Elevated plasma D-dimer levels are associated with risk of future incident venous thromboembolism

Running head: D-dimer and incident venous thromboembolism

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ABSTRACT

Background: D-dimer, a global biomarker for activation of the coagulation and fibrinolysis systems, is useful in assessing individual risk of venous thromboembolism (VTE) recurrence. However, there is limited information on the association between D-dimer and risk of a first lifetime VTE event.

Objectives: To investigate the association between plasma D-dimer levels and risk of future incident VTE.

Methods: A population-based nested case-control study, comprising 414 VTE patients and 843 randomly selected age- and sex-matched controls, was derived from the Tromsø Study (1994-2007). D-dimer was measured in plasma samples collected at cohort baseline (1994-95). Odds ratios (ORs) for VTE with 95% confidence intervals (CIs) were estimated according to quartile cut-offs of D-dimer levels determined in controls.

Results: The risk of VTE increased across quartiles of D-dimer levels (P_{trend}=0.014) in the age- and sex-adjusted model. Participants with plasma D-dimer levels in the highest quartile (≥152 ng/mL) had an OR for VTE of 1.65 (95% CI 1.14-2.40) compared with those in the lowest quartile (<94 ng/mL). The ORs were marginally attenuated after additional adjustment for body mass index (BMI) (OR 1.51, 95% CI 1.04-2.20) and C-reactive protein (CRP) (OR 1.34, 95% CI 0.90-1.98). Similar results were obtained for VTE subgroups, i.e. deep vein thrombosis, pulmonary embolism, and provoked/unprovoked events.

Conclusion: Our results indicate that elevated plasma D-dimer levels are associated with increased risk of incident VTE. However, the attenuation of risk estimates upon additional adjustment for BMI and CRP suggests that D-dimer partly reflects underlying conditions associated with obesity and an inflammatory state.

Keywords: D-dimer; inflammation; obesity; venous thromboembolism; deep vein thrombosis

INTRODUCTION

Venous thromboembolism (VTE), a term used to collectively name deep vein thrombosis (DVT) and pulmonary embolism (PE), is a multicausal disease occurring in 1-2 per 1000 individuals annually [1]. VTE is associated with serious short- and long-term complications including post-thrombotic syndrome, post-PE syndrome, recurrence and death [1-3]. Despite an increase in thromboprophylaxis use, the incidence of VTE has not changed or has even slightly increased during the last decades [4-6]. To mitigate the health burden of VTE, there is a need to provide insights into novel biomarkers in order to improve risk stratification and pursue targeted VTE prevention.

D-dimer, a fibrin degradation product, is a global biomarker for activation of the coagulation and fibrinolysis systems and seems also to reflect activation of inflammatory pathways [7]. A D-dimer value below cut-off in patients with low clinical probability of VTE is used in clinical algorithms to exclude further radiological procedures in patients with suspected VTE [8, 9]. In addition, multiple studies have found that elevated levels of D-dimer measured mainly after stopping anticoagulant treatment are associated with increased risk of recurrence in patients with unprovoked VTE [10-12]. Conversely, only a few studies have investigated the association between baseline D-dimer levels and the risk of a future first lifetime VTE in the general population [13-16]. In these studies, a higher baseline D-dimer value was associated with increased risk of incident VTE [13-16].

Several established risk factors for VTE have been shown to influence D-dimer levels, including obesity, cancer, inflammatory diseases, and genetic factors (e.g. factor V Leiden and prothrombin G20210A) [13, 14, 17-19]. Of note, data from a genome-wide association study revealed that the proportion of variation in plasma D-dimer explained by genetic variants located in hemostatic factor genes was modest [20]. Thus, a high D-dimer may

particularly reflect acquired underlying conditions that predispose to VTE, such as obesity and inflammatory diseases [14, 18, 19]. However, whether a state of chronic low-grade inflammation explains, at least in part, the association between D-dimer and incident VTE remains unclear. In the present study, we therefore aimed to investigate the association between baseline plasma D-dimer levels and incident VTE in a population-based nested case-control study while adjusting for high-sensitivity C-reactive protein (CRP), which is considered a sensitive downstream marker of inflammation. Further, we extended this investigation to specific VTE subgroups (i.e. DVT, PE, and provoked and unprovoked VTE) and assessed the potential association of D-dimer with VTE over time by taking into account the time elapsed between blood sampling at baseline and the occurrence of VTE events.

METHODS

Study population

The Tromsø Study is a population-based cohort with repeated health surveys of the residents of Tromsø in Norway, details of which have been described elsewhere [21]. Briefly, all inhabitants aged ≥25 years living in the municipality of Tromsø were invited to take part in the fourth survey (Tromsø 4, conducted in 1994-1995), and a total of 27,158 subjects participated (77% response rate). These participants were followed from the inclusion date in the survey (1994-1995) until an incident VTE, migration, death, or end of follow-up (September 1, 2007), whatever came first. All potential first lifetime VTE events were identified by a thorough search of the hospital discharge diagnosis registry, the radiology procedure registry and the autopsy registry from the University Hospital of North Norway (UNN), which is the only provider of hospital care in the region of Tromsø. Trained personnel confirmed and recorded each VTE event by extensively reviewing medical records, as

previously described [22]. A VTE was confirmed based on the presence of signs and symptoms of DVT or PE in combination with objective confirmation by imagining methods, which resulted in treatment initiation [22].

There were 462 individuals who developed a first lifetime VTE during the follow-up period (1994-2007). To establish a nested case-control study, for each VTE case, two age- and sex-matched controls (n=924), who were alive at the index date of the thrombotic event, were randomly sampled from the parent cohort [23, 24]. From this population, we excluded 48 cases and 81 controls due to insufficient quality of plasma samples. Therefore, 414 VTE cases and 843 controls were included in the final analysis of the nested case-control study (Fig. 1). In this design, the temporal sequence between exposure and outcome is preserved, since D-dimer was measured in blood samples collected at inclusion of the parent cohort in 1994-95. The regional committee for medical and health research ethics approved the study, and all participants provided written informed consent.

Classification of VTE events

A VTE was classified as provoked or unprovoked depending on the presence of provoking factors closely preceding the VTE diagnosis. A VTE event was defined as provoked if one or more of the following provoking factors were present: trauma, surgery, or an acute medical condition (acute infection, acute myocardial infarction, or acute ischemic stroke) within 8 weeks before the event, active cancer at the time of VTE diagnosis, immobilization (confinement to a wheelchair or bed rest for longer than 3 days within the past 8 weeks, or long distance travel lasting 4 hours or longer in the past 14 days), or other factors described as provoking in the medical record by a physician (e.g. intravascular catheter).

Baseline measurements

Weight (to the nearest 0.5 kg) and height (to the nearest cm) were measured with subjects wearing light clothing and no shoes [22]. Body mass index (BMI) was calculated as weight divided by the square of height in meters (kg/m^2). Baseline information on history of previous cancer and arterial cardiovascular disease (CVD) events (i.e. myocardial infarction, angina pectoris, stroke and transient ischemic attack) was obtained from a self-administered questionnaire [23, 24].

Blood sampling and storage

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 as anticoagulant (K_3-EDTA 40 µL, 0.37 mol/L per tube), as previously described elsewhere [23, 24]. We prepared platelet-poor plasma by centrifugation at 3000*g* for 10 minutes at room temperature. The supernatant was then transferred into cryovials (Greiner Bio-One, Nürtingen, Germany) in 1-mL aliquots and stored at -80°C.

Measurements of D-dimer and high-sensitivity CRP

To measure D-dimer and high-sensitivity CRP in plasma**,** samples were initially thawed in a water bath at 37°C for 5 minutes and then subjected to centrifugation at 13500*g* for 2 minutes in order to obtain platelet-free plasma [24]. D-dimer was measured by an enzyme-immunoassay (EIA) method, in which a monoclonal antibody (S4H9) [25] was used for coating together with a monoclonal horseradish peroxidase-conjugated antibody for detection (ab24474, Abcam, Cambridge, United Kingdom), as previously described [26]. Parallel diluted samples of known concentration were used as standards. The intra- and

inter-assay coefficients of variation of D-dimer were 2.1% and 4.3%, respectively. High-sensitivity CRP was measured by EIA using commercially available reagents (R&D Systems, Minneapolis, MN), with intra- and inter-assay coefficients of variation of 2.6% and 9.1%, respectively [24].

Statistical analysis

Statistical analyses were performed using Stata version 16 (StataCorp LLC, Texas, USA) and R version 4.0.5 (The R Foundation for Statistical Computing, Vienna, Austria). D-dimer was categorized according to quartile cut-offs determined in controls (<94, 94-119, 119-152, ≥152 ng/mL). We used unconditional logistic regression to estimate odds ratios (ORs) for VTE with 95% confidence intervals (CIs) according to quartiles of D-dimer, and the lowest quartile served as the reference category. P-values for linear trend across increasing quartiles of D-dimer levels were calculated. The association between D-dimer levels and VTE was adjusted for age and sex in model 1. BMI and inflammation, as reflected by high-sensitivity CRP, can influence both D-dimer levels [14, 18, 19] and VTE risk [27, 28], thereby acting as potential confounders in the association between D-dimer and VTE. We therefore added BMI to a second model and high-sensitivity CRP was further included in a third model.

In addition, separate analyses were conducted for the VTE subgroups (i.e. DVT, PE± DVT, provoked and unprovoked events). Cancer and arterial CVD have been reported to be associated with both D-dimer levels [17, 29] and VTE risk [30-32], so we assessed the association between D-dimer and overall VTE after excluding participants with self-reported history of arterial CVD or cancer at cohort baseline.

Results based only on baseline measurement of D-dimer could be affected by regression dilution bias due to the long follow-up time in the parent cohort [33]. To address

this, we took into account the time elapsed between blood sampling at baseline for D-dimer measurement and the occurrence of VTE events. For overall VTE, we performed analyses that restricted the maximum follow-up time for the VTE cases, while keeping all controls in the analyses [23, 24]. The logistic regression analyses on time restrictions were set to require the occurrence of at least 10 events of VTE. The ORs were generated at every time point a new VTE occurred and plotted as a function of this maximum time.

RESULTS

The distribution of baseline characteristics across quartiles of plasma D-dimer levels is described in Table 1. The mean age and BMI, median CRP levels, and the proportion of subjects with self-reported history of arterial CVD increased across D-dimer quartiles. The proportion of men was somewhat lower in the highest two quartiles compared with the two lowest quartiles. The proportion of participants with self-reported history of cancer was low and showed no consistent trend across quartiles of D-dimer. The characteristics of the VTE patients are displayed in Table 2. The mean age at the time of the VTE event was 67.8 ± 13.6 years, 48.3% were men, and most of the VTE patients presented with DVT (62.6%) and provoked events (58.2%).

The ORs for VTE across quartiles of plasma D-dimer are shown in Table 3. In the age- and sex-adjusted model, the ORs for overall VTE increased across D-dimer quartiles (P_{trend} = 0.014). Participants with plasma D-dimer levels in the highest quartile (≥152 ng/mL) had an OR for VTE of 1.65 (95% CI 1.14-2.40) compared with those with D-dimer in the lowest quartile (<94 ng/mL). Risk estimates for overall VTE were marginally attenuated after adding BMI to model 2 (OR 1.51, 95% CI 1.04-2.20), and a further attenuation was noted when CRP was added to model 3 (OR 1.34, 95% CI 0.90-1.98).

Next, we analyzed the association between D-dimer levels and thrombosis risk in VTE subgroups (Table 3). In the age- and sex-adjusted model, the risk estimates increased with increasing D-dimer levels in DVT (P_{trend} = 0.012) and unprovoked VTE (P_{trend} = 0.025), with ORs of 1.80 (95% CI 1.16-2.80) and 1.64 (95% CI 1.00-2.70) for the highest vs the lowest quartile, respectively. Compared with the lowest quartile, the ORs for the highest quartile were 1.44 (95% CI 0.83-2.48) for PE and 1.69 (95% CI 1.05-2.71) for provoked VTE. Similar to the main analysis for overall VTE, further adjustment for BMI and CRP attenuated the risk estimates in the subgroup analyses (Table 3) and in the sensitivity analysis where subjects with self-reported history at baseline of arterial CVD and cancer were excluded (Supplemental Tables 1-2).

To assess the possibility of underestimating the true association because of regression dilution bias, we estimated ORs (highest vs lowest quartile of D-dimer) for overall VTE as a function of time between blood sampling and VTE events. As depicted in Fig. 2, the ORs for VTE comparing the highest vs lowest quartile of D-dimer were higher with shortened time between blood sampling and VTE events, especially within the first 3 years of follow-up.

DISCUSSION

In this nested case-control study derived from the general population, we investigated whether plasma D-dimer levels were associated with incident VTE. The ORs for VTE increased across quartiles of D-dimer, and participants with D-dimer levels in the highest quartile had an almost 1.7-fold increased ORs for VTE compared with those in the lowest quartile in models adjusted for age and sex. However, the risk estimates were attenuated upon additional adjustment for BMI and CRP. Similar findings were observed for VTE subgroups and when participants with self-reported history of arterial CVD or cancer at

baseline were excluded. For the regression dilution analysis, the OR for VTE by plasma D-dimer levels (highest vs lowest quartile) appeared to increase with shortened time between blood sampling and VTE events. Our findings suggest that the association between D-dimer and risk of incident VTE is partially explained by body fat, as reflected by BMI, and an underlying inflammatory state, as reflected by CRP levels.

D-dimer has been extensively investigated as a biomarker for risk of recurrence in patients with unprovoked VTE. In previous systematic reviews, the prognostic value of D-dimer related to recurrent VTE was assessed in patients who had completed the initial anticoagulant treatment after an unprovoked event [10, 11]. Results from these studies indicated that an elevated D-dimer was associated with increased risk of recurrent VTE and could be useful to assess the individual risk of recurrence [10-12]. Additionally, results from the Tromsø study showed that D-dimer measured at VTE diagnosis had the ability to discriminate patients with high and low risk of recurrence [34].

In contrast to recurrent VTE, fewer studies have evaluated the association between baseline D-dimer and incident VTE, especially in the general population. To the best of our knowledge, this is the first study to stratify on DVT and PE and to perform a detailed analysis on the role of inflammation when assessing the association between D-dimer and risk of incident VTE in a population-based cohort. The relationship of baseline D-dimer with incident VTE was initially addressed in the Longitudinal Investigation of Thromboembolism Etiology (LITE), which is a pooled study composed of two community-based cohorts, the Atherosclerosis Risk in Communities (ARIC) Study and the Cardiovascular Health Study (CHS). Using a nested case-control design that included 307 VTE cases and 616 controls, investigators found that participants with higher D-dimer levels had an increased risk of VTE in models adjusted for age, race, sex, BMI, factor V Leiden, prothrombin G20210A, and

factor VIII [13]. The authors later expanded plasma D-dimer measurements in the ARIC population and found that D-dimer levels were related to increased risk of future VTE in a dose-response manner even with more than 10 years of follow-up, in analyses adjusted for age, race, and sex [14]. Additionally, results from the Multi-Ethnic Study of Atherosclerosis (MESA), a prospective cohort with a median of 14 years of follow-up, showed that elevated D-dimer levels were associated with increased risk of incident VTE independently of age, sex, race/ethnicity, education, field center, BMI, diabetes, and estimated glomerular filtration rate [16]. In a nested case-control study (215 VTE cases and 867 controls) derived from the Women's Health Initiative hormone trials, which comprised postmenopausal women, Cushman et al. investigated several biomarkers with regards to risk of future VTE [35]. The authors found that high plasma D-dimer levels were associated with increased risk of VTE in analyses adjusted for age, race, BMI, treatment assignment, self-reported VTE and hysterectomy at screening. Finally, the predictive ability of D-dimer was evaluated in the Framingham Heart Study, a cohort comprising 3120 participants, of whom 139 experienced an incident VTE during a median follow-up of 16 years. Among several tested biomarkers, higher D-dimer levels were associated with increased risk of VTE in multivariable-adjusted models [15]. Our results are in line with the previous studies [13-16, 35], as we found that higher D-dimer levels were associated with an increased risk of incident VTE, but according to our results, such an association was partly explained by BMI and inflammation. In fact, none of the aforementioned studies [13-16, 35] made adjustment for low-grade systemic inflammation, as assessed by CRP levels.

D-dimer is a global biomarker for activation of the coagulation and fibrinolysis systems and its levels are influenced by both environmental and genetic factors [36, 37]. Indeed, twin studies have found a wide range of heritability estimates for D-dimer levels,

spanning from 23% to 65% in Northern Europeans [36-39]. Further, data from a genome-wide association study revealed that D-dimer levels were in part explained by genetic variants located in hemostatic factor genes that encode key procoagulant factors (tissue factor, factor V, and fibrinogen). However, these genetic variants accounted for only 1.8% (range 0%-4.2%) of the total variance in D-dimer phenotype [20], suggesting that environmental factors play an important role in determining D-dimer levels.

An elevated D-dimer can be a marker of acquired VTE risk factors. Obesity, often assessed by an increased BMI, is a well-known risk factor for VTE [27]. Folsom et al. showed in the ARIC study that BMI increased across quintiles of D-dimer [14], similarly to what we observed in our analysis. Additionally, in a twin study, Ariens et al. found that BMI had a small but significant effect on D-dimer concentration, explaining 2.7% of its variance [36]. When we added BMI to our regression models, D-dimer remained associated with overall VTE across quartiles, but risk estimates were somewhat attenuated, implying the presence of confounding due to body fat. Upon additional adjustment for CRP, the risk estimates for overall VTE decreased further, and similar effects were observed for all VTE subgroups and in sensitivity analyses. As CRP is a sensitive downstream marker of inflammation, the risk attenuation by adjustment for CRP may indicate that D-dimer reflects underling conditions that increase the risk of VTE. In fact, diseases associated with chronic low-grade inflammation, such as cancer, autoimmune and inflammatory diseases, chronic infections and kidney disease, have shown to be associated with higher D-dimer levels [17-19, 40, 41] and increased risk of VTE [30, 42-44]. As an elevated D-dimer may reflect the sum of several underlying conditions that increase the risk of VTE, D-dimer could potentially be useful as a global biomarker to identify those at an especially high risk of incident VTE. However, it is important to address that our study was not designed to investigate the ability of D-dimer to

discriminate subjects at high and low risk of future incident VTE. Thus, future prediction studies aimed at assessing cut-off values of D-dimer that could be applied to aid risk stratification of a first lifetime VTE would be warranted.

Strengths of this study include the nested case-control study design, where a large population of VTE cases and controls were selected from the same population-based cohort. D-dimer was measured in blood samples collected at cohort inclusion (1994-95) from subjects who had no prior history of VTE and were not suspected of having an acute VTE at blood sampling. Hence, given the prospective nature of our study design, we could make assumptions on the temporal sequence between D-dimer and incident VTE. Further, since there is only one hospital in the study area that provides VTE diagnostics and treatment, there was a low likelihood of VTE cases being missed or misclassified. Some limitations of this study merit attention. Blood samples were drawn in 1994-1995 and stored for more than 20 years before analyses, and this could potentially have affected D-dimer 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. In addition, intra-individual variation in D-dimer levels during follow-up could have contributed to attenuation of the true association [33]. The latter is likely, as ORs for VTE by high levels of D-dimer were higher with shortened time between blood sampling and VTE events. However, this analysis should be interpreted with caution because there were few events within the first years after blood sampling. Because information on prothrombotic genotypes, such as factor V Leiden and prothrombin G20210A mutation, was only available for a small proportion of the study population, we could not adjust for these factors when investigating the association between D-dimer and incident VTE. As previously described,

genetic variants accounted for only a small proportion of the total variance in D-dimer levels [20]. Moreover, results from the ARIC study showed that risk estimates for VTE across quintiles of D-dimer were only marginally attenuated after additional adjustment for common prothrombotic genotypes [14]. Taken together, adjustment for prothrombotic genotypes would most likely not influence our risk estimates notably. Finally, the majority of study participants were white, and therefore caution is needed when extrapolating our findings to other ethnicities.

In conclusion, we found that elevated plasma levels of D-dimer were associated with increased risk of future incident VTE. However, the attenuation of risk estimates upon adjustment for BMI and CRP suggests that D-dimer partly reflects underlying conditions associated with obesity and an inflammatory state.

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CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTIONS

E-S Hansen analyzed data, interpreted the results, and drafted the manuscript. F. Rinde and M. S. Edvardsen interpreted the results and revised the manuscript. K. Hindberg provided statistical support, interpreted the results, and revised the manuscript. N. Latysheva, P. Aukrust, T. Ueland and A. E. Michelsen performed laboratory analyses 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, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

REFERENCES

1. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrøm J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost. 2007;5(4):692-9.
2. Schulman S, Lindmarker P, Holmstrom M, Larfars G, Carlsson A, Nicol P, et al. Post-thrombotic syndrome, recurrence, and death 10 years after the first episode of venous thromboembolism treated with warfarin for 6 weeks or 6 months. J Thromb Haemost. 2006;4(4):734-42.
3. Klok FA, van der Hulle T, den Exter PL, Lankeit M, Huisman MV, Konstantinides S. The post-PE syndrome: a new concept for chronic complications of pulmonary embolism. Blood Rev. 2014;28(6):221-6.
4. Heit JA, Crusan DJ, Ashrani AA, Petterson TM, Bailey KR. Effect of a near-universal hospitalization-based prophylaxis regimen on annual number of venous thromboembolism events in the US. Blood. 2017;130(2):109-14.
5. Arshad N, Isaksen T, Hansen JB, Braekkan SK. Time trends in incidence rates of venous thromboembolism in a large cohort recruited from the general population. Eur J Epidemiol. 2017;32(4):299-305.
6. Münster AM, Rasmussen TB, Falstie-Jensen AM, Harboe L, Stynes G, Dybro L, et al. A changing landscape: Temporal trends in incidence and characteristics of patients hospitalized with venous thromboembolism 2006-2015. Thromb Res. 2019;176:46-53.
7. Lippi G, Franchini M, Targher G, Favaloro EJ. Help me, Doctor! My D-dimer is raised. Ann Med. 2008;40(8):594-605.
8. Wells PS, Anderson DR, Rodger M, Forgie M, Kearon C, Dreyer J, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. N Engl J Med. 2003;349(13):1227-35.
9. Righini M, Perrier A, De Moerloose P, Bounameaux H. D-Dimer for venous thromboembolism diagnosis: 20 years later. J Thromb Haemost. 2008;6(7):1059-71.
10. Bruinstroop E, Klok FA, Van De Ree MA, Oosterwijk FL, Huisman MV. Elevated D-dimer levels predict recurrence in patients with idiopathic venous thromboembolism: a meta-analysis. J Thromb Haemost. 2009;7(4):611-8.
11. Verhovsek M, Douketis JD, Yi Q, Shrivastava S, Tait RC, Baglin T, et al. Systematic review: D-dimer to predict recurrent disease after stopping anticoagulant therapy for unprovoked venous thromboembolism. Ann Intern Med. 2008;149(7):481-90, w94.
12. Douketis J, Tosetto A, Marcucci M, Baglin T, Cushman M, Eichinger S, et al. Patient-level meta-analysis: effect of measurement timing, threshold, and patient age on ability of D-

dimer testing to assess recurrence risk after unprovoked venous thromboembolism. Ann Intern Med. 2010;153(8):523-31.
13. Cushman M, Folsom AR, Wang L, Aleksic N, Rosamond WD, Tracy RP, et al. Fibrin fragment D-dimer and the risk of future venous thrombosis. Blood. 2003;101(4):1243-8.
14. Folsom AR, Alonso A, George KM, Roetker NS, Tang W, Cushman M. Prospective study of plasma D-dimer and incident venous thromboembolism: The Atherosclerosis Risk in Communities (ARIC) Study. Thromb Res. 2015;136(4):781-5.
15. Puurunen MK, Enserro D, Xanthakis V, Larson MG, Benjamin EJ, Tofler GH, et al. Biomarkers for the prediction of venous thromboembolism in the community. Thromb Res. 2016;145:34-9.
16. Evensen LH, Folsom AR, Pankow JS, Hansen JB, Allison MA, Cushman M, et al. Hemostatic factors, inflammatory markers and risk of incident venous thromboembolism: The Multi-Ethnic Study of Atherosclerosis. J Thromb Haemost. 2021;19(7):1718-28.
17. Douma RA, van Sluis GL, Kamphuisen PW, Söhne M, Leebeek FW, Bossuyt PM, et al. Clinical decision rule and D-dimer have lower clinical utility to exclude pulmonary embolism in cancer patients. Explanations and potential ameliorations. Thromb Haemost. 2010;104(4):831-6.
18. Ingegnoli F, Fantini F, Favalli EG, Soldi A, Griffini S, Galbiati V, et al. Inflammatory and prothrombotic biomarkers in patients with rheumatoid arthritis: effects of tumor necrosis factor-alpha blockade. J Autoimmun. 2008;31(2):175-9.
19. Hayat M, Ariëns RA, Moayyedi P, Grant PJ, O'Mahony S. Coagulation factor XIII and markers of thrombin generation and fibrinolysis in patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol. 2002;14(3):249-56.
20. Smith NL, Huffman JE, Strachan DP, Huang J, Dehghan A, Trompet S, et al. Genetic predictors of fibrin D-dimer levels in healthy adults. Circulation. 2011;123(17):1864-72.
21. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: the Tromso Study. Int J Epidemiol. 2012;41(4):961-7.
22. Braekkan SK, Borch KH, Mathiesen EB, Njølstad I, Wilsgaard T, Hansen JB. Body height and risk of venous thromboembolism: The Tromsø Study. Am J Epidemiol. 2010;171(10):1109-15.
23. Høiland, II, Liang RA, Braekkan SK, Pettersen K, Ludviksen JK, Latysheva N, et al. Complement activation assessed by the plasma terminal complement complex and future risk of venous thromboembolism. J Thromb Haemost. 2019;17(6):934-43.
24. Liang RA, Høiland, II, Ueland T, Aukrust P, Snir O, Hindberg K, et al. Plasma levels of mannose-binding lectin and future risk of venous thromboembolism. J Thromb Haemost. 2019;17(10):1661-9.
25. Bennick A, Haddeland U, Brosstad F. D-dimer specific monoclonal antibodies react with fibrinogen aggregates. Thromb Res. 1996;82(2):169-76.
26. Hansen ES, Hindberg K, Latysheva N, Aukrust P, Ueland T, Hansen JB, et al. Plasma levels of growth differentiation factor 15 are associated with future risk of venous thromboembolism. Blood. 2020;136(16):1863-70.
27. Borch KH, Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Stormer J, et al. Anthropometric measures of obesity and risk of venous thromboembolism: the Tromso study. Arterioscler Thromb Vasc Biol. 2010;30(1):121-7.
28. Horvei LD, Grimnes G, Hindberg K, Mathiesen EB, Njolstad I, Wilsgaard T, et al. C-reactive protein, obesity, and the risk of arterial and venous thrombosis. J Thromb Haemost. 2016;14(8):1561-71.

29. Kruskal JB, Commerford PJ, Franks JJ, Kirsch RE. Fibrin and fibrinogen-related antigens in patients with stable and unstable coronary artery disease. N Engl J Med. 1987;317(22):1361-5.
30. Timp JF, Braekkan SK, Versteeg HH, Cannegieter SC. Epidemiology of cancer-associated venous thrombosis. Blood. 2013;122(10):1712-23.
31. Rinde LB, Lind C, Småbrekke B, Njølstad I, Mathiesen EB, Wilsgaard T, et al. Impact of incident myocardial infarction on the risk of venous thromboembolism: the Tromsø Study. J Thromb Haemost. 2016;14(6):1183-91.
32. Rinde LB, Smabrekke B, Mathiesen EB, Lochen ML, Njolstad I, Hald EM, et al. Ischemic Stroke and Risk of Venous Thromboembolism in the General Population: The Tromso Study. J Am Heart Assoc. 2016;5(11):e004311.
33. Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, et al. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. American journal of epidemiology. 1999;150(4):341-53.
34. Bjori E, Johnsen HS, Hansen JB, Braekkan SK. D-dimer at venous thrombosis diagnosis is associated with risk of recurrence. J Thromb Haemost. 2017;15(5):917-24.
35. Cushman M, Larson JC, Rosendaal FR, Heckbert SR, Curb JD, Phillips LS, et al. Biomarkers, menopausal hormone therapy and risk of venous thrombosis: The Women's Health Initiative. Res Pract Thromb Haemost. 2018;2(2):310-9.
36. Ariens RA, de Lange M, Snieder H, Boothby M, Spector TD, Grant PJ. Activation markers of coagulation and fibrinolysis in twins: heritability of the prethrombotic state. Lancet. 2002;359(9307):667-71.
37. Williams FMK, Carter AM, Kato B, Falchi M, Bathum L, Surdulescu G, et al. Identification of quantitative trait loci for fibrin clot phenotypes: the EuroCLOT study. Arterioscler Thromb Vasc Biol. 2009;29(4):600-5.
38. Bladbjerg EM, De Maat MPM, Christensen K, Bathum L, Jespersen J, Hjelmborg J. Genetic influence on thrombotic risk markers in the elderly - a Danish twin study. J Thromb Haemost. 2006;4(3):599-607.
39. Peetz D, Victor A, Adams P, Erbes H, Hafner G, Lackner KJ, et al. Genetic and environmental influences on the fibrinolytic system: a twin study. Thromb Haemost. 2004;92(2):344-51.
40. Huang MJ, Wei RB, Wang Y, Su TY, Di P, Li QP, et al. Blood coagulation system in patients with chronic kidney disease: a prospective observational study. BMJ open. 2017;7(5):e014294.
41. Borges AH, O'Connor JL, Phillips AN, Baker JV, Vjecha MJ, Losso MH, et al. Factors associated with D-dimer levels in HIV-infected individuals. PLoS One. 2014;9(3):e90978-e.
42. Ocak G, Vossen CY, Verduijn M, Dekker FW, Rosendaal FR, Cannegieter SC, et al. Risk of venous thrombosis in patients with major illnesses: results from the MEGA study. J Thromb Haemost. 2013;11(1):116-23.
43. Grainge MJ, West J, Card TR. Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study. Lancet. 2010;375(9715):657-63.
44. Tichelaar YI, Kluin-Nelemans HJ, Meijer K. Infections and inflammatory diseases as risk factors for venous thrombosis. A systematic review. Thromb Haemost. 2012;107(5):827-37.

TABLES

Table 1. Distribution of baseline characteristics of the study population (n=1257) across quartiles of plasma D-dimer levels

	D-dimer levels (ng/mL)			
	Quartile 1 <94	Quartile 2 94-119	Quartile 3 119-152	Quartile 4 ≥152
n	288	319	319	331
Age, years	51.8±13.0	58.2±13.1	63.3±12.2	66.8±12.1
Sex, men	49.3 (142)	53.6 (171)	44.5 (142)	41.4 (137)
BMI, kg/m^2	25.3±3.5	26.1±4.0	27.0±4.5	27.1±4.7
hsCRP, mg/L	0.8 (0.5-1.3)	1.3 (0.6-2.5)	1.5 (0.8-2.8)	2.2 (1.1-3.8)
Cancer[a]	2.8 (8)	5.0 (16)	6.3 (20)	4.2 (14)
CVD[b]	8.0 (23)	11.9 (38)	20.4 (65)	22.1 (73)

Continuous variables are shown as mean (± standard deviation) or median (25th percentile -75th percentile). Categorical variables are shown as percentages with numbers in brackets.

[a]Self-reported history of cancer at baseline. [b]Self-reported history of arterial cardiovascular disease (myocardial infarction, angina, stroke) at baseline. BMI, body mass index; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein.

Table 2. Characteristics of the venous thromboembolism (VTE) events (n=414)

Characteristics	
Age at VTE (years)	67.8±13.6
Sex (males)	48.3 (200)
Deep vein thrombosis	62.6 (259)
Pulmonary embolism	37.4 (155)
Unprovoked	41.8 (173)
Provoked VTE	58.2 (241)
Surgery/trauma	22.5 (93)
Cancer	21.5 (89)
Immobilization	18.1 (75)
Acute medical condition	15.7 (65)
Other factors	4.1 (17)

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 quartiles of plasma D-dimer levels

			OR (95% CI)		
D-dimer (ng/mL)	Controls	Cases	Model 1	Model 2	Model 3
Overall VTE					
<94	210	78	Ref.	Ref.	Ref.
94-119	211	108	1.41 (0.99-2.01)	1.36 (0.95-1.94)	1.27 (0.89-1.83)
119-152	212	107	1.43 (0.99-2.06)	1.31 (0.91-1.90)	1.20 (0.83-1.76)
≥152	210	121	1.65 (1.14-2.40)	1.51 (1.04-2.20)	1.34 (0.90-1.98)
P for trend			0.014	0.056	0.225
Deep vein thrombosis					
<94	210	48	Ref.	Ref.	Ref.
94-119	211	66	1.44 (0.94-2.20)	1.40 (0.91-2.14)	1.32 (0.85-2.03)
119-152	212	68	1.54 (0.99-2.38)	1.43 (0.92-2.22)	1.33 (0.85-2.09)
≥152	210	77	1.80 (1.16-2.80)	1.65 (1.06-2.58)	1.50 (0.94-2.39)
P for trend			0.012	0.040	0.115
Pulmonary embolism					
<94	210	30	Ref.	Ref.	Ref.
94-119	211	42	1.36 (0.81-2.28)	1.30 (0.77-2.18)	1.19 (0.70-2.01)
119-152	212	39	1.27 (0.74-2.18)	1.15 (0.66-1.98)	1.02 (0.58-1.78)
≥152	210	44	1.44 (0.83-2.48)	1.28 (0.74-2.22)	1.10 (0.62-1.94)
P for trend			0.270	0.514	0.936
Provoked VTE					
<94	210	40	Ref.	Ref.	Ref.
94-119	211	72	1.78 (1.15-2.77)	1.72 (1.10-2.67)	1.64 (1.05-2.57)
119-152	212	61	1.50 (0.94-2.40)	1.41 (0.88-2.25)	1.33 (0.82-2.14)
≥152	210	68	1.69 (1.05-2.71)	1.55 (0.96-2.49)	1.43 (0.87-2.34)
P for trend			0.106	0.228	0.415
Unprovoked VTE					
<94	210	38	Ref.	Ref.	Ref.
94-119	211	36	1.00 (0.61-1.65)	0.97 (0.59-1.60)	0.87 (0.52-1.46)
119-152	212	46	1.37 (0.84-2.24)	1.23 (0.74-2.02)	1.08 (0.65-1.80)
≥152	210	53	1.64 (1.00-2.70)	1.47 (0.89-2.43)	1.23 (0.73-2.08)
P for trend			0.025	0.085	0.297

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 high-sensitivity C-reactive protein.

FIGURES

Figure 1. Flowchart of the study population. The flowchart shows the nested case-control study derived from the fourth survey (Tromsø 4) of the Tromsø Study, conducted in 1994-1995. Venous thromboembolism (VTE)

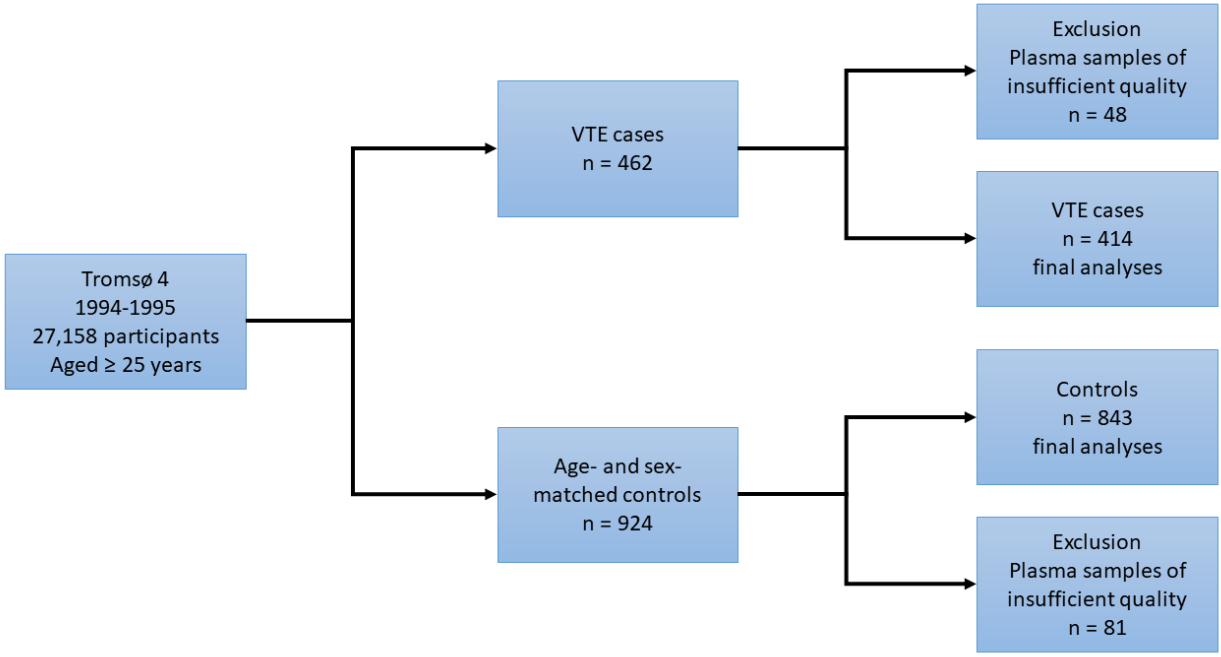
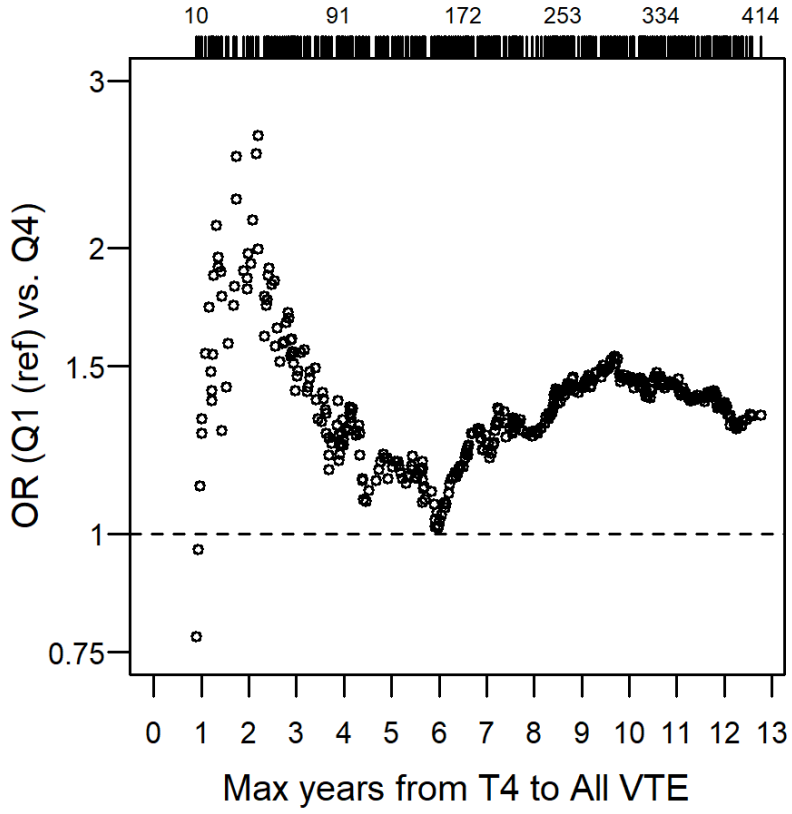


Figure 2. Plots of estimated odds ratios (ORs) for overall venous thromboembolism (VTE) as a function of time between blood sampling in Tromsø 4 (T4, 1994-95) and VTE events. Participants with D-dimer plasma levels in the highest quartile (Q4) were compared with those with D-dimer levels in the lowest quartile (Q1, reference). ORs were adjusted for age, sex, body mass index and high-sensitivity C-reactive protein. Risk estimates were not statistically significant at a *P*-value <0.05. The number of VTE events are shown above the plots.



Elevated plasma D-dimer levels are associated with risk of future incident venous thromboembolism

Running head: D-dimer and incident venous thromboembolism

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SUPPLEMENTAL TABLES

Supplemental Table 1. Odds ratios (OR) with 95% confidence interval (CI) for overall venous thromboembolism according to quartiles of plasma D-dimer levels in participants without self-reported cardiovascular disease at baseline (n=1058)

			OR (95% CI)		
D-dimer (ng/mL)	Controls	Cases	Model 1	Model 2	Model 3
<94	196	69	Ref.	Ref.	Ref.
94-119	183	98	1.54 (1.06-2.24)	1.48 (1.01-2.15)	1.42 (0.97-2.07)
119-152	167	87	1.52 (1.02-2.25)	1.39 (0.93-2.07)	1.30 (0.87-1.96)
≥152	165	93	1.65 (1.10-2.48)	1.49 (0.99-2.25)	1.38 (0.90-2.11)
P for trend			0.027	0.100	0.241

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 high-sensitivity C-reactive protein.

Supplemental Table 2. Odds ratios (OR) with 95% confidence interval (CI) for overall venous thromboembolism according to quartiles of plasma D-dimer levels in participants without self-reported cancer at baseline (n=1199)

			OR (95% CI)		
D-dimer (ng/mL)	Controls	Cases	Model 1	Model 2	Model 3
<94	206	74	Ref.	Ref.	Ref.
94-119	200	103	1.46 (1.02-2.10)	1.41 (0.98-2.03)	1.31 (0.90-1.89)
119-152	201	98	1.42 (0.98-2.07)	1.30 (0.89-1.90)	1.17 (0.79-1.73)
≥152	205	112	1.62 (1.11-2.37)	1.46 (0.99-2.14)	1.26 (0.84-1.88)
P for trend			0.026	0.111	0.423

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 high-sensitivity C-reactive protein.