertase), which in turn generate C3a and C3b [13]. Notably, this amplification through the alternative pathway accounts for more than 80% of the terminal complement activation regardless of the initiating pathway [12,14,15].

Extensive crosstalk between the complement and coagulation systems suggests a potential role of the complement system in the pathogenesis of thrombosis [16–18]. A few prospective observational studies have investigated the association between individual components of the complement system and risk of VTE. In a large, Danish population-based cohort [19], subjects with plasma complement C3 levels in the highest tertile had a 30% higher risk of VTE compared with subjects in the lowest tertile. Similarly, in a nested case-control study, we recently reported that subjects with C5 levels in the highest tertile had a 45% increased risk of future VTE compared with those with C5 levels in the lowest reference tertile [20]. Furthermore, we reported that high plasma levels of the final activation product of the complement cascade, the terminal C5b-9 complex, was associated with increased risk of VTE, and unprovoked events in particular [21], and that low levels of mannose-binding lectin (MBL), a major pattern recognition molecule of the lectin pathway, was associated with a lower risk of VTE [22].

In the present study, we aimed to investigate whether plasma levels of CFB and CFD, and the specific activation product of the alternative pathway, C3bBbP, were associated with risk of future VTE in a nested case-control study derived from a general population.

2.1 Methods

2.1.1 Study population

We conducted a nested case-control study based on the fourth survey of the Tromsø Study (1994-95). The Tromsø study is a single-center population-based cohort with repeated health surveys of the inhabitants of the municipality of Tromsø, Norway [23]. In Tromsø 4, all inhabitants aged ≥25 years were invited, and 27,158 (77% of the eligible) participated. These were followed until an incident VTE, migration, death, or the end of follow-up (September 1, 2007).

Incident VTE events were identified by searching the hospital discharge registry, the radiology registry, and the autopsy registry at the University Hospital of North Norway (UNN). The UNN is the exclusive provider of diagnostic radiology and hospital care in the study region. The process of VTE identification and adjudication in the Tromsø study has been described in detail earlier [24]. Briefly, trained personnel reviewed the medical record of each potential case, and the adjudication criteria included a combination of sign and symptoms of VTE, a thrombus confirmed by radiology, a recorded diagnosis of PE or DVT, and initiation of anticoagulant treatment (unless contraindications were specified). All events were classified according to clinical presentation (DVT or PE) and presence of provoking factors. In patients presenting with concurrent PE and DVT, the event was classified as PE. The following factors were defined as provoking: surgery or trauma (within 8 weeks prior to the event), acute medical conditions (myocardial infarction, ischemic stroke, or major infections), active cancer, marked immobilization (bedrest ≥ 3 days, confinement to wheelchair, or long-distance travel (≥ 4 h within the previous 14 days), or other provoking factor(s) described in the medical records.

There were 462 VTE events during the follow-up period (1994-2007). For each case, two age- and sex-matched controls (n=924) were sampled from the source population. Controls

had to be alive at the date of the VTE event for the corresponding case. Due to missing or insufficient quality of plasma samples, 82 cases and 120 controls were excluded, leaving 380 cases and 804 controls in our final analytic sample. The study was approved by the Regional Committee for Medical and Health Research, and all participants provided written informed consent.

2.1.2 Baseline assessments

Baseline information was collected by trained personnel, and included physical examinations, blood samples and self-administrated questionnaires. Height and weight were measured with participants wearing light clothes and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Information on history of cardiovascular disease and cancer was collected through the questionnaires.

2.1.3 Laboratory methods

At baseline, non-fasting blood samples were obtained from an antecubital vein into 5-mL vacutainers (Becton Dickinson, le Point de Claix, France) with EDTA (K_3-EDTA 40 µL, 0.37 mol/L per tube) as an anticoagulant. Samples were centrifuged at 3000 x *g* in room temperature for 10 minutes to prepare platelet poor plasma. The supernatant was then transferred to cryovials (Greiner Laboratechnik, Nütringen, Germany) in 1-mL aliquots and stored at -80°C until analysis.

Measurements of CFB, CFD and C3bBbP, as well as C-reactive protein (CRP), were performed at the Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Norway. Samples were thawed in a 37°C water bath for 5 minutes, and then

centrifuged at 13,500 x *g* for 2 minutes. Plasma levels of CRP, CFB, CFD and C3bBbP were measured in duplicates by enzyme-immunoassays (EIA). The method for C3BbP and CFB has been described in detail previously [25,26]. CRP and CFD levels were measured using commercially available reagents (R&D Systems, Minneapolis, Minnesota, United States). All EIAs were performed in a 384-format using the combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, Vermont, United States) dispenser/washer (EL406). Absorption was read at 450 nm with a wavelength correction set to 540 nm using an EIA plate reader (Synergy H1 Hybrid, BioTek).

2.1.4 Statistical analyses

Statistical analyses were carried out in Stata version 16 (StataCorp LLC, College Station, TX, USA) and R version 4.1.2 (The R Foundation for Statistical Computing, Vienna, Austria). Exposures (CFB, CFD, C3bBbP) and CRP were divided into quartiles according to cut-off levels in the control population.

Baseline characteristics by quartiles of CFB were expressed as means ± standard deviation (SD), medians (25^{th}-75^{th} percentile) or percentages (count), as appropriate. Unconditional logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to quartiles of CFD, CFB, C3bBbP and log(C3bBbP)/log(CFB), with the first quartile serving as the reference category. Analyses were performed for total VTE, and for subgroups of clinical presentation (PE or DVT) and presence of provoking factors (unprovoked or provoked). The ORs were estimated in three different models: Age- and sex-adjusted (Model 1), with additional adjustment for BMI (Model 2) and additional adjustment for CRP (Model 3).

Since fluctuations in plasma biomarker levels during follow-up may introduce regression dilution bias and underestimate associations [27], we additionally ran the analyses with restricted time from baseline to incident VTE, while keeping all controls in the analyses. The regression analysis was set to require at least 10 events, and ORs were estimated at every time point when a new VTE occurred and plotted as a function of this maximum time.

3.1 Results

Characteristics of the cases, controls, and overall are shown in Table 1. The mean age of inclusion overall was 60.4 ± 13.9 years. The proportion of obese, the levels of C3bBbP, and hsCRP was higher among the cases whereas the proportion of smokers was higher among the controls.

Characteristics of the VTE events are shown in Table 2. The mean age at diagnosis was 67.5 ± 14 years, and 52.4% were women. 62.9% presented as DVTs and 37.1% as PE. 60.0% were classified as provoked VTE.

The ORs for VTE according to quartiles of CFB and CFD are shown in Tables 3 and 4, respectively. Neither CFB nor CFD was associated with a risk of overall, provoked, or unprovoked VTE. The ORs were essentially similar across adjustment models. Results of subgroup analyses according to clinical presentation resembled those for total VTE, both for CFB (Supplementary Table 1) and CFD (Supplementary Table 2).

The ORs for VTE according to quartiles of C3bBbP are shown in Table 5. The OR for overall VTE increased across quartiles of C3bBbP (Model 1). For C3bBbP, the OR for Q4 versus Q1 was 1.29 (95% CI 0.91-1.83) in the age- and sex-adjusted model (Model 1). The association was attenuated after adjustment for BMI (Model 2 OR 1.15; 95% CI 0.80-1.64), whereas further adjustment for CRP (Model 3) did not influence the ORs. The association was driven by provoked events, with an OR for Q4 versus Q1 of 1.68 (95% CI 1.08-2.64) in the age-, sex- and BMI-adjusted model (Model 2). Subgroup analyses revealed stronger associations for provoked DVT (Model 2 OR 1.84; 95% CI 1.08-3.16;), while the corresponding OR for provoked PE was 1.46 (95% CI 0.73-2.94; Supplementary Table 3).

To explore whether the fraction of converted CFB could be a better indicator of VTE risk, we also investigated the association of the ratio C3bBbP:CFB with VTE (Table 6). In the

age- and sex-adjusted model, the OR for Q4 versus Q1 was 1.33 (95% CI 0.93-1.89) for overall VTE (Model 1), and 1.41 (95% CI 0.92-2.17) for provoked VTE. The ORs were marginally attenuated after adjustment for BMI and CRP. Subgroup analyses revealed that the association was solely explained by DVT and particularly provoked DVT, with corresponding ORs of 1.61 (95% 1.04-2.49) and 1.77 (95% CI 1.05-2.99), respectively.

To investigate the possibility of underestimated associations because of regression dilution, we estimated the ORs for VTE in subjects in the highest versus lowest quartiles (Q4 vs. Q1) of C3bBbP as a function of time between blood sampling and the VTE event (Figure 1). The ORs for total and provoked VTEs were higher when blood sampling was close to the VTE events, indicating some regression dilution over time.

4.1 Discussion

In the present study, we found no association between factors B and D of the alternative complement pathway and future risk of VTE. We did not find an association between the alternative pathway activation product C3bBbP and overall VTE, but high levels of C3bBbP were associated with risk of future provoked VTE in a subgroup analysis. Furthermore, the C3bBbP:CFB ratio did not show any stronger association with VTE than plasma C3bBbP levels alone.

Results from previous studies provide growing evidence for an association of several components of the complement system with the risk of VTE. In a Danish population-based cohort investigating C3, a central mediator of complement activation through all three pathways, those with C3 levels in the highest tertile had 31% higher VTE risk compared with those in the lowest quartile when potential confounding by age, sex, body mass index and chronic inflammation, assessed by CRP, was taken into account [19]. Furthermore, C3-deficient mice displayed platelet inhibition, prolonged bleeding, and reduced thrombus incidence and size compared to wild-type mice in a flow-restricted inferior vena cava model [28]. Recently, we showed that subjects with high plasma C5 levels had higher VTE risk compared to those with low C5 levels, and the risk was of similar magnitude as the VTE risk associated with plasma C3 levels [20]. Moreover, C5-deficient mice showed reduced fibrin formation, reduced exposure of negatively charged phospholipids on adherent leukocytes and reduced clot burden compared to wild-type mice in a flow-restricted inferior vena cava model [28]. We recently reported that low plasma levels of MBL were associated with lower risk of VTE [22]. Even though the mechanism for the relationship between plasma MBL and VTE risk is unknown, it is reasonable to assume that MBL-associated serine proteases (MASPs) are involved. When MBL recognize an appropriate sugar-containing membrane surface on perturbed host cell, MASPs will be activated [29] and promote procoagulant effects [30–32], and may thereby

contribute to thrombosis formation [33,34]. Finally, in a nested case-control study derived from the general population, we reported that increased complement activation, assessed by plasma levels of the terminal complement complex, was associated with VTE risk, and risk of unprovoked VTE in particular [21].

The associations between CFB or CFD and risk of VTE have, to our knowledge not previously been investigated. Results from previous studies suggest that components of the alternative pathway are associated with the prevalence, severity, and prognosis of arterial cardiovascular diseases. Cross-sectional studies have reported elevated plasma CFB in patients with coronary artery disease [35] and symptomatic aortic stenosis [26]. CFB was also associated with a poorer prognosis in patients with aortic stenosis [26]. CFD has been reported to be a risk marker for myocardial infarction, but not for stroke [36], and CFD was found to be elevated in patients with chronic heart failure compared with health controls [37]. In our nested case-control study derived from the general population, we found that neither plasma CFB nor CFD levels were associated with future risk of VTE.

Previous studies have demonstrated that CFD is a rate-limiting factor for activation of the alternative complement pathway [38], and ex-vivo experiments have demonstrated increased formation of C3bBbP with increasing levels of CFD [37]. We found a parallel increase in plasma CFB but not CFD across categories of plasma C3bBbP levels. Even though CFD and CFB levels were not associated with VTE risk, we found that specific activation of the alternative complement pathway, assessed by plasma C3bBbP, was associated with a higher risk of future provoked VTE, albeit only in a subgroup analysis. The association was only marginally affected by adjustments for demographic factors (age, sex, and BMI) and chronic inflammation, assessed by plasma CRP.

As plasma levels of modifiable risk factors fluctuating over time is expected to underestimate the true risk in prospective studies with long-term follow-up [39], we accordingly

demonstrated that the ORs for VTE among subjects with high compared to low plasma C3bBbP levels increased markedly with shorter time between blood sampling and VTE (i.e., the ORs were higher in the first years of follow-up). The ORs did vary slightly over time, and some of the variation is likely due to random fluctuations (as there will be some degree of imprecision in the estimates, particularly when there are few events). Since the confidence intervals were wide and overlapping (also for comparison of ORs with CIs generated early and late in the study period), there was no significant time-varying effect.

Even though the risk of total VTE by high plasma C3bBbP levels was present when time between blood sampling and development of VTE was short, the most consistent association was observed for the relationship between plasma C3bBbP levels and provoked VTE events. This implies that elevated C3bBbP yields a higher risk of VTE mainly in the presence of other provoking risk factors. Interpreted in the context of the thrombosis potential model, it suggests that elevated plasma C3bBbP increases the baseline risk of thrombosis, but not sufficiently to independently exceed the thrombosis threshold [40]. This contrasts our previous findings on the relationships between elevated plasma C5, MBL, and TCC levels and VTE risk, where associations were particularly strong for unprovoked events [20–22].

Dysfunctional regulation of the alternative complement pathway has innumerable of causes, leading to enhanced activation and increased C3bBbP generation. Most important are genetic mutations of the components, including gain of function mutations in C3 and CFB, or loss of function of CFH, CFI or MCP (CD46) [41]. In addition, individuals occasionally develop autoantibodies against the alternative C3 convertase, called nephritic factors (NeFs), which stabilize the convertase and enhance the activation [42]. It was out of scope of the present study to make a further investigation into possible genetic or molecular mechanisms behind the dysfunction of the patients in this study.

Strengths of our study include the population-based nested case-control design, with VTE-patients and controls derived from the same source population. As the UNN is the only hospital in the study region, a near complete VTE registry can be anticipated. Additionally, the prospective design of our study allows for assumption on the temporal sequence between exposures and outcome and limits the chance of reverse causation. Some limitations of the study also merit consideration. Blood samples from all participants were drawn at baseline in 1994-95 and stored at -80°C for up to 22 years. Although the long storage time could influence plasma levels of complement factors and C3bBbP, this would be similar in cases and controls and thus if any, lead to underestimation of the true effect due to non-differential misclassification. Plasma levels of the exposure variables were only measured at baseline, and individual changes during follow-up may have further introduced regression dilution and underestimation associations [27,39]. Accordingly, when ORs were plotted as a function of time, there was evidence of regression dilution. The many statistical comparisons in our study also increase the likelihood of chance findings, so we must interpret the association between C3bBbP levels and risk of future provoked VTE with caution. Unfortunately, we did not have information on renal function, and could therefore not adjust for it. However, since chronic kidney disease is a modest risk factor for VTE present in only 10% of the population [43], we would not consider renal function to be a strong confounder in this setting. As in all observational studies, potential presence of residual confounding could not be ruled out. Finally, most of our study population was Caucasian, and caution is warranted if findings are extrapolated to other ethnic groups.

4.1.2 Conclusions

In conclusion, the results from our population-based nested case-control study imply that plasma levels of the alternative complement pathway, CFB, CFD, and C3bBbP, are not associated with risk of future VTE, but that specific activation of the alternative complement pathway, assessed by plasma C3bBbP, may be associated with risk of provoked VTE.

Addendum

Funding: This study was supported by an independent grant from Stiftelsen Kristian Gerhard Jebsen, the Norwegian Council on Cardiovascular Disease, the Odd Fellow Foundation, the Simon Fougner Hartmann Family Fund, and the Northern Norway Regional Health Authority (HNF1473-19).

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Tables

Table 1. Distribution of baseline characteristics for cases, controls, and overall. If not otherwise specified, values are means ± standard deviations or percentages with numbers in parentheses.

	Cases (n = 380)	**Controls (n = 804)**	**Overall (n = 1184)**
Age at inclusion	60.3 ± 14.0	60.5 ± 13.9	60.4 ± 13.9
Female	52.4% (199)	54.7% (440)	54.0% (639)
BMI	27.1 ± 4.6	26.0 ± 4.6	26.4 ± 4.3
Obese*	22.6% (86)	13.9% (112)	16.7% (198)
CFB (mg/mL)**	1.0 ± 0.4	1.0 ± 0.4	1.0 ± 0.4
CFD (μg/mL)**	4.3 ± 1.6	4.2 ± 1.6	4.3 ± 1.6
C3bBbP (CAU/mL)**	14.8 ± 4.9	14.4 ± 4.7	14.5 ± 4.8
hsCRP, (μg/mL), median	1.4 ± 1.4	1.1 ± 1.3	1.2 ± 1.4
Smokers	30.3% (115)	32.5% (261)	31.8% (376)
CVD history†	15.8% (60)	15.7% (126)	15.7% (186)
Diabetes†	3.7% (14)	3.9% (31)	3.8% (45)

Abbreviations: BMI, body mass index; hsCRP, high-sensitivity C-reactive protein

†Self-reported history of diabetes or cardiovascular diseases (myocardial infarction, angina pectoris or stroke) at baseline.

*BMI ≥ 30 kg/m2
**Data from control population in visit 1 and shipment 1 (n=483)

Table 2 Characteristics of the venous thromboembolism (VTE) events (n=380).

Age (years), mean ± SD	67 ± 14
Female, % (n)	52.4 (199)
PE, % (n)	37.1 (141)
DVT, % (n)	62.9 (239)
Provoked, % (n)	60.0 (228)
Unprovoked, % (n)	40.0 (152)

DVT, deep vein thrombosis; PE, pulmonary embolism; SD, standard deviation

Table 3 Odds ratios (ORs) with 95 % confidence intervals (CI) for venous thromboembolism (VTE) according to quartiles of complement factor B (CFB).

All VTE	**cases**	**controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	91	208	1 (reference)	1 (reference)	1 (reference)
Q2	104	203	1.17 (0.83-1.64)	1.16 (0.82-1.63)	1.13 (0.80-1-60)
Q3	90	195	1.06 (0.74-1.51)	1.00 (0.69-1.43)	0.95 (0.66-1.37)
Q4	95	198	1.10 (0.77-1.59)	1.03 (0.71-1.48)	0.96 (0.66-1.41)
Provoked VTE					
Q1	56	208	1 (reference)	1 (reference)	1 (reference)
Q2	56	203	1.01 (0.67-1.54)	1.01 (0.66-1.54)	1.00 (0.66-1.53)
Q3	57	195	1.06 (0.69-1.62)	1.01 (0.66-1.54)	0.98 (0.63-1.51)
Q4	59	198	1.06 (0.69-1.65)	1.01 (0.65-1.56)	0.97 (0.62-1.53)
Unprovoked VTE					
Q1	35	208	1 (reference)	1 (reference)	1 (reference)
Q2	48	203	1.41 (0.87-2.27)	1.40 (0.86-2.26)	1.35 (0.83-2.20)
Q3	33	195	1.05 (0.62-1.76)	0.98 (0.58-1.65)	0.91 (0.54-1.55)
Q4	36	198	1.15 (0.68-1.96)	1.06 (0.62-1.80)	0.95 (0.55-1.65)

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein

Table 4 Odds ratios (ORs) with 95 % confidence intervals (CI) for venous thromboembolism (VTE) according to quartiles of complement factor D (CFD).

All VTE	**cases**	**controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	105	203	1.00	1.00	1.00
Q2	79	201	0.75 (0.53-1.07)	0.73 (0.51-1.04)	0.74 (0.52-1.05)
Q3	98	200	0.94 (0.67-1.33)	0.89 (0.63-1.26)	0.88 (0.62-1.24)
Q4	98	200	0.96 (0.67-1.37)	0.86 (0.60-1.23)	0.85 (0.59-1.22)
Provoked VTE					
Q1	62	203	1.00	1.00	1.00
Q2	50	201	0.81 (0.53-1.23)	0.79 (0.51-1.21)	0.79 (0.52-1.21)
Q3	56	200	0.89 (0.59-1.35)	0.85 (0.56-1.29)	0.84 (0.55-1.28)
Q4	60	200	0.94 (0.62-1.44)	0.86 (0.56-1.33)	0.85 (0.55-1.32)
Unprovoked VTE					
Q1	43	203	1.00	1.00	1.00
Q2	29	201	0.67 (0.40-1.13)	0.65 (0.39-1.09)	0.66 (0.39-1.10)
Q3	42	200	1.02 (0.63-1.65)	0.96 (0.59-1.55)	0.93 (0.57-1.51)
Q4	38	200	0.99 (0.60-1.65)	0.86 (0.51-1.44)	0.84 (0.50-1.42)

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein

Table 5 Odds ratios (ORs) with 95 % confidence intervals (CI) for venous thromboembolism (VTE) according to quartiles of C3bBbP.

All VTE	**cases**	**controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	83	204	1 (reference)	1 (reference)	1 (reference)
Q2	80	199	0.99 (0.69-1.42)	0.97 (0.67-1.40)	0.97 (0.67-1.40)
Q3	111	199	1.38 (0.97-1.94)	1.28 (0.90-1.82)	1.27 (0.89-1.80)
Q4	106	202	1.29 (0.91-1.83)	1.15 (0.80-1.64)	1.12 (0.78-1.60)
Provoked VTE					
Q1	39	204	1 (reference)	1 (reference)	1 (reference)
Q2	57	199	1.49 (0.95-2.35)	1.49 (0.94-2.35)	1.49 (0.94-2.35)
Q3	61	199	1.60 (1.02-2.50)	1.53 (0.97-2.41)	1.52 (0.96-2.40)
Q4	71	202	1.83 (1.18-2.83)	1.68 (1.08-2.64)	1.67 (1.06-2.62)
Unprovoked VTE					
Q1	44	204	1 (reference)	1 (reference)	1 (reference)
Q2	23	199	0.54 (0.31-0.93)	0.52 (0.30-0.90)	0.52 (0.30-0.90)
Q3	50	199	1.18 (0.75-1.86)	1.09 (0.69-1.73)	1.07 (0.67-1.69)
Q4	35	202	0.81 (0.50-1.32)	0.70 (0.43-1.16)	0.67 (0.41-1.11)

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein

Table 6 Odds ratios (ORs) with 95 % confidence intervals (CI) for venous thromboembolism (VTE) according to quartiles of log(C3bBbP)/log(CFB)s.

All VTE	**cases**	**controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	81	202	1 (reference)	1 (reference)	1 (reference)
Q2	88	201	1.10 (0.77-1.58)	1.07 (0.74-1.55)	1.09 (0.76-1.58)
Q3	105	199	1.33 (0.93-1.90)	1.29 (0.90-1.85)	1.31 (0.91-1.88)
Q4	106	202	1.33 (0.93-1.89)	1.26 (0.88-1.81)	1.29 (0.90-1.86)
Provoked VTE					
Q1	50	202	1 (reference)	1 (reference)	1 (reference)
Q2	48	201	0.99 (0.64-1.55)	0.97 (0.62-1.53)	0.98 (0.63-1.54)
Q3	63	199	1.35 (0.88-2.07)	1.33 (0.86-2.05)	1.34 (0.87-2.07)
Q4	67	202	1.41 (0.92-2.16)	1.35 (0.88-2.08)	1.37 (0.89-2.12)
Unprovoked VTE					
Q1	31	202	1 (reference)	1 (reference)	1 (reference)
Q2	40	201	1.27 (0.76-2.11)	1.22 (0.73-2.04)	1.26 (0.75-2.11)
Q3	42	199	1.31 (0.78-2.19)	1.25 (0.74-2.10)	1.28 (0.76-2.15)
Q4	39	202	1.20 (0.71-2.02)	1.14 (0.68-1.92)	1.19 (0.70-2.01)

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein

Figure 1 Plot of estimated odds ratios (ORs) for overall venous thromboembolism (VTE), including deep vein thrombosis (DVT). The ORs are plotted as a function of time since blood sampling in Tromsø 4 (T4, 1994-95) to VTE. Subjects with plasma C3bBbP levels in the highest quartile (Q4) were compared to those with in the lowest quartile (Q1, reference category). Analyses were adjusted for age, sex, body mass index (BMI), and high-sensitive C-reactive protein (CRP). The cumulated number of VTE events are shown above the plot.

SUPPLEMENTARY MATERIAL

Supplementary Table 1 Odds ratios (ORs) with 95 % confidence intervals (CI) for deep vein thrombosis (DVT) and pulmonary embolism (PE) according to quartiles of complement factor B (CFB).

All DVT	**n cases**	**n controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	60	208	1 (reference)	1 (reference)	1 (reference)
Q2	68	203	1.16 (0.78-1.73)	1.16 (0.77-1.73)	1.12 (0.75-1.68)
Q3	58	195	1.04 (0.68-1.57)	0.97 (0.64-1.48)	0.91 (0.59-1.40)
Q4	53	198	0.94 (0.61.1.45)	0.87 (0.56-1.36)	0.80 (0.50-1.25)
p for trend			0.684	0.426	0.232
Provoked DVT					
Q1	38	208	1 (reference)	1 (reference)	1 (reference)
Q2	39	203	1.05 (0.64-1.71)	1.05 (0.65-1.72)	1.03 (0.63-1.69)
Q3	39	195	1.08 (0.66-1.78)	1.02 (0.62-1.68)	0.97 (0.58-1-61)
Q4	32	198	0.87 (0.51-1.48)	0.83 (0.49-1.42)	0.77 (0.44-1.34)
p for trend			0.678	0.525	0.359
Unprovoked DVT					
Q1	22	208	1 (reference)	1 (reference)	1 (reference)
Q2	29	203	1.35 (0.75-2.43)	1.35 (0.75-2.43)	1.29 (0.71-2.34)
Q3	19	195	0.95 (0.49-1.82)	0.88 (0.46-1.70)	0.82 (0.42-1.59)
Q4	21	198	1.06 (0.55-2.04)	0.96 (0.49-1.86)	0.85 (0.43-1.68)
p for trend			0.864	0.613	0.388
All PE					
Q1	31	208	1 (reference)	1 (reference)	1 (reference)
Q2	36	203	1.18 (0.70-1.98)	1.17 (0.69-1.97)	1.16 (0.69-1.97)
Q3	32	195	1.10 (0.64-1.89)	1.05 (0.61-1.80)	1.04 (0.60-1.80)
Q4	42	198	1.42 (0.84-2.41)	1.33 (0.78-2.28)	1.31 (0.76-2.28)
p for trend			0.245	0.374	0.423
Provoked PE					
Q1	18	208	1 (reference)	1 (reference)	1 (reference)
Q2	17	203	0.95 (0.47-1.89)	0.94 (0.47-1.89)	0.96 (0.48-1.92)
Q3	18	195	1.02 (0.51-2.07)	0.98 (0.49-1.96)	1.00 (0.50-2.02)
Q4	27	198	1.46 (0.75-2.83)	1.37 (0.70-2.66)	1.42 (0.72-2.81)
p for trend			0.240	0.340	0.301
Unprovoked PE					
Q1	13	208	1 (reference)	1 (reference)	1 (reference)
Q2	19	203	1.50 (0.72-3.13)	1.50 (0.72-3.14)	1.46 (0.70-3.06)
Q3	14	195	1.21 (0.55-2.68)	1.15 (0.52-2.54)	1.09 (0.49-2.43)
Q4	15	198	1.32 (0.59-2.96)	1.25 (0.56-2.80)	1.14 (0.49-2.64)
p for trend			0.640	0.770	0.957

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein

Supplementary Table 2 Odds ratios (ORs) with 95 % confidence intervals (CI) for deep vein thrombosis (DVT) and pulmonary embolism (PE) according to quartiles of complement factor D (CFD).

All DVT	**n cases**	**n controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	62	203	1 (reference)	1 (reference)	1 (reference)
Q2	55	201	0.90 (0.59-1.36)	0.86 (0.57-1.31)	0.87 (0.57-1.32)
Q3	66	200	1.09 (0.73-1.64)	1.03 (0.68-1.55)	1.01 (0.67-1.52)
Q4	56	200	0.94 (0.61-1.44)	0.84 (0.54-1.30)	0.82 (0.53-1.28)
p for trend			0.985	0.616	0.543
Provoked DVT					
Q1	38	203	1 (reference)	1 (reference)	1 (reference)
Q2	36	201	0.96 (0.58-1.58)	0.92 (0.56-1.52)	0.93 (0.56-1.54)
Q3	40	200	1.06 (0.65-1.74)	1.02 (0.62-1.68)	1.00 (0.61-1.65)
Q4	34	200	0.90 (0.53-1.52)	0.83 (0.49-1.42)	0.82 (0.48-1.40)
p for trend			0.805	0.615	0.563
Unprovoked DVT					
Q1	24	203	1 (reference)	1 (reference)	1 (reference)
Q2	19	201	0.80 (0.42-1.50)	0.77 (0.40-1.45)	0.76 (0.40-1.45)
Q3	26	200	1.13 (0.62-2.07)	1.05 (0.57-1.92)	1.01 (0.55-1.86)
Q4	22	200	1.02 (0.53-1.93)	0.85 (0.44-1.64)	0.83 (0.43-1.60)
p for trend			0.720	0.871	0.780
All PE					
Q1	43	203	1 (reference)	1 (reference)	1 (reference)
Q2	24	201	0.55 (0.32-0.94)	0.55 (0.32-0.94)	0.55 (0.32-0.94)
Q3	32	200	0.74 (0.44-1.23)	0.70 (0.42-1.16)	0.69 (0.41-1.15)
Q4	42	200	0.99 (0.60-1.63)	0.89 (0.53-1.47)	0.88 (0.53-1.46)
p for trend			0.879	0.753	0.724
Provoked PE					
Q1	24	203	1 (reference)	1 (reference)	1 (reference)
Q2	14	201	0.57 (0.29-1.14)	0.57 (0.29-1.14)	0.57 (0.29-1.14)
Q3	16	200	0.63 (0.32-1.24)	0.59 (0.30-1.17)	0.60 (0.30-1.18)
Q4	26	200	1.00 (0.54-1.87)	0.88 (0.47-1.66)	0.89 (0.47-1.67)
p for trend			0.958	0.715	0.726
Unprovoked PE					
Q1	19	203	1 (reference)	1 (reference)	1 (reference)
Q2	10	201	0.52 (0.24-1.16)	0.52 (0.23-1.15)	0.52 (0.24-1.15)
Q3	16	200	0.89 (0.44-1.81)	0.84 (0.41-1.72)	0.82 (0.40-1.68)
Q4	16	200	0.97 (0.46-2.03)	0.88 (0.41-1-84)	0.86 (0.41-1.81)
p for trend			0.855	0.916	0.860

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein

Supplementary Table 3 Odds ratios (ORs) with 95 % confidence intervals (CI) for deep vein thrombosis (DVT) and pulmonary embolism (PE) according to quartiles of C3bBbP.

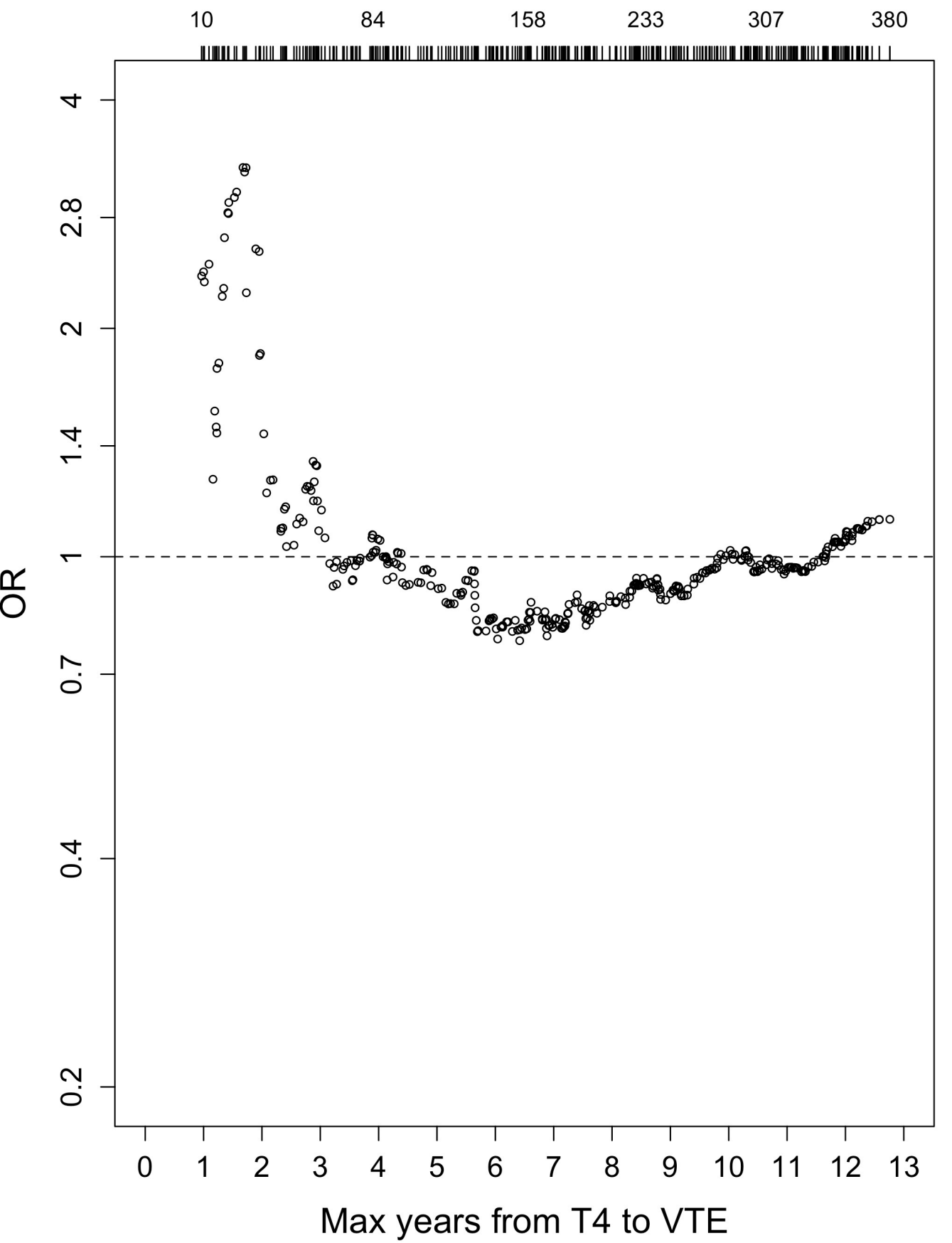
All DVT	**n cases**	**n controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	50	204	1 (reference)	1 (reference)	1 (reference)
Q2	47	199	0.97 (0.62-1.50)	0.96 (0.61-1.50)	0.96 (0.61-1.50)
Q3	74	199	1.52 (1.01-2.92)	1.46 (0.96-2.21)	1.43 (0.95-2.17)
Q4	68	202	1.38 (0.91-2.09)	1.26 (0.82-1.92)	1.22 (0.79-1.87)
p for trend			0.034	0.112	0.157
Provoked DVT					
Q1	25	204	1 (reference)	1 (reference)	1 (reference)
Q2	36	199	1.48 (0.86-2.55)	1.52 (0.87-2.64)	1.51 (0.87-2.62)
Q3	40	199	1.64 (0.96-2.81)	1.64 (0.95-2.83)	1.62 (0.94-2.80)
Q4	47	202	1.90 (1.13-3.21)	1.84 (1.08-3.16)	1.80 (1.05-3.10)
p for trend			0.017	0.030	0.040
Unprovoked DVT					
Q1	25	204	1 (reference)	1 (reference)	1 (reference)
Q2	11	199	0.46 (0.22-0.95)	0.43 (0.21-0.91)	0.43 (0.21-0.91)
Q3	34	199	1.41 (0.81-2.46)	1.30 (0.74-2.28)	1.27 (0.72-2.22)
Q4	21	202	0.86 (0.46-1.58)	0.73 (0.39-1.36)	0.70 (0.37-1.31)
p for trend			0.581	0.973	0.910
All PE					
Q1	33	204	1 (reference)	1 (reference)	1 (reference)
Q2	33	199	1.03 (0.61-1.73)	0.99 (0.58-1.67)	0.98 (0.58-1.67)
Q3	37	199	1.15 (0.69-1.91)	1.05 (0.63-1.76)	1.04 (0.62-1.75)
Q4	38	202	1.16 (0.70-1.92)	1.02 (0.61-1.70)	1.00 (0.60-1.69)
p for trend			0.501	0.898	0.937
Provoked PE					
Q1	14	204	1 (reference)	1 (reference)	1 (reference)
Q2	21	199	1.52 (0.75-3.07)	1.45 (0.71-2.95)	1.45 (0.72-2.95)
Q3	21	199	1.51 (0.74-3.05)	1.37 (0.67-2.79)	1.38 (0.68-2.82)
Q4	24	202	1.69 (0.85-3.37)	1.46 (0.73-2.94)	1.48 (0.73-2.98)
p for trend			0.169	0.366	0.352
Unprovoked PE					
Q1	19	204	1 (reference)	1 (reference)	1 (reference)
Q2	12	199	0.66 (0.31-1.39)	0.64 (0.30-1.36)	0.63 (0.30-1.35)
Q3	16	199	0.87 (0.44-1.75)	0.82 (0.41-1.66)	0.80 (0.39-1.61)
Q4	14	202	0.76 (0.37-1.55)	0.68 (0.33-1.40)	0.65 (0.31-1.35)
p for trend			0.592	0.403	0.342

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein

Supplementary Table 4 Odds ratios (ORs) with 95 % confidence intervals (CI) for deep vein thrombosis (DVT) and pulmonary embolism (PE) according to quartiles of log(C3bBbP)/log(CFB).

All DVT	**n cases**	**n controls**	**OR model 1**	**OR model 2**	**OR model 3**
Q1	44	202	1 (reference)	1 (reference)	1 (reference)
Q2	51	201	1.17 (0.75-1.84)	1.16 (0.74-1.84)	1.19 (0.75-1.88)
Q3	74	199	1.73 (1.12-2.66)	1.70 (1.10-2.63)	1.74 (1.12-2.69)
Q4	70	202	1.61 (1.04-2.49)	1.57 (1.01-2.43)	1.61 (1.04-2.51)
p for trend			0.010	0.017	0.012
Provoked DVT					
Q1	27	202	1 (reference)	1 (reference)	1 (reference)
Q2	30	201	1.14 (0.65-1.99)	1.15 (0.66-2.03)	1.18 (0.67-2.07)
Q3	45	199	1.76 (1.04-2.97)	1.78 (1.04-3.03)	1.82 (1.06-3.11)
Q4	46	202	1.77 (1.05-2.99)	1.76 (1.03-3.00)	1.81 (1.06-3.08)
p for trend			0.012	0.014	0.011
Unprovoked DVT					
Q1	17	202	1 (reference)	1 (reference)	1 (reference)
Q2	21	201	1.22 (0.62-2.40)	1.17 (0.59-2.29)	1.20 (0.61-2.37)
Q3	29	199	1.68 (0.88-3.20)	1.58 (0.83-3.03)	1.63 (0.85-3.12)
Q4	24	202	1.37 (0.71-2.66)	1.30 (0.67-2.53)	1.36 (0.70-2.66)
p for trend			0.250	0.3285	0.271
All PE					
Q1	37	202	1 (reference)	1 (reference)	1 (reference)
Q2	37	201	1.01 (0.61-1.67)	0.96 (0.58-1.59)	0.97 (0.58-1.61)
Q3	31	199	0.85 (0.50-1.45)	0.83 (0.49-1.40)	0.83 (0.49-1.42)
Q4	36	202	0.98 (0.59-1.64)	0.92 (0.55-1.53)	0.93 (0.55-1.56)
p for trend			0.803	0.637	0.669
Provoked PE					
Q1	23	202	1 (reference)	1 (reference)	1 (reference)
Q2	18	201	0.82 (0.43-1.58)	0.77 (0.37-1.48)	0.76 (0.39-1.47)
Q3	18	199	0.85 (0.44-1.66)	0.81 (0.42-1.58)	0.81 (0.42-1.58)
Q4	21	202	0.98 (0.52-1.85)	0.90 (0.48-1.71)	0.89 (0.47-1.70)
p for trend			0.979	0.815	0.798
Unprovoked PE					
Q1	14	202	1 (reference)	1 (reference)	1 (reference)
Q2	19	201	1.32 (0.64-2.71)	1.27 (0.61-2.62)	1.30 (0.62-2.69)
Q3	13	199	0.87 (0.39-1.94)	0.85 (0.38-1.90)	0.87 (0.39-1.94)
Q4	15	202	1.00 (0.46-2.16)	0.95 (0.44-2.06)	0.99 (0.46-2.15)
p for trend			0.724	0.647	0.710

Model 1: adjusted for age and sex; Model 2: Model 1 + body mass index; Model 3: Model 2 + C-reactive protein


10
84
158
233
307
380
4
2.8
2
1.4
1
0.7
0.4
0.2
OR
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Max years from T4 to VTE