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# D-dimer for diagnosis and risk assessment first and recurrent venous thromboembolism

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# Summary

Venous thromboembolism (VTE) is a collective term for deep vein thrombosis (DVT) and pulmonary embolism. D-dimer is a biomarker for coagulation- and fibrinolysis activation and has played a central part in the diagnostic work-up of VTE for decades. Today, D-dimer has several application areas, reflecting different aspects of the disease. In the present thesis, we investigated i) the safety and efficiency of D-dimer as a stand-alone test to rule out the diagnosis in patients with suspected DVT, ii) the association between plasma D-dimer levels and incident VTE and the influence of obesity and inflammation on this relationship, and iii) the impact of D-dimer measured at the time of VTE diagnosis on the risk of recurrence.

In paper I, individuals with suspected DVT referred to the University Hospital of North Norway were included. Based on the DVT diagnosis at the initial visit or during the subsequent three months, we retrospectively estimated the number of DVT patients that would have been undetected if D-dimer as a stand-alone test to rule out DVT had been the diagnostic strategy in use. In the study, we found that D-dimer may be applied as a stand-alone test to safely exclude proximal DVT (failure rate 0.6%) in outpatients and potentially simplify and increase the efficiency of the diagnostic work-up.

In paper II, we used a population-based nested case-control study of VTE cases and matched controls derived from the Tromsø study. D-dimer was measured in plasma samples from the cohort baseline, and odds ratios for VTE were estimated according to quartiles of D-dimer. We showed that higher baseline plasma levels of D-dimer were associated with an increased risk of future incident VTE (OR 1.65) and that the D-dimer levels may partly reflect underlying conditions related to obesity and an inflammatory state.

In paper III, we included patients with incident symptomatic VTE from the TROLL registry at Østfold Hospital. All recurrent events during follow-up were recorded, and cumulative incidences of recurrence were estimated according to D-dimer levels ≤1900 ng/mL (≤25th percentile) and >1900 ng/mL at the time of diagnosis. In the study, we demonstrated that D-dimer in the lowest quartile, measured at the diagnosis, may identify patients at low risk of recurrent VTE (five-year cumulative incidence 14.3%), in whom anticoagulant therapy may be stopped after the initial therapy phase.

Our findings show the diversity of D-dimer and its central part in the diagnostics and risk prediction of VTE recurrence. The proposed strategies in this thesis have the potential to improve the current management of VTE.

# Sammendrag

Venøs tromboembolisme (VTE) er en samlebetegnelse på dyp venetrombose (DVT) og lungeemboli. D-dimer, en blodprøve for koagulasjon og fibrinolyse, har vært en sentral del av VTE-diagnostikken i flere tiår. Blodprøven D-dimer har flere bruksområder og kan dermed reflektere ulike aspekter ved VTE. I denne avhandlingen har vi undersøkt i) om det er trygt og effektivt å bruke D-dimer uten samtidig bruk av risikoskår for å utelukke DVT hos pasienter med mistenkt DVT, ii) sammenhengen mellom D-dimer-nivå og førstegangs VTE og hvordan overvekt og inflammasjon påvirker denne sammenhengen, og iii) betydningen av D-dimer målt ved diagnosetidspunktet på risikoen for residiverende VTE.

I artikkel I inkluderte vi pasienter henvist til Universitetssykehuset Nord-Norge med mistenkt DVT. Basert på de faktiske DVT-diagnosene ved det første besøket eller i løpet av de påfølgende tre månedene, estimerte vi hvor mange DVTer som ville ha blitt oversett (udiagnostisert) hvis D-dimer alene hadde vært den diagnostiske strategien. I artikkelen fant vi at D-dimer alene er trygt for å utelukke proksimal DVT hos ikke-innlagte pasienter og at denne strategien har potensiale til å forenkle og øke effektiviteten av utredningen.

I artikkel II utførte vi en befolkningsbasert nøstet kasus-kontroll studie med VTE-kasuser og tilhørende matchende kontroller fra Tromsøundersøkelsen. D-dimer ble målt i blodprøver som var tatt ved inklusjon og odds ratioer for VTE ble beregnet utfra D-dimer-kvartiler. I studien fant vi at høyere D-dimer ved inklusjon er assosiert med økt risiko for førstegangs VTE, og at D-dimer-nivåene delvis reflekterer underliggende tilstander relatert til overvekt og inflammasjon.

I artikkel III inkluderte vi pasienter med førstegangs symptomatisk VTE fra TROLL-registeret ved Sykehuset Østfold. Alle tilfeller av residiverende VTE i oppfølgingstiden ble registrert, og den samlede insidensen av residiv ble estimert etter henholdsvis D-dimer ≤1900 ng/mL (≤25 persentilen) og >1900 ng/mL på diagnosetidspunktet. I studien fant vi at D-dimer i laveste kvartil, målt på diagnosetidspunktet, trolig kan identifisere pasienter med lav risiko for residiverende VTE. Hos disse kan blodfortynnende behandling trolig stoppes etter den første behandlingsfasen.

Funnene i denne avhandlingen viser bredden i bruken av D-dimer og dens viktige plass både i diagnostikken av førstegangs DVT og prediksjonen av residiverende hendelser. De foreslåtte strategiene i avhandlingen har potensiale til å forbedre dagens håndtering av VTE.

# List of papers

The thesis is based on the following papers:

I. D-dimer as a stand-alone test to rule out deep vein thrombosis
Fridtjof B. Rinde, Synne G. Frønæs, Waleed Ghanima, Anders Vik, John-Bjarne Hansen, Sigrid K. Brækkan
*Thrombosis Research 2020; 191: 134-139*

II. Elevated plasma D-dimer levels are associated with risk of future incident venous thromboembolism
Ellen-Sofie Hansen, Fridtjof B. Rinde, Magnus S. Edvardsen, Kristian Hindberg, Nadezhda Latysheva, Pål Aukrust, Thor Ueland, Annika E. Michelsen, John-Bjarne Hansen, Sigrid K. Brækkan, Vânia M. Morelli
*Thrombosis Research 2021; 208: 121-126*

III. Low D-dimer levels at diagnosis of venous thromboembolism are associated with reduced risk of recurrence: data from the TROLL registry
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*Manuscript*

# Abbreviations

APC – Activated Protein C

ARIC – Atherosclerosis Risk in Communities

BMI – Body Mass Index

CI – Confidence Interval

COPD – Chronic Obstructive Pulmonary Disease

CRP – C-Reactive Protein

CTEPH – Chronic Thromboembolic Pulmonary Hypertension

CTPA – Computed Tomography Pulmonary Angiography

CUS – Compression Ultrasound

DOAC – Direct Oral Anticoagulants

DVT – Deep Vein Thrombosis

ELISA – Enzyme-Linked Immunosorbent Assay

F – Factor

FEU – Fibrinogen Equivalent Units

FGG – Fibrinogen Gamma Gene

FVL – Factor V Leiden

GWAS – Genome-Wide Association Studies

HR – Hazard Ratio

IR – Incidence Rate

ISTH – International Society on Thrombosis and Haemostasis

MAR – Missing At Random

MCAR – Missing Completely At Random

MI – Myocardial Infarction

MNAR – Missing Not At Random

OR – Odds Ratio

PE – Pulmonary Embolism

PTS – Post-Thrombotic Syndrome

RCT – Randomized Controlled Trial

RR – Relative Risk

SNP – Single Nucleotide Polymorphism

SSC – Scientific and Standardization Committee

TF – Tissue Factor

TFPI – Tissue Factor Pathway Inhibitor

TROLL - Venous Thrombosis Registry in Østfold Hospital

UNN – University Hospital of North Norway

VTE – Venous thromboembolism

VQ – Ventilation-perfusion

# 1 Introduction

Deep vein thrombosis (DVT) is the formation of a blood clot, i.e., a thrombus, in the deep veins, most often in the large veins of the lower extremities (1). Other more unusual sites of thrombosis do also occur, such as in the upper extremities, abdominal or cerebral veins (2). A DVT can obstruct the blood flow, causing common signs and symptoms of DVT, including pain, swelling, and redness of the affected limb. Pulmonary embolism (PE) primarily occurs when all, or parts, of a DVT break away and travel with the venous bloodstream to the lungs, where it lodges and blocks the blood flow (1). Signs and symptoms of PE may be diffuse and unspecific but commonly include dyspnea, tachypnea, coughing, or chest pain. The mechanistic link between DVT and PE was first proposed by Rudolf Virchow, and due to shared underlying pathology, the two conditions are now collectively referred to as venous thromboembolism (VTE) (3-5).

Historically, DVT has been identified solitary in around two-thirds of the VTE events, while one-third of the VTE events have been PE with or without DVT (6, 7). However, due to an increase in the incidence of PE over the last decades, data from more recent studies have shown a more half-and-half distribution of DVT and PE (8-10). The two conditions often present simultaneously, though frequently clinically silent in one of the locations (11). Studies have demonstrated that the origin of the emboli remains undetected in up to half of PE patients (12-14). This may indicate that PE could have other etiological sources than a thrombus originating from the deep veins. Studies based on autopsy and echocardiographic examinations have identified thrombus formation in the right atrium of patients with atrial fibrillation (15, 16). Thus, PEs may have a cardiac origin, such as the right atrium in atrial fibrillation patients (17-20). Other potential sources include de novo formation of thrombi in the pulmonary circulation, potentially due to local inflammatory processes or hypoxia (13, 21, 22), as observed during the COVID-19 pandemic (23).

VTE is a major cause of morbidity and mortality (24). Fortunately, the disease may be prevented and treated by anticoagulant drugs (25). While anticoagulant therapy is highly efficient in preventing thrombosis, the treatment is also associated with an increased risk of bleeding (26, 27). The first anticoagulant used for treating VTE was heparin, discovered in 1916 (28). Heparin was highly effective, and in a study from 1950, heparin was found to reduce VTE mortality at the time from 18% to 0.4% (29). However, the drug had some limitations, such as parenteral administration and treatment restrictions to the inpatient setting. The

introduction of the oral anticoagulant warfarin, a vitamin K antagonist, allowed treatment after hospitalization (30, 31). As the full anticoagulant effect of warfarin usually occurs after a few days, the combination of warfarin and heparin was early found beneficial (31). In the mid-90s, low-molecular-weight heparin was introduced (32). The subcutaneously administered drug further simplified the anticoagulant treatment and reduced the need for hospital stay (33, 34). Today, an additional group of anticoagulant drugs, the direct oral anticoagulants (DOAC), have been developed and further simplified the treatment of VTE. The DOACs are now increasingly replacing vitamin K antagonists, mainly due to the lower bleeding risk, as well as practical considerations, such as standardized dosing and reduced need for monitoring (35). Lately, DOACs have also been found sufficiently safe and efficient in the majority of cancer patients. Consequently, DOAC is now increasingly replacing low-molecular-weight heparin in this patient group as well (36-40).

## 1.1 Epidemiology of venous thromboembolism

VTE is the third most common cardiovascular disease after myocardial infarction (MI) and stroke (41), with an estimated overall incidence of 1 to 2 per 1000 persons per year in Western countries (7, 42, 43). Although VTE can occur in all ages, the annual incidence increases exponentially with age from 1 per 10 000 in young adults to 1 per 100 in the elderly (44-48). The incidence of VTE differs across ethnicity as well (49, 50). Individuals with African ancestry have the highest incidence, followed by persons of Caucasian origin. The lowest incidence rate is observed in individuals with Asian ancestry (43, 49, 50).

Across different study populations, several studies have reported an increased incidence of VTE in the last decades (48, 51, 52). For example, the Tromsø study observed a 27% increase in age-adjusted incidence rates (IR) of VTE from 1996 to 2012, mainly due to increased incidence of PE (48). In a nationwide population-based cohort study of all hospital-diagnosed VTEs in Denmark, there was a 20% increased IR of VTE from 2006 to 2015, due to a 78% increased incidence of PE (8). Improved detection of the disease through more accessible and sensitive diagnostic methods and increased awareness of VTE, may partly explain the increasing incidence (48, 53). However, in the same period, the incidence of MI and ischemic stroke rapidly decreased despite the same diagnostic development (54-56). Thus, diagnostic improvement may only explain some of the increase in the incidence of VTE, and the substantial rise in VTE-related risk factors, such as cancer, obesity, and increasing age, have likely contributed to the observed development (8, 57).

VTE is associated with short- and long-term complications, contributing to a major health burden in society (58-60). Post-thrombotic syndrome (PTS) is the most frequent long-term complication in DVT patients. The condition affects 20-50% of patients with a proximal DVT (61-63). Due to the impaired circulation of the leg, PTS causes swelling, pain, chronic limb fatigue, and paresthesia (61). After an episode of acute PE, half of the patients may suffer from functional limitations or decreased quality of life, commonly referred to as post-PE syndrome (64, 65). One of the most severe long-term complications of PE, chronic thromboembolic pulmonary hypertension (CTEPH), is a rare condition, affecting 1-4% of all PE patients surviving the acute event (66, 67). An incomplete resolution of the PE with residual thrombi in the pulmonary circulation may cause pulmonary vascular disease of major pulmonary arteries, such as increased pulmonary vascular resistance, progressive pulmonary hypertension, and in the worst case right ventricular failure (68, 69). Signs and symptoms of CTEPH are unspecific, but in patients with a history of PE, CTEPH should be considered if the patient has persistent dyspnea, right ventricular dysfunction, or perfusion defects (69). Several studies have shown a relationship between VTE and permanent work-related disability (59, 70). For example, in a Danish nationwide population-based cohort study, VTE was associated with a 2- to 3-fold increased risk of subsequent work-related disability pensions compared to the general population (70).

VTE is a major cause of mortality, and epidemiological models estimated around 500 000 VTE-related deaths across the European Union in 2004 (population of 450 million) (24, 71). The 1-year mortality of VTE is estimated at approximately 20% (45, 72). Moreover, the overall mortality rates at 1-year are higher for VTE than the corresponding rates for MI (72, 73). In comparison, in patients with ST-segment elevation MI and patients with non-ST-segment elevation MI the 1-year cumulative mortality rate is around 9% and 12%, respectively (73). Survival after a VTE event varies according to the site of the thrombus (45, 74). The reported mortality rate is highest in PE patients, and the 30-day mortality is approximately 3-fold higher in PE compared to isolated DVT (45, 75). The mortality rate, however, seems to be declining, potentially reflecting improved management of the disease and increased detection of smaller, less severe thrombi (76).

## 1.2 Pathophysiology of venous thromboembolism

Hemostasis is the physiological process of stopping the bleeding in response to vessel injury (77). This is a crucial mechanism to limit hemorrhage while maintaining normal blood flow elsewhere in the circulation (78). Under normal conditions, the endothelium of the vessel wall contains an anticoagulant surface to preserve the blood in a fluid state. However, in the presence of an injury, the subendothelial matrix is exposed and subsequent activation of the hemostasis processes occurs within seconds. Hemostasis consists of two main mechanisms, primary and secondary hemostasis (78). In the initial phase after an injury, i.e., the primary hemostasis, a platelet plug is formed through activation and aggregation of platelets. The platelet plug is then strengthened and stabilized through the activation of the coagulation cascade and the formation of fibrin, i.e., the secondary hemostasis (77, 78). In this rapid response, the generation of fibrin and the aggregation of platelets occur simultaneously.

The coagulation cascade consists of the extrinsic and intrinsic pathway, also known as the tissue factor (TF) and contact pathway, respectively. The two pathways coalescence in a common pathway and the formation of thrombin. The extrinsic pathway is initiated by TF, expressed by activated monocytes, subendothelial matrix, monocyte-derived microvesicles, and probably activated endothelial cells (79). TF forms a complex with factor VIIa, which further initiates a proteolytic cascade of activating FX to FXa. The intrinsic pathway is initiated through the activation of FXIIa, the exposure of subendothelial collagen, cellular RNA, and polyphosphate released from activated platelets or bacteria (79). The activated FXIIa causes a chain of activation of the coagulation factors FXI, FIX, and FVIII, culminating in FXa. Through the activation of FXa and the subsequent formation of the FXa-FVa prothrombin complex, the extrinsic and intrinsic pathway adjoins in a cascade of thrombin activation and the formation of fibrin, known as the common pathway (80).

Hemostasis is, under normal conditions, a carefully balanced process between the pathological states of hypocoagulability and hypercoagulability (77). The coagulation system is regulated in several steps through the cascade. Depending on FXa, tissue factor pathway inhibitor (TFPI) binds to the TF-FVII/FVIIa complex and prohibits its activation of FX and FIX (81). Thus, TFPI prevents further TF-induced activation of FXa and additional FXa can only be produced through the intrinsic pathway. Antithrombin, one of the most critical natural anticoagulants circulating in human plasma, prevents intravascular thrombus formation by inhibiting, among other things, thrombin (82). Due to its anticoagulant effect, the heparin-binding domain of antithrombin is utilized in the prevention of thrombosis with anticoagulant

therapy (33, 83). Another major natural anticoagulant is the protein C pathway. Activated protein C inactivates FVa and FVIIIa in the presence of protein S. Through their inhibition of these central co-factors, the activated protein C and protein S inhibit further thrombin generation and thereby contribute to the regulation and prevention of thrombus formation (84).

VTE is a complex, multifactorial disease, and the development of a VTE occurs as a combination of many simultaneous events and factors (85). Simplified, these factors can be categorized into the following three groups: hypercoagulability, changes in blood flow (stasis and turbulence), and endothelial dysfunction (Figure 1) (86). These groups of thrombogenic factors are also known as Virchow's triad, after the German physician Rudolf Virchow, and forms the basis of our pathophysiological understanding of VTE (87, 88).

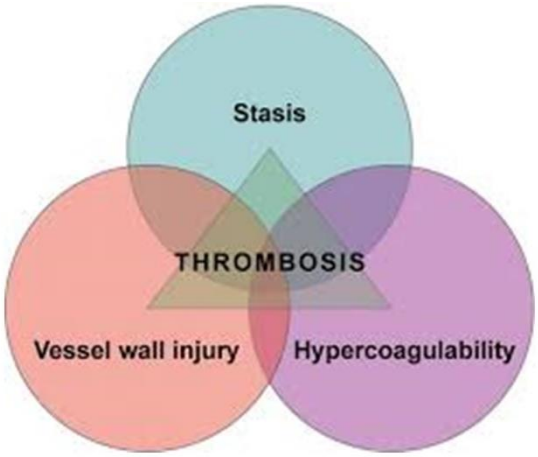


**Figure 1.** Virchow's triad.
(Adapted from Kyrle & Eichinger, Blood 2009)

The initiation of a thrombus is thought to occur in the pocket sinuses of venous valves, as these are particularly prone to low blood flow and stasis (Figure 2) (79, 88, 89). This is supported by radiology and autopsy studies, showing that these pockets frequently contain thrombi (90-92). The underlying mechanism is likely attributed to local stasis and hypoxia due to vortex blood flow, causing accumulation of coagulation factors and activation of platelets, leukocytes, and endothelial cells (86, 93, 94). Furthermore, an animal study has shown that rapid development of hypoxia in the pocket of the venous valves after only hours of stasis, potentially activates the coagulation cascade, causing a hypercoagulable environment (95). By moving the blood past the venous valves, the skeletal muscle pump usually counteracts stasis and thereby prevents the formation of DVT. However, several factors and conditions may inhibit or overload this mechanism, such as mechanical compression (e.g., pelvic tumor or pregnancy), immobilization (e.g., prolonged bed rest, neurological deficits), chronic medical conditions (e.g., congestive heart failure), and hyperviscosity (e.g., polycythemia) (92, 96).

Under normal conditions, the endothelium contributes as an antithrombotic and profibrinolytic surface thereby preventing the formation of thrombi. However, under pathological conditions, such as injury of the vessel wall or changes in protein expression, the

endothelial surface is converted into a procoagulant state (88, 97). The changes in protein expression may be induced by hypoxia, inflammation, or altered blood flow, causing activation of the endothelium and triggering thrombus development (88, 92). Activated endothelium is thought to downregulate the expression of antithrombotic factors, such as endothelial protein C receptors and thrombomodulin, and upregulate the expression of TF (86, 98). Consequently, the imbalance of pro- and anticoagulant factors may explain the mechanism of thrombus formation (86). In addition, hypoxia is also thought to increase the expression of P-selectin on endothelium, resulting in the recruitment of leukocytes, platelets, and leukocyte-derived TF-containing microvesicles, all potentially contributing to activation of the coagulation cascade in some cases (88, 99, 100).

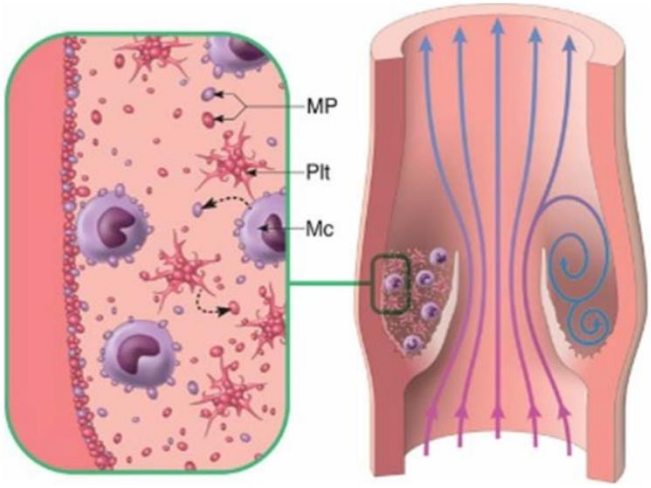


**Figure 2.** The venous valvular sinus as a predilection site for DVT initiation. Blood is trapped in a vortex of the valve pockets, and the resultant hypoxia activates the venous endothelium, leading to the recruitment and binding of leukocytes, especially monocytes (Mc), platelets (Plt) and TF-positive microparticles (MP). Consequently, TF from activated monocytes and microparticles may activate the coagulation cascade and initiate thrombosis formation.

Increased levels of procoagulant proteins, often characterized as hypercoagulability, leads to increased thrombin generation and are associated with a higher risk of thrombosis (88, 101, 102). The pathophysiological mechanism may differ, and hypercoagulability can be categorized as inherent or acquired. Inherent hypercoagulability is due to prothrombotic genotypes increasing the quantity or activity of proteins that promote coagulation, e.g., prothrombin 20210A and Factor V Leiden mutation (FVL), or genotypes decreasing the quantity of proteins that inhibit the process, e.g., Protein S and Protein C (85, 89). Acquired hypercoagulability is often secondary to other conditions, resulting in increased circulating TF and other procoagulant proteases or decreased activity of anticoagulant proteins (99, 103). Cancer, chronic inflammation, surgery, and pregnancy are all examples of such conditions that may lead to acquired hypercoagulability (99, 103).

## 1.3 Diagnosis of venous thromboembolism

As VTE is associated with a considerable risk of morbidity and mortality (24), the diagnosis and treatment of the disease are of great importance. Signs and symptoms of VTE may be diffuse and nonspecific. Making the diagnosis purely based on the clinical presentation may therefore be challenging. In patients with VTE, the risk of morbidity and mortality justifies the use of anticoagulant therapy (25). However, in subjects without a thrombus, the treatment introduces an unnecessary risk of bleeding (104). Therefore, objective assessment and confirmation of VTE are highly important, as both under- and overdiagnosis of the disease can entail considerable risks. To evaluate the need for objective testing, the assessment of pretest probability is applied as an initial step to stratify patients into low-, intermediate- and high-risk groups (105), as further described in section 1.6.3 of this thesis.

Venography was introduced over a half-century ago and has since been accepted as the reference standard within diagnostics of DVT (106, 107). Later, other imaging techniques were introduced and all approaches with a post-test probability of having VTE comparable to venography were considered acceptable to rule out DVT (107, 108). Today, compression ultrasound (CUS) is the first-line imaging test (109, 110). In short, two different approaches for CUS are applied, a proximal or a whole-leg test. With a proximal CUS, only the popliteal and common femoral veins are examined. This approach is generally faster but less sensitive to distal thrombi (111). Therefore, an initial negative proximal CUS should be followed by a serial proximal CUS after one to two weeks, at least in patients with an intermediate to high pretest probability (112-114). A whole-leg scan is a more thorough examination, where the calf veins are examined in addition. The more comprehensive scan is also efficient in detecting distal DVTs, and a single negative test is sufficient to rule out DVT in most patients (112, 114). As whole-leg CUS is more extensive, the strategy may potentially discover distal thrombi with uncertain clinical impacts (108, 111). The management and treatment of distal DVTs are heavily debated (115, 116). The second update of the 9$^{th}$ edition of the American College of Chest Physicians guidelines suggests serial whole-leg testing to rule out proximal extension over the treatment of distal DVTs with anticoagulants (114, 117). In the American Society of Hematology guidelines from 2018, serial whole-leg CUS is recommended in patients with a high pretest probability and an initial negative test (112).

Computed Tomography Pulmonary Angiography (CTPA) is the method of choice when imaging is required in patients with a suspected PE (118). It has high accuracy, and particularly in patients with a low to intermediate pretest probability, a negative CTPA test alone is

sufficient to exclude PE (118). Another imaging modality for PE is the ventilation-perfusion (VQ) scan. Compared to CTPA, a VQ scan have considerably less radiation exposure (40, 119). A normal VQ scan can exclude PE. However, in half of the cases, VQ scan results are inconclusive, and the test's clinical utility is limited (108). Pulmonary angiography has traditionally been used for diagnostics and exclusion of PE (120), but the invasive procedure involves a considerable risk of complications. After thorough validation of CTPA's accuracy, pulmonary angiography is now rarely performed (108).

## 1.4 Risk factors of incident venous thromboembolism

VTE is a multifactorial disease, caused by a combination of predisposing genetic factors and environmental exposures (85, 121). The VTE risk changes over time and depends on the accumulation and combination of inherent and acquired risk factors. Our present understanding of the causality of VTE is best illustrated by the *thrombosis potential model* (Figure 3), first described by professor Rosendaal in 1999 (85). As the model demonstrates, the development of VTE depends on the combination of factors leading to a thrombosis potential exceeding a thrombosis threshold. For instance, a major transient provoking factor, such as surgery, may by itself not cause a thrombosis potential exceeding a patient's thrombosis threshold. However, as the risk of VTE increases with age (45), the same provoking factor may later in life lead to thrombosis, as the combined risk of surgery and age results in a thrombosis potential crossing the threshold.

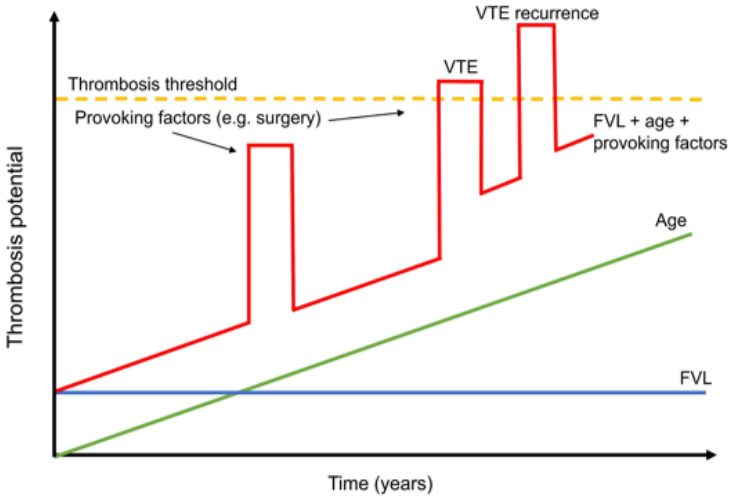


**Figure 3.** The thrombosis potential model. The blue line represents intrinsic factors that are stable over time such as inherited risk factors, e.g., Factor V Leiden (FVL), and the green line represents the effect of a risk factor that increases over time, like age. The red line represents the cumulated thrombosis potential taking the combined effect of intrinsic factors, factors increasing over time, and provoking factors into account (Adapted from Rosendaal, Lancet 1999).

A risk factor is defined as everything that affects the incidence of disease occurrence (121). Discovering and investigating new risk factors are essential to improve our causal understanding of the disease and thereby increase the possibility of intervention and prophylaxis. Epidemiological studies have shown that both acquired, e.g., cancer, and genetic, e.g., FVL, risk factors contribute to the development of VTE (88).

Depending on the appearance of environmental exposures at the time of VTE diagnosis, VTE can be defined as provoked or unprovoked (122). In contrast to clinical risk factors, provoking factors have an instant and short-term effect on the VTE risk, potentially leading to an acute episode of VTE (122). Provoking factors include exposures such as surgery, pregnancy, infection, and immobilization. Conversely, an event occurring without any acute provoking factor(s) is classified as an unprovoked event (122). Studies have reported a proportion of unprovoked events ranging from 25-45% (43, 48). Obesity can be used as an example to explain the differences between clinical risk factors and provoking factors. As obesity increases the probability of a VTE episode over time, it is considered a clinical risk factor for VTE (123-125). However, as obesity only increases the baseline risk for VTE and does not trigger an acute event by itself, obesity is not recognized as a provoking factor. The same applies to other clinical risk factors, such as hereditary thrombophilia and age (122). As some factors, such as progressive metastatic cancer, may work as both a clinical risk factor and a provoking factor for the event, the distinction between the terms may not always be well-defined (122).

A further distinction of risk factors into transient and persistent is important as it may impact the duration of anticoagulant treatment (Figure 4). As transient provoking factors will subsequently be removed or cease, the thrombosis potential will drop, and the risk for a recurrent VTE will be heavily reduced (126). With a persistent provoking factor or an unprovoked event, the thrombosis potential would not drop, resulting in a persistently increased risk for a recurrent event.

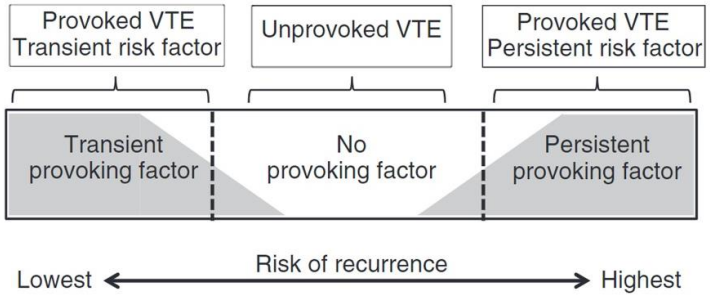


**Figure 4.** The conceptual framework for provoked and unprovoked VTE. The extreme left represents a major transient risk factor that is fully reversible, e.g., one week after major elective surgery. The extreme right represents a major persistent risk factor that is irreversible, e.g., patient with metastatic cancer. The dashed lines represent the point along a continuum that separates risk factors (transient or persistent) that are prognostically unimportant and important for recurrent VTE. (Kearon et al., J Thromb Haemost 2016).

Transient factors are classified as “major” or “minor” depending on their influence on the VTE risk (Table 1). Major transient risk factors are defined as factors that

occur within three months before the VTE that are associated with half the risk of recurrence after cessation of anticoagulant therapy or a greater than ten-fold increased risk of having a first VTE (122). Minor transient risk factors occur within two months before the VTE that are associated with half the risk of recurrence or a 3- to 10-fold-increased risk of having a first VTE (122).

**Table 1.** Definitions for 'VTE provoked by a transient risk factor'.

| Major transient risk factor | Minor transient risk factor |
|---|---|
| A risk factor is considered 'major' if it has been shown to be associated with<br>1) half the risk of recurrent VTE after stopping anticoagulant therapy (compared with if there was no transient risk factor), when the risk factor occurred up to 3 months before the VTE; or<br>2) a greater than 10-fold increase in the risk of having a first VTE. | A risk factor is considered 'minor' if it has been shown to be associated with:<br>1) half the risk of recurrent VTE after stopping anticoagulant therapy (compared with if there was no transient risk factor), when the risk factor occurred up to 2 months before the VTE; or<br>2) a 3 to 10-fold increase in the risk of having a first VTE. |
| Examples:<br>• Surgery with general anesthesia for greater than 30 min.<br>• Confined to bed in hospital for at least three days with an acute illness.<br>• Cesarean section. | Examples:<br>• Surgery with general anesthesia for less than 30 min.<br>• Admission to hospital for less than three days with an acute illness.<br>• Pregnancy or puerperium.<br>• Confined to bed out of hospital for at least three days with an acute illness. |

(Adapted from Kearon et al., J Thromb Haemost 2016).

### 1.4.1 Inherited risk factors

Inheritance is a central part of the VTE risk, and 50-60% of risk variation may be attributed to genetic factors (127-131). VTE inheritance has been proposed to follow a multifactorial non-Mendelian inheritance model, where multiple genetic factors contribute to the increased risk (132-134). Studies have shown that individuals with a sibling with VTE have a 2.5-fold increased risk compared to the general population (129, 130, 132). Several genetic mutations are thought to influence the incidence of VTE, and over a hundred independent genomic loci significantly associated with VTE risk have been identified so far (135). Most known genetic risk factors involve mutations in the coagulation system and can often be classified as either *gain-of-function* or *loss-of-function* mutations (136).

*Loss-of-function mutations*

Antithrombin is a natural anticoagulant and prevents thrombus formation through the inhibition of central enzymes in the coagulation cascade, such as thrombin and FXa (137). **Antithrombin**

**deficiency** causes a considerably increased risk of VTE, and among the known inherited thrombophilia, antithrombin deficiency leads to the highest risk of VTE (137-139). While homozygous antithrombin deficiency is rarely compatible with life, heterozygous carriers have a 10-fold increased VTE risk (137, 138). However, this type of inherited thrombophilia is rare and only occurs in 0.2% of the general population (140). Antithrombin deficiency results from mutations in the SERPINC1 gene coding for antithrombin, and more than 130 different mutations in this gene are reported (141).

Activated **protein C** binds to its cofactor **protein S** to limit the coagulation cascade by inhibiting FVa and FVIIIa (142, 143). Accordingly, conditions with decreased protein C or protein S levels lead to increased VTE risk (144). Heterozygous protein C deficiency is associated with an 8-fold increased risk compared to non-carriers (138). Protein C and S deficiency are rare conditions, with less than 1% carriers in the population (145, 146). The reduced level of circulating protein C occurs due to several mutations in the promoter region of the PROC gene, while mutations in the PROS1 gene may cause a deficiency of protein S (147-150).

*Gain-of-function mutations*

In gain-of-function mutations, prothrombotic genotypes increase the quantity or activity of proteins promoting coagulation (85, 89). The rs6025 mutation, commonly known as **FVL**, is a frequent genetic variation in the FV gene. The gain-of-function mutation has a prevalence of approximately 5% in the Caucasian population (151, 152) and occurs in 20% of all VTE cases (134, 153). The FVL mutation causes resistance to activated protein C (APC), thereby reducing the inactivation of FVa (153, 154). Heterozygous carriers are associated with a 2- to 7-fold increased risk, while 10- to 80-fold increased risk is seen in homozygous carriers (133, 141, 152, 153).

A mutation in part of the prothrombin gene, **prothrombin 20210A** (rs17199963), causes an overproduction of prothrombin, leading to a hypercoagulable state, increasing the risk of VTE (155). In addition, the mutation inhibits the inactivation of FVa by APC, thereby causing APC resistance (156, 157). The variation has a prevalence of 2% in the population (136, 141) and occurs almost exclusively in Caucasians (134). Carrying the prothrombin 20210A mutation is associated with a 2- to 3-fold increased risk of VTE (140, 158). As both FVL and prothrombin 20210A are relatively common in the population, double heterozygosity

of the two mutations is not rare, and the combination is associated with a 20-fold increased risk of VTE compared to individuals with neither mutation (134, 159).

The most common prothrombotic genotype variation is **non-O blood type**, with a 1.5- to 2.0-fold increased risk for VTE (160-162). Non-O blood types are associated with increased plasma levels of von Willebrand factor and FVIII, two plasma factors associated with increased VTE risk (163-165). However, non-O blood has also been shown to be associated with the risk of VTE independently of FVIII (166). The **fibrinogen gamma gene** (FGG) encodes for one of three polypeptides creating the fibrinogen molecule, the γ polypeptide (167). Fibrinogen is an essential component of the hematological system (168). Catalyzed by thrombin, fibrinogen is cleaved and converted into fibrin, with subsequent formation of a fibrin clot. The fibrinogen γ chain is mainly transcripted to the Aγ. However, an alternative spliced form may occur, creating a γ' chain (169). This alternative γ' chain is thought to contribute to thrombin inhibition, as an important part of the antithrombin's anticoagulant effect (141). Reduced levels of γ' are associated with an increased incidence of VTE (141), and individuals with a specific mutation in FGG, the rs2066865 SNP, have a 2.4-fold increased risk of VTE (168).

*Genome-wide association studies and modern discoveries of genetic variants*

Since the first discovery of antithrombin deficiency in 1965, due to a cluster of VTE in a Norwegian family (170), the discovery of new genetic variations associated with VTE has developed and advanced. During the 1990s, the candidate gene approach was applied, discovering genetic variations such as the FVL (151, 171). As a result of development within genotyping technology over the last decades, new genotypes associated with VTE have been investigated through genome-wide association studies (GWAS) (171). In contrast to candidate gene studies, where specific genes or areas of the genome are studied, GWAS are conducted without any prior hypotheses (172). Where candidate gene studies had a generally smaller sample size and were limited to only a few genetic variants, GWAS has the opportunity to test for association in hundreds of thousands of single nucleotide polymorphisms (SNPs) (173-175). However, the first GWAS primarily confirmed the influence of known genetic risk factors, and novel discovered genes have only a modest effect on the VTE risk (160, 171, 176).

A single genetic biomarker is unable to predict the risk of VTE (177). Based on the genetic variation strongest associated with VTE, de Haan et al. constructed a genetic risk prediction model for an incident VTE consisting of five SNP, the FVL, FII, FXI, FGG, and ABO genes (178). The 5-SNP risk score demonstrated a similar discriminative accuracy as a

score consisting of 31 SNP. In addition, the 5-SNP score was combined with a nongenetic risk score improving the clinical accuracy, indicating a potential clinical value of genetic testing in VTE prediction in high-risk populations (178).

### 1.4.2 Acquired risk factors

A strong age gradient for the risk of VTE is observed, indicating **aging** as one of the most prevalent and substantial risk factors for VTE (179, 180). In the general population, the incidence of first-time VTE is 1-2 per 1000 person-years (7, 42, 43). However, in the population of 85 years and older, VTE is considerably more common, with an incidence of almost 1 per 100 person-years (44, 45). The mechanism for the increased risk is not fully understood. The higher prevalence of conventional risk factors in the older population, such as cancer, immobilization, and the presence of medical conditions, has been hypothesized as one explanation (179). Age-specific factors mainly present in the older population, such as frailty, endothelial dysfunction, and venous insufficiency, may also partly explain the increased risk. In addition, the elderly have increased plasma levels of the hemostatic factors associated with an increased risk of VTE (181).

**Obesity** is a well-established risk factor for VTE. Obese subjects have a 2- to 3-fold increased risk of first VTE compared with lean subjects (123-125, 182). Body mass index (BMI) has been the most widely used anthropometric measure for obesity when studying the association with VTE. However, studies have shown that other measures of obesity, such as total body fat, waist circumference, hip circumference, and waist-hip ratio, are all associated with VTE as well (183, 184). The pathophysiological mechanism behind the association between obesity and VTE is not fully discovered. The strong association between obesity and known atherosclerotic risk factors, e.g., hypercholesterolemia, hypertension, diabetes mellitus type 2, has been used to partly explain the association between obesity and arterial thrombosis, i.e., MI, ischemic stroke, and peripheral arterial disease (182). However, the same association between VTE and atherosclerotic risk factors has not been established (185). Consequently, other hypotheses have been proposed. As abdominal obesity causes increased intra-abdominal pressure (186-188), stasis may play a key role (87, 125). Furthermore, chronic inflammation due to the release of proinflammatory proteins in adipose tissue, e.g., IL-6 or TNF-α, may be a plausible mechanism (189, 190). The relationship and crosstalk between inflammation and the coagulation system are well established (191-193). However, the role of chronic low-grade

inflammation and the risk of VTE is disputed, as the results from studies assessing this association are not consistent (165, 194-197).

**Body height** is an anthropometric measure associated with an increased risk of VTE. Those with tall stature have the highest VTE risk (123, 198, 199). The association is particularly pronounced in those with long legs (199). In the LITE study, participants with the longest legs had a 59% greater risk for VTE than those with the shortest (199). The underlying mechanism is speculated to be partly due to greater venous pressure, stasis, and higher venous flow rate in tall individuals, causing endothelial damage and the development of VTE (188, 199, 200). Alternatively, the association may be due to a large venous surface and thereby a greater area for thrombus development (199). As the number of venous valves is directly associated with the risk of VTE, an increased number of valves in taller persons may partly explain the relationship as well (201).

Since first noted by Bouillaud in 1823 and later described by Trousseau in 1865 (202-204), the relationship between **cancer** and VTE has been repeatedly investigated (205-212). Today, cancer is established as a major risk factor for VTE (213). Cancer patients have a 4- to 7-fold increased risk of VTE (214-216). Furthermore, malignancy has a prevalence of approximately 20% in VTE patients (43, 217, 218). The risk of cancer-associated thrombosis varies over the natural history of cancer, with the highest risk during hospitalization and the development of metastatic disease (219, 220). The site of the tumor is associated with the VTE incidence as well, and certain types of cancer, such as pancreatic, brain, ovary, kidney, stomach, and lung cancer, have the highest rates of VTE (219, 220). The pathophysiology behind cancer-associated VTE is multifactorial (221, 222). Tumor cells may have abnormal production of procoagulant proteins, especially TF, as well as the expression of inhibitors for the fibrinolytic system (218, 223). In addition, a further hypercoagulable state occurs due to the release of inflammatory cytokines and neutrophil extracellular traps (219, 223-225). Cancer-associated VTE may also result from vessel wall injury due to tumor invasion or venous stasis due to direct compression of nearby blood vessels (226).

Presumably, as a result of venous stasis, **immobilization** is associated with an increased risk of VTE (227). In a meta-analysis including 36 cohort studies, the pooled VTE risk was estimated to be almost 90% increased among immobilized medical patients compared to non-immobilized patients (228). Immobilization may occur due to different causes and the risk of VTE vary across the causes of immobilization. In outpatients, the highest risk is found for

immobilization due to orthopedic conditions (i.e., cast or external fixation), or neurologic diseases (i.e., paralysis or paresis due to brain, spinal cord, or neuromuscular disease or injury) (229). Immobilization due to prolonged travel is considered a minor risk factor, with an estimated odds ratio (OR) of 1.2 (229).

Women of reproductive age have about twice the incidence of VTE compared to men of the same age (230). This sex difference is partly explained by increased thrombogenicity due to **pregnancy** and **puerperium** (45, 230). VTE occurs in 1-2 per 1000 pregnancies (231, 232), and pregnant women have a 5-fold increased risk compared to non-pregnant women of the same age (233). Thus, VTE is a considerable complication of maternal health, with PE as one of the leading causes of maternal morbidity in western countries (234, 235). The increased risk is mediated by physiological induced hypercoagulability and increased venous stasis in the lower limbs, especially due to compression of the left iliac vein (236).

An additional explanation for increased risk in women of reproductive age is the use of **contraceptives** (45, 230). Combined oral contraceptives are commonly used and often the preferred form of birth control worldwide (237). However, the use of combined oral contraceptives increases the risk of VTE (238). Women using combined oral contraceptives have a 4-fold higher incidence of VTE than non-users (238). The risk varies across different oral contraceptives and according to the type of progesterone and dose of estrogen in use (238, 239). The risk of VTE is most pronounced during the first year of use, especially the initial six months (239, 240). Studies have shown that women who withdraw oral contraceptives and later re-introduced have a similar risk of VTE as first-time users (240, 241).

VTE is a common complication in **surgical** patients, and PE is considered one of the most frequent causes of preventable death in these patients (242, 243). Surgical patients have a 4- to 22-fold increased risk of VTE in the first three months after surgery (215, 244, 245). The incidence of VTE varies considerably across procedures, with orthopedic surgery, invasive neurosurgical procedures, and major vascular procedures as the interventions associated with the highest VTE risk (242, 243). The incidence of VTE after surgery also depends on patient-related factors, such as age and history of VTE (7, 179, 180). Risk assessment scores based on intervention and patient characteristics, such as the Caprini score or the Rogers score, have been developed and are routinely recommended in several procedures (243, 246-249).

Several **medical conditions** are associated with an increased incidence of VTE (250). For instance, the link between arterial and venous thrombosis has been thoroughly investigated (89, 251-253). Several studies support a bidirectional association between arterial thrombotic diseases and VTE, and both MI and ischemic stroke are associated with an increased risk of subsequent VTE (254, 255). The pathophysiological mechanism is not yet fully understood, but shared risk factors, indirect factors, or a direct causal relationship have been proposed (121). However, studies argue against a strong impact of shared atherosclerotic risk factors, and the relationship may have an alternative explanation (254, 256-258). As seen during the COVID-19 pandemic, infections may contribute to hypercoagulability (259-261). In both hospitalized and non-hospitalized patients, acute infections are associated with an increased risk of VTE (55, 262, 263). Chronic obstructive pulmonary disease (COPD) is considered a moderate risk factor for VTE, and patients with severe COPD have a 60% higher risk of VTE than those with normal airflow (264). The risk is particularly pronounced during an acute exacerbation, and studies have observed a PE prevalence of 15-30% in COPD patients with an exacerbation (265-268). The risk related to COPD is assumed to be mediated through conventional risk factors, such as infection, immobilization, stasis, and right ventricular heart failure (264, 265).

Of all VTE cases, 40-60% are associated with **hospitalization** (6, 24, 269, 270). The overall age- and sex-adjusted VTE incidence is more than 100-fold higher among hospitalized patients compared to community residents (271). Thus, hospitalization is recognized as a major risk factor for VTE. Hospitalization is often accompanied by prolonged immobilization, which may partly explain the increased risk (228, 272). However, in a recent case-crossover study, hospitalization was found as a trigger for VTE in the absence of immobilization (272). The increased risk due to hospitalization is compounded (273) and represents a combination of the cause of the hospital admission (e.g., cancer, trauma, acute medical conditions), hospital-related factors (e.g., infection, surgery, immobilization), and the patient-related factors (e.g., age, heredity, obesity) (269, 272, 274).

## 1.5 Biomarkers and venous thromboembolism

The term biological marker, or biomarker, is defined by the World Health Organization as "*any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease*" (275). Biomarkers are used for several clinical and research purposes, and can be classified depending on their application, such as diagnostic,

predictive, prognostic, and therapeutic (276, 277). A diagnostic biomarker enables the detection of disease. A predictive marker may predict the risk of disease or the response to a treatment or therapeutic intervention. A prognostic biomarker may assess the likely course of a disease or outcome. Finally, a therapeutic marker may provide information on potential targets of treatments. In managing VTE, biomarkers are currently used as a part of the diagnostics work-up, estimating the risk of recurrent VTE and guiding the duration of therapy.

The biomarker D-dimer was first introduced over 30 years ago as a part of the diagnostics of VTE (278). D-dimer is still a cornerstone of the diagnostic work-up and the most recognized biomarker in the assessment of VTE (279). With high sensitivity, D-dimer is excellent for excluding the disease. However, the low specificity of the biomarker requires additional tests to confirm the diagnosis. Therefore, novel biomarkers are searched for to reduce the requirement of objective testing (280).

## 1.6 D-dimer

During thrombus formation, factor XIIIa creates covalent bindings between the D domains of two fibrin molecules, creating cross-linked fibrin (278, 281, 282). Through the breakdown of the thrombus, the enzyme plasmin cleaves cross-linked fibrin, producing fibrin degradation products (Figure 5). The covalently bonded D domains, i.e., the D-dimer units, are one of several fragments produced through fibrinolysis. Thus, the D-dimer fragments reflect the processes of thrombus formation and fibrinolysis. Elevated D-dimer levels may therefore be seen in the presence of thrombosis, such as in VTE patients (278, 283). However, elevated D-dimer levels are unspecific and may reflect various conditions, such as old age, pregnancy, or cancer (278, 284).

D-dimer was first proposed as part of the diagnostics of PE (285, 286) and DVT (287, 288) almost 30 years ago. Since then, the biomarker has also been applied to the diagnostic work-up of other diseases. For instance, D-dimer is essential in diagnosing and monitoring patients with disseminated intravascular coagulation (289) and acute aortic dissection (290, 291). Furthermore, some studies have shown that D-dimer levels could predict severe outcomes for patients with COVID-19 (292-294).

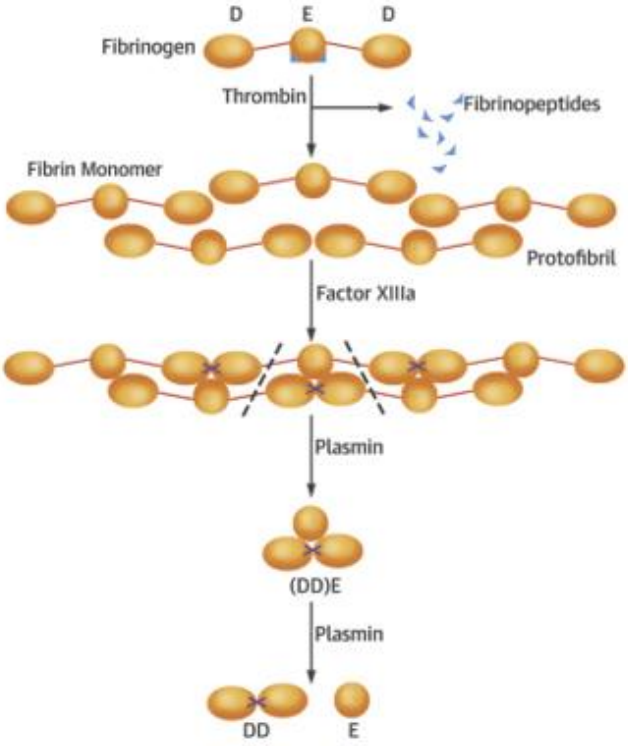


**Figure 5.** D-dimer formation (Weitz et al., J. Am. Coll. Cardiol. 2017)

### 1.6.1 Measuring D-dimer

The D-dimer levels are measured by monoclonal antibodies detecting specific epitopes for the D-dimer units (281). These epitopes are absent on fibrinogen and non-cross-linked fragments of fibrin, thus ensuring the measurement of only the D-dimer fragments (278). Different D-dimer assays use distinctive methods to detect the antibody complex. Due to its high sensitivity, the enzyme-linked immunosorbent assay (ELISA) is considered the gold standard within D-dimer assays (278, 281). The technique uses coated wells with antibodies to capture the D-dimer antigen before a second enzyme-linked antibody is added to create a colorimetric reaction in the presence of fibrin-related antigens (282). Despite its high sensitivity, the classic microplate ELISA is not regularly used in clinical practice due to its time-consuming design and the requirement of specialized personnel. Therefore, other techniques for real-time analysis have been introduced, such as enzyme-linked immunofluorescence assays, latex assays, and whole-blood assays (281). Later, second-generation latex-enhanced immunoturbidimetric assays were developed, such as the STA Lia assays used at the University hospital of North Norway (295). These assays are fully automatic, quantitative assays, using agglutination and photometric analysis to measure the D-dimer levels (281, 296). In the presence of D-dimer antigens, antibody-coated latex beads will cause agglutination and change the degree of light

absorption in the following photometric analysis (278, 281). The method gives a rapid result with comparable operating characteristics to the ELISA-based assays (281, 297).

The reporting units of the D-dimers have no current standardization and vary depending on the testing method (282). Therefore, two different systems of units are used, both D-dimer units and fibrinogen equivalent units (FEU) (296). While D-dimer units use purified D-dimer fragments as calibration, the calibration of FEU is obtained from purified fibrinogen clotted in the presence of factor XIIIa (278).

### 1.6.2 D-dimer as a diagnostic marker for VTE

As described in section 1.3 of this thesis, a VTE diagnosis generally requires verification with objective imaging testing, such as CUS and CTPA (110, 114, 118). As signs and symptoms of VTE may be diffuse and non-specific, and VTE is a highly prevalent differential diagnosis, excluding VTE by imaging testing in all patients with suspected VTE would be a resource-demanding task. In addition, exposure to radiation with CTPA is associated with an increased risk of cancer (298, 299). Therefore, current guidelines recommend using clinical pretest probability scores to assess the need for further diagnostic work-up (110, 114, 300). Over the years, several clinical probability assessment scores have been developed, both for DVT and PE (301-305). Common for these clinical algorithms are the use of signs and symptoms of VTE in combination with D-dimer to evaluate whether VTE can be safely ruled out or whether further diagnostic imaging is needed.

In DVT diagnostics, the pretest probability assessment is usually performed by the Wells score (301, 306). The original score was based on signs, symptoms, and risk factors for DVT and classified patients into three risk categories (low, moderate, or high probability). A later modification of the Wells score additionally incorporated previous documented DVT in the risk model (Table 2) (302). The modified Wells score dichotomizes the groups into two categories (DVT unlikely or likely) based on the total score ($<2$ or $\geq 2$ points) (302). In patients with a “DVT unlikely” pretest probability based on the modified Wells score, the D-dimer level should be measured (109, 110). With the combination of a low Wells score and a D-dimer level $<500$ ng/mL, DVT can be ruled out with a negative predictive value of 98-99% (307).

**Table 2.** Modified Wells score. Two-level, clinical model for predicting the pretest probability of DVT.

| Clinical Characteristic | Score |
|---|---|
| Active cancer (patient receiving treatment for cancer within the previous 6 mo or currently receiving palliative treatment) | 1 |
| Paralysis, paresis, or recent plaster immobilization of the lower extremities | 1 |
| Recently bedridden for 3 days or more, or major surgery within the previous 12 wk requiring general or regional anesthesia | 1 |
| Localized tenderness along the distribution of the deep venous system | 1 |
| Entire leg swollen | 1 |
| Calf swelling at least 3 cm larger than that on the asymptomatic side (measured 10 cm below tibial tuberosity) | 1 |
| Pitting edema confined to the symptomatic leg | 1 |
| Collateral superficial veins (nonvaricose) | 1 |
| Previously documented deep-vein thrombosis | 1 |
| Alternative diagnosis at least as likely as deep-vein thrombosis | -2 |

(Adapted from Wells et al., N Eng J Med 2003).

Due to heterogeneity within D-dimer assays, each manufacturer has defined its fixed cut-off for a positive test. Traditionally, this threshold has been chosen intentionally low to reduce the likelihood of a false negative result, i.e., increasing the sensitivity (278). However, by increasing the test's sensitivity, the specificity decreases, and the number of healthy patients with a positive test, i.e., false positive, will increase. As an alternative to a fixed threshold, varying cut-off levels according to clinical probability or special populations have been proposed to increase the negative predictive value (304, 308). For instance, in the YEARS algorithm for PE, patients with a low pretest probability require a higher D-dimer level (1000 ng/mL FEU) to be referred for imaging testing, compared with patients with a high pretest probability (500 ng/mL FEU) (304). As D-dimer increases with age (308-310), another proposed strategy is to adjust the D-dimer threshold according to age. In this approach, the patient's age is multiplied by 10 ng/mL to find their age-adjusted cut-off (308). For example, a 63-year-old person would have an age-adjusted cut-off of 630 ng/mL FEU, instead of the traditional cut-off of 500 ng/mL FEU.

Despite the central role of the Wells score in the diagnostic work-up of suspected DVT, the score has several limitations. The subjective assessment of some of the score items may introduce misclassification (311), and several studies have shown poor adherence and lack of correct implementation of the score in daily clinical practice (312-315). In addition, as many of today's clinics are organized to be as efficient as possible, standard blood samples, including

D-dimer, may be obtained before clinical evaluation. Consequently, D-dimer is potentially interpreted before calculating the Wells score, contrary to the intention behind the clinical decision rule (316-318). As an alternative, the use of D-dimer as a stand-alone test to rule out VTE has been proposed (319-321). A strategy that relies entirely on D-dimer to determine the need for objective imaging may simplify and optimize the efficiency of the diagnostic work-up and reduce the number of imaging tests. Frønæs et al. investigated the safety of D-dimer as a stand-alone test to rule out DVT in 913 outpatients referred with suspected DVT (319). Of the 298 (33%) patients with a negative D-dimer, only one patient was diagnosed with DVT, yielding a failure rate of 0.3%. These findings indicated that fixed D-dimer as a stand-alone test could safely exclude DVT while requiring fewer CUS than a combined approach of D-Dimer and Wells score. However, these findings have not been validated and further studies are needed.

### 1.6.3 Intrinsic D-dimer and risk of first VTE

Plasma D-dimer levels in healthy subjects have been associated with an increased risk of incident VTE (322) and coronary heart disease (323, 324). The intrinsic D-dimer level has therefore been proposed as a potential marker of inherent hypercoagulability. The relationship between plasma D-dimer and the future risk of VTE was first addressed prospectively in the LITE study (325). The LITE study consisted of two cohort studies, the Atherosclerosis Risk in Communities (ARIC) Study and the Cardiovascular Health Study. The study used a nested case-control design, including 307 VTE cases and 616 controls. In the LITE study, individuals with the highest D-dimer levels (quintile 5, $\geq$ 277.8 ng/mL) had a 3-fold increased risk of VTE compared to those with the lowest levels (quintile 1, <68.6 ng/mL), when adjusted for age, race, sex, BMI, FVL, prothrombin 20210A, and elevated FVIII (325). Later, using the ARIC cohort with more than ten years of follow-up, a dose-response relationship between plasma D-dimer levels and the risk of future VTE was found (326). The association has further been confirmed in a nested case-control study including 215 VTE cases and 867 controls derived from the Women's Health Initiative hormone trials (327). In the Framingham Heart Study, a cohort study of 3120 participants and 139 VTE cases, higher D-dimer levels were one of the investigated biomarkers associated with increased risk of VTE (328).

The D-dimer levels may be influenced by several established risk factors, such as cancer and obesity (278, 284). Studies have also shown an association between D-dimer levels and

genetic factors, e.g., FVL and prothrombin G20210A (325, 329). However, findings from a GWAS study indicated that only a modest proportion of the proposed variation in plasma D-dimer could be explained by genetic variants located in hemostatic factors genes (330). Thus, the relationship between intrinsic D-dimer levels and future risk of VTE may particularly reflect acquired underlying conditions predisposing for VTE. One example of such a condition is inflammation, which influences both the risk of VTE and the plasma D-dimer level (193, 278). The impact of chronic low-grade inflammation, often assessed by C-reactive protein (CRP), on the association between plasma D-dimer and incident VTE remains unclear. Further, the association across subgroups of VTE, i.e., PE, DVT, provoked, and unprovoked VTE, and the potential association of D-dimer with VTE over time has not been investigated.

### 1.6.4 D-dimer and recurrent venous thromboembolism

The risk of recurrence is high after a first VTE. Up to 30-40% of VTE patients experience a recurrence within ten years following the first event (60, 331-333). Despite anticoagulant treatment, the recurrence risk is highest in the initial 6 to 12 months following an incident event (72, 332). Although the risk declines thereafter, the risk of recurrence never returns to the baseline VTE risk observed in the general population (60, 75). Accordingly, VTE may be regarded as a chronic disease (7, 332, 333).

Secondary prophylaxis with anticoagulants is highly effective in preventing recurrence (26, 334), though at the expense of increased risk of bleeding (335). In patients at high risk of recurrence, prolonged treatment is necessary, and the subsequent increased bleeding risk can be justified. However, in patients where short-term anticoagulation is sufficient to prevent VTE recurrence, extended anticoagulant treatment introduces unnecessary bleeding risk. The challenge lies in identifying patients who may benefit from extended thromboprophylaxis but with minimal risk of bleeding complications. Likewise, to avoid excessive exposure to bleeding risk, it is desirable to identify subjects with a low risk of VTE recurrence in whom short-term treatment with anticoagulants would be sufficient.

D-dimer is an established biomarker for recurrent VTE, and elevated D-dimer after discontinuation of the anticoagulant treatment is associated with a 2- to 4-fold increased risk of recurrence (336-340). Thus, D-dimer levels are proposed as a helpful tool to guide the duration of anticoagulant therapy (109). The current suggested strategies are based on D-dimer levels measured during or after cessation of the anticoagulant treatment, where a positive D-dimer

may indicate the need for prolonged therapy (341, 342). Further, several prediction models use clinical risk factors in combination with D-dimer to stratify patients according to the risk of recurrent VTE, such as DASH (343), Vienna prediction model (344), and HERDOO2 (345).

The currently recommended strategies are based on D-dimer levels after discontinuation of the anticoagulant treatment and require additional blood samples and revisits to the clinics (343-345). As D-dimer is commonly used in the diagnostic work-up of patients with suspected VTE (302), D-dimer assessment at the time of diagnosis is widely available. Using the D-dimer level assessed before initiating anticoagulant therapy is potentially less resource-demanding and may be a preferable approach. In a study of 454 patients with first-time VTE, Bjøri et al. showed that D-dimer measured at the time of VTE diagnosis could identify patients at low risk of recurrent VTE (295). While patients with a D-dimer level >1500 ng/mL had an estimated 10-year cumulative incidence of 35%, the cumulative incidence was only 14% among patients with D-dimer levels ≤1500 ng/mL. The study findings are promising but need to be further investigated and confirmed in a larger study population.

## 1.7 Summary and rationale for the present thesis

D-dimer is currently applied as a diagnostic marker for VTE in combination with pretest probability, usually performed by the Wells score. Despite the central role of the Wells score, the pretest probability score has several limitations. The subjective assessment of some of the score items may introduce misclassification (311), and several studies have shown poor adherence and lack of correct implementation of the score in daily clinical practice (312-315). Consequently, the use of D-dimer as a stand-alone test to rule out VTE has been proposed (319-321). A strategy that relies entirely on D-dimer to determine the need for objective imaging may simplify and optimize the efficiency of the diagnostic work-up and reduce the number of imaging tests. One study has previously investigated the strategy with promising results (319), but these findings are not validated. Therefore, in the present thesis, we investigated the safety and efficiency of D-dimer as a stand-alone test in a larger cohort of unselected DVT patients.

Intrinsic D-dimer levels are proposed as a clinical risk factor for future VTE, and some studies indicate an association between higher baseline D-dimer values and increased risk of incident VTE (325, 326, 328). However, the association is limited investigated and only addressed in a few studies (325, 326, 328). Further, a genetic study revealed that only a modest proportion of variation in the plasma D-dimer levels might be explained by genetic variants

located in the hemostatic genes (330). Thus, increased plasma D-dimer may particularly reflect acquired conditions. In the present thesis, we investigated the association between inherent D-dimer and the risk of future incident VTE. In addition, we assessed the influence of the acquired conditions inflammation and obesity and extended the investigation to different subgroups of VTE.

D-dimer is associated with an increased risk of recurrent VTE in several studies and may guide the appropriate duration of anticoagulant therapy (336, 337, 342, 346). The currently proposed strategies are based on D-dimer levels measured after discontinuation of the anticoagulant treatment (343-345), an approach requiring additional blood samples and revisits to the clinics. Alternatively, using D-dimer levels at the time of diagnosis is potentially less resource-demanding and may be a preferable approach. In a study by Bjori et al., the authors showed that D-dimer measured at the time of VTE diagnosis could identify patients at low risk of recurrence (295). The findings of the study are promising but need to be further investigated and confirmed in another, larger study population. Therefore, the present thesis investigated the impact of D-dimer measured at the time of VTE diagnosis on the risk of recurrence.

# 2 Aim of the thesis

The specific aims of this thesis were:

A. To assess the safety and efficiency of applying D-dimer as a stand-alone test in a larger population of unselected outpatients with a suspected DVT.

B. To investigate the association between plasma D-dimer levels and incident VTE in a population-based nested case-control study while adjusting for high-sensitivity C-reactive protein.

C. To investigate the impact of D-dimer measured at the time of VTE diagnosis on the risk of recurrence in a large cohort of patients with a first-time VTE.

# 3 Methods

## 3.1 D-dimer as a stand-alone test to rule out deep vein thrombosis

### 3.1.1 Study population

In paper I, data was collected from consecutive outpatients referred with a suspected DVT to the Emergency Department of the University Hospital of North Norway (UNN) in Tromsø, Norway, between 2008 and 2018. UNN is the sole provider of all VTE diagnostic procedures and VTE-related healthcare in the Tromsø region, serving a local population of 127 000 inhabitants. A total of 2003 patients were referred with a suspected DVT during the 10-year period. Patients with a permanent address outside the hospital's catchment area, who could not be followed for three months, were excluded. The hospital is the only clinic providing diagnostic and therapeutic management of VTE within a radius of 250 km. Thus, the likelihood of a complete follow-up of patients living in this catchment area is high.

### 3.1.2 Exposure and outcome assessment

**D-dimer** was assessed at the time of diagnosis as a part of the diagnostics work-up in patients with suspect VTE. For the diagnostic purpose, a positive D-dimer was defined as levels ≥500 ng/mL. All blood samples were analyzed at the Department of Clinical Chemistry at UNN, and the D-dimer levels were assessed by the immunoturbidimetric methods of STA®-Liatest® D-Di Plus (Stago Diagnostics, Asnieres, France).

Patients diagnosed with **DVT** at the Emergency Department at UNN were recorded as a DVT event. The diagnosis was verified and confirmed by whole-leg CUS, assessing all veins of the affected extremity for compressibility. Non-compressibility was the main criterion for DVT, but a confident gray-scale visualization of the thrombus was also considered diagnostic. In patients where a CUS was impractical (e.g., if leg casting or excessive subcutaneous tissue or fluid prevented adequate assessment of compressibility) or the result of the CUS was uncertain, venography was performed instead or as an additional test. All patients referred with suspected DVT to the Emergency Department were followed for three months after their visit by close review of their medical records. In patients where DVT was ruled out at baseline, a diagnosis of DVT occurring in the following three months was considered an undetected and misclassified event from their first visit. DVT occurring after the initial three months follow-up period was considered a new event, i.e., incident or recurrent DVT.

The **failure rate** was used to express the safety of the diagnostic strategy of interest. The failure rate was defined as the proportion of patients who did not meet the criteria for undergoing CUS defined by the chosen strategy, i.e., negative tests, but still were diagnosed with DVT. The amount of **required CUS** indirectly expressed the efficiency of the strategy, defined as the proportion of patients who met the criteria for undergoing CUS for the chosen approach, i.e., positive tests.

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

### 3.2.1 Study population

The Tromsø study is a single-center, prospective, population-based study with repeated health surveys of the inhabitants of Tromsø, Norway (347). The study was started in 1974. Since then, over four decades later, six additional surveys have been conducted. The latest survey, Tromsø 7, was completed in 2016. The study's long follow-up, high attendance rate, longitudinal design, and single-center follow-up are some of the many strengths of the study. In paper II, the study population consisted of participants from the fourth survey of the Tromsø study. The study had a high attendance rate, with inclusion of 77% of those invited and a total of 27,158 individuals participating. The study participants were followed from the date of inclusion until an incident VTE, emigration, death, or end of follow-up (September 1, 2007), whichever came first. In total, 462 subjects experienced a VTE event during the follow-up period (1994-2007). With a nested case-control study design, each case was assigned two age- and sex-matched controls, randomly sampled among the remaining participants in Tromsø 4, who were alive at the index date of the VTE event (n=924). Due to the insufficient quality of plasma samples, 48 cases and 81 controls were excluded. In total, 414 VTE cases and 843 controls were included in the final analysis of the nested case-control study.

### 3.2.2 Exposure and outcome assessment

Intrinsic measurement of **D-dimer** was analyzed from stored plasma samples in all subjects with a VTE event in the Tromsø study, collected at inclusion in Tromsø 4 (1994-1995). D-dimer was measured by enzyme-immunoassay using a monoclonal antibody (s4H9) (348) for coating together with a monoclonal horseradish peroxidase-conjugated antibody for detection

(ab24474, Abcam, Cambridge, United Kingdom). Parallel diluted samples of known concentration were used as standards.

**High-sensitivity CRP** was measured by enzyme-immunoassay using commercially available reagents (R&D Systems, Minneapolis, MN) in a 384 format using the combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT) 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, Winooski, VT).

All incident **VTE** events were identified by searching the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry at the University Hospital of North Norway. UNN is the sole provider of all in- and outpatient VTE-related diagnostic procedures and VTE-related healthcare in the Tromsø region. The relevant discharge codes were ICD-9 codes 325, 415.1, 451, 452, 453, 671.3, 671.4, and 671.9 for 1994–1998, and ICD-10 codes I26, I80, I81, I82, 167.2, O22.5, O87.1 and O87.3 for the period 1999–2012. Trained personnel reviewed the medical record for each potential VTE case and extracted information for case validation. A VTE event was adjudicated when the presence of clinical signs and symptoms of DVT or PE were combined with objective confirmatory tests, i.e., compression ultrasonography, venography, spiral computed tomography, perfusion–ventilation scan, pulmonary angiography, or autopsy, and resulted in a VTE diagnosis that required treatment with anticoagulants (low molecular weight heparin, warfarin, or DOAC), thrombolytic therapy, or vascular surgery, unless contraindications were specified. For cases derived from the autopsy registry, a VTE event was only recorded when the autopsy record indicated PE as the sole cause of death or as a significant contributing cause of death.

The VTE events were categorized as **provoked** or **unprovoked**, determined by the presence of known provoking factors at the time of diagnosis. Provoking factors in the Tromsø study were defined as recent surgery or trauma within the previous eight weeks, acute medical conditions, i.e., acute myocardial infarction, ischemic stroke, or major infectious disease, active cancer, immobilization (i.e., bed rest >3 days, wheelchair use, or long-distance travel exceeding 4 hours within the last 14 days prior to the event), or any other factor specifically described by a physician in the medical records.

## 3.3 Low D-dimer levels at diagnosis of venous thromboembolism are associated with reduced risk of recurrence: : data from the TROLL registry

In paper III, the study population comprised patients enrolled in the Venous Thrombosis Registry in Østfold Hospital (TROLL) registry, a large cohort study of VTE patients. Between January 1, 2005, and April 30, 2020, all consecutive patients diagnosed and treated for VTE at the Østfold Hospital were enrolled in the TROLL registry. Østfold Hospital, located in Østfold county, Norway, serves a local population of approximately 317 000 inhabitants. Inclusion required symptomatic lower limb DVT or PE. Patients diagnosed with both DVT and PE were categorized as PE. Patients with a permanent address outside the hospital's catchment area were not included to increase the likelihood of a complete follow-up. A total of 3586 patients with an incident VTE were eligible.

### 3.3.1 Exposure and outcome assessment

**D-dimer** was assessed at the time of diagnosis as a part of the diagnostics work-up in patients with suspect VTE. For the diagnostic purpose, a positive D-dimer was defined as levels ≥500 ng/mL. D-dimer was assessed by STA®-Liatest® D-Di Plus assay and analyzed at the Department of Clinical Chemistry at Østfold Hospital. In the study, we divided the population into quartiles based on the D-dimer levels (quartile 1, ≤1900 ng/mL; quartile 2, 2000-3500 ng/mL; quartile 3, 3600-8200 ng/mL; quartile 4, >8200 ng/mL). Since a previous study had shown a threshold effect at the lowest quartile (295), we merged the upper three quartiles yielding two final categories (≤1900 ng/mL and >1900 ng/mL).

In the TROLL registry, all in- and outpatients diagnosed with **VTE** (i.e., distal DVT, proximal DVT, or PE) were referred to and followed up by the hospital's outpatient clinic for thrombosis patients. All recurrent events in patients included in the TROLL registry were verified and recorded by the outpatient clinic. In addition, the hospital discharge diagnosis registry was searched to identify all potentially remaining recurrent events. The potential events identified by the discharge diagnosis registry were verified and recorded by reviewing the patients' medical records. Recurrent events included distal DVT, proximal DVT and/or PE (fatal and non-fatal). For fatal PE, the diagnosis was determined by imaging performed shortly before death or autopsy. Individuals without recurrence who died of unknown or uncertain causes during follow-up were not considered as recurrent events.

The VTE events were categorized as **provoked** or **unprovoked**, determined by the presence of known provoking factors at the time of diagnosis. In the TROLL registry, an event was defined as provoked by the presence of one or more of the following factors: recent surgery or trauma, immobilization due to medical conditions, paralysis of the lower extremities, or long-distance travel (>4 hours) within the previous 12 weeks, or any other factor explicitly described as being provoking in the medical records.

# 4 Main results

## 4.1 Paper I – D-dimer as a stand-alone test to rule out deep vein thrombosis

Current guidelines recommend the use of clinical decision rules, such as Wells score, in combination with D-dimer to assess the need for objective imaging to rule out DVT. However, the clinical decision rule has limitations, and the use of D-dimer as a stand-alone test has been suggested. We aimed to assess the safety and efficiency of D-dimer as a stand-alone test to rule out DVT in outpatients referred with suspected DVT, using collected data from consecutive outpatients referred to our hospital with suspected DVT in 2008-2018. D-dimer levels were analyzed using STA® Liatest® D-Di assay. D-dimer as a stand-alone test was theoretically applied in retrospect, and the number of misdiagnosed events were estimated as if such an approach had been initially used. All patients were followed for three months. Of 1765 included patients, 293 (16.6%) were diagnosed with DVT. A total of 491 patients (27.8%) had a negative D-dimer (<500 ng/mL). Of these, nine were diagnosed with DVT, yielding a failure rate for D-dimer as a stand-alone test of 1.8% (95% CI, 0.8%-3.5%). The majority of the misdiagnosed patients had a distal DVT (6/9). In analyses restricted to proximal DVTs, the failure rate was 0.6% (95% CI, 0.1%-1.8%). D-dimer as a stand-alone approach reduced the required ultrasounds from 81.8% to 72.2%. In conclusion, D-dimer as a stand-alone test may be safe for excluding proximal DVT. The strategy has the potential to simplify and increase the efficiency of the diagnostic work-up in patients with suspected DVT.

## 4.2 Paper II – Elevated plasma D-dimer levels are associated with risk of future incident venous thromboembolism

D-dimer, a global biomarker for activation of the coagulation and fibrinolysis systems, is useful in assessing individual risk of VTE recurrence. However, there is limited information on the association between D-dimer and the risk of an incident VTE event. In the study, we sought to investigate the association between plasma D-dimer levels and the risk of future incident VTE. With a nested case-control study, derived from the Tromsø study (1994-2007), 414 VTE patients and 843 randomly selected age- and sex-matched controls were included. D-dimer was measured in plasma samples collected at cohort baseline (1994–95). OR for VTE with 95% confidence intervals (CIs) were estimated according to quartile cut-offs of D-dimer levels determined in controls. The risk of VTE increased across quartiles of D-dimer levels (Ptrend = 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 BMI (OR 1.51, 95% CI 1.04–2.20) and 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. Our results indicate that elevated plasma D-dimer levels are associated with an increased risk of incident VTE. However, the attenuation of the risk estimates upon additional adjustment for BMI and CRP suggests that D-dimer partly reflects underlying conditions associated with obesity and an inflammatory state.

## 4.3 Paper III – Low D-dimer levels at diagnosis of venous thromboembolism are associated with reduced risk of recurrence: data from the TROLL registry

VTE is a severe disease with a high risk of recurrence. It has been suggested that D-dimer levels at the time of VTE diagnosis can be used to identify patients at low risk of recurrent VTE. We therefore aimed to investigate the impact of D-dimer measured at the time of VTE diagnosis on the risk of recurrence in a large cohort of patients with an incident VTE. In the TROLL registry, we included 2585 non-cancer associated patients with incident symptomatic VTE. All recurrent events during follow-up were recorded, and the cumulative incidences of recurrence were estimated according to D-dimer levels ≤1900 ng/mL (≤25$^{th}$ percentile) and >1900 ng/mL. During a median follow-up of 3.3 years, 395 patients experienced a recurrent VTE. The one- and five-year cumulative incidence of recurrence was 2.9% (95% CI, 1.8-4.6) and 11.4% (95% CI, 8.7-14.8) in those with D-dimer ≤1900 ng/mL, and 5.0% (95% CI, 4.0-6.1) and 18.3% (95% CI, 16.2-20.6) in those with D-dimer>1900 ng/mL, respectively. In patients with unprovoked VTE, the five-year cumulative incidence was 14.3% (95% CI, 10.3-19.7) in the ≤1900 ng/mL category, and 20.2% (95% CI, 17.3-23.5 95%) in the >1900 ng/mL category. In a cox regression model adjusting for age and sex, patients with a D-dimer ≤1900 ng/mL had a 39% lower risk of recurrence (HR 0.61, 95% CI 0.47-0.79) compared to patients with a D-dimer >1900 ng/mL. Our findings indicate that D-dimer levels within the lowest quartile, measured at the time of VTE diagnosis, were associated with reduced lower recurrence risk. The findings imply that D-dimer levels measured at the time of diagnosis may be used to identify VTE patients at low risk of recurrent VTE.

# 5 General discussion

## 5.1 Methodological considerations

### 5.1.1 Study design

The aim of epidemiological studies is mainly to contribute to understanding the frequency, pattern, and causes of disease in populations (349). Different study designs can be viewed as tools to achieve these aims. Study design can be classified based on three commonly used classifications: 1) descriptive or analytic studies, 2) retrospective or prospective studies, and 3) observational or experimental studies. Traditionally, based on these three classifications and inherent design features (e.g., numbers of groups, sampling method, measurement before and after intervention), study designs have been divided into five main groups: case series, cross-sectional, case-control, cohort, and trials, all with different strengths and limitations (349).

A cohort study follows a sample or an entire population to assess the relationship between exposure and an outcome (349). Information on exposures and patients' characteristics are collected from each participant at inclusion and potentially through subsequent follow-ups. The participants are followed from enrolment until an outcome of interest or other censoring events, such as migration, death, or end of the study period, occur. Participants are classified according to the exposure of interest, and the probability of developing the outcome in the exposed group is compared with the non-exposed group. (350). Paper III was based on a cohort of VTE patients at Østfold Hospital, the TROLL registry. The cohort consisted of over 2500 patients with incident VTE, recruited from 2005 to 2020. In the paper, we investigated the relationship between the exposure, low D-dimer at diagnosis, and the outcome, recurrent VTE. The prospective design of the cohort study allowed the estimation of absolute and relative risk, and we estimated both cumulative incidences (absolute risk) and hazard ratios (relative risk) of recurrence according to exposure levels. The temporal sequence of the exposure and outcome avoids the risk of reverse causation (350). Further, the cohort of unselected/consecutive VTE patients recruited from the general population yields a high degree of generalization of the result, i.e., increasing the study's external validity. With six-fold as many patients as in a previous study on the subject, the considerable sample size reduced the risk of type II error, i.e., rejecting a true association, and allowed reliable analyses also in subsets of the study population. One of the major methodological challenges with a cohort study is the time-consuming design, as seen in the TROLL registry with participants recruited for over 15 years.

The study is also vulnerable to changes in exposure during follow-up, confounding, and potential biases, as further discussed in detail in the following sections of this thesis. The TROLL registry's recruitment from the general population yields a high external validity, but the approach may be inefficient in investigating the incidence of a latent or rare outcome. However, as the recurrence rate of VTE is fairly high this design was well suited for our study purpose (295, 344, 345).

In paper I, a cross-sectional design was applied, and four diagnostic strategies of interest were investigated. In the study, individuals with suspected DVT referred to UNN were included. The different diagnostic strategies of interest were then theoretically applied based on the actual DVT diagnosis at the initial visit or during the subsequent three months, i.e., an event occurring during this time was considered missed at the initial diagnostic work-up. The criteria that would have led to referral for radiological imaging for each approach were used, and the proportion of DVT patients not detected by the specific diagnostic strategy was estimated retrospectively. This retrospective approach entailed no additional risk for the participants, as they were managed according to the current guidelines. However, a limitation of the study design and the retrospective approach is the lack of clinical aspects of implementing D-dimer as a stand-alone test. In everyday clinical practice, the physician's assessments and clinical judgment would affect the management of the patients. Unfortunately, this aspect would not be assessed by these retrospective estimates. Therefore, prospective studies are required to investigate the utility of D-dimer as a stand-alone test in a real-life setting.

Paper II is based on a case-control design within a cohort study, a design termed nested case-control study. In a nested case-control study, the cohort participants' characteristics and exposures are assessed at inclusion (351). The cases and controls are then identified at a later time (Figure 7). Study participants developing the outcome of interest, e.g., VTE, are defined as cases. The control group is randomly assembled from the parent cohort, usually matched on the time of outcome (i.e., controls have to be alive and under cohort follow-up at the time the case occurs), age, or sex (352). As cases and controls are recruited from the same source population, the chance of selection bias is low. Further, the nested case-control design preserves the temporal sequence between exposure and outcome, thus preventing reverse causation. Finally, as only a small proportion of the parent cohort is analyzed, the design may be more cost-effective (352). For instance, in paper II the cost of the study was reduced as intrinsic D-dimer only was assessed in the roughly 1200 cases and controls, and not the whole parent cohort

of almost 30,000. As for cohort studies, nested case-control studies may be vulnerable to the influence of confounding and changes in exposure during follow-up.

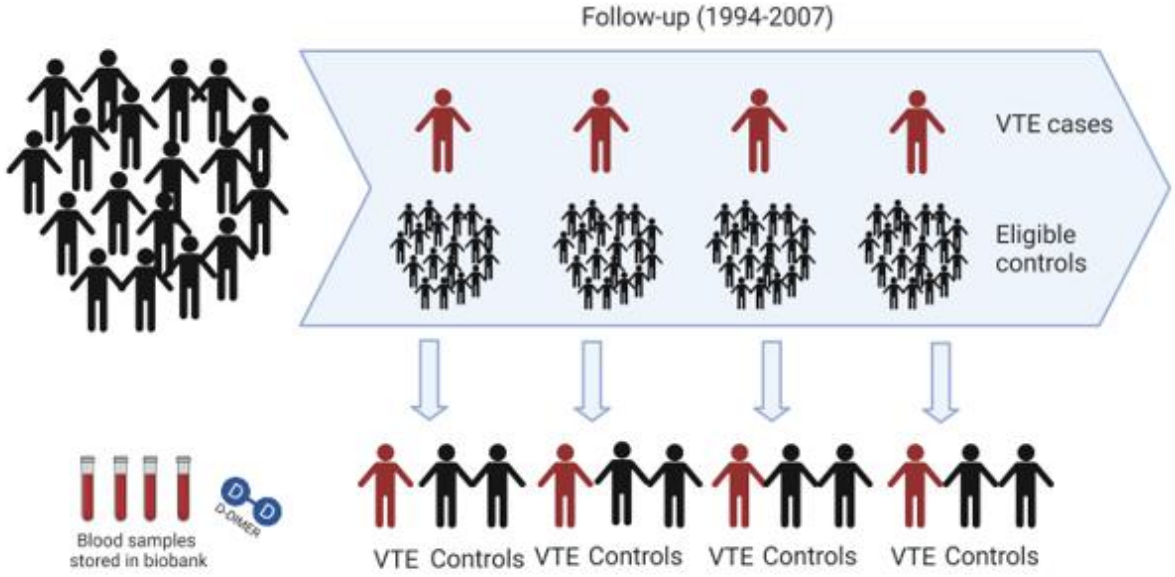


**Figure 7.** Graphical illustration of the nested case-control design in the Tromsø study.

### 5.1.2 Generalizability and external validity

External validity, or generalizability, describes to what extent the results from a study can be directly applied to other populations (353). The internal validity of a study concerns to which extent the results are valid for the defined population studied (354). As population-based cohort studies are recruited from the general population, a broad spectrum of participants is included, and higher generalizability is achieved (350). Nevertheless, the introduction of selection bias in the recruitment and/or follow-up period may limit the generalizability of population-based cohorts. High participation rates and minimal loss to follow-up are important aspects of a cohort study to ensure high external validity.

The participants in the Tromsø study are recruited from the general population and generally have a high attendance rate, with over 75% in the first five surveys (347). The sixth survey, conducted in 2007-2008, had a slightly lower attendance rate (66%) due to a lower attendance rate among the relatively young participants (347). A decline in the participation rate for epidemiological studies in recent years is a common trend seen in other studies (355). For example, the attendance rate in the ARIC study declined from almost 100% at the baseline enrolment to 80% at the fifth exam (356). In the Tromsø study, as in most health surveys, the participation rate was lower among the younger and the older population (347, 355). In addition, the participation rate was lower among men than women (347, 355). The lower participation

rate of the young and the elderly may affect the generalization of our findings in these age groups. However, as the differences are small, these are not likely to have a practical impact on our results.

Further, in the studies from UNN and Østfold Hospital, study participants were recruited among suspected or confirmed VTE patients. These studies were more clinical-oriented, and consecutive patients referred to the respective hospitals were enrolled. The results of these studies are therefore highly generalizable and represent the population and clinical setting of interest. However, as these studies were conducted at a single hospital, the validity to other clinics and countries may differ. For instance, the study population consisted primarily of participants of Caucasian origin, reducing its generalizability regarding different ethnicities. Furthermore, a selection of patients agreeing to enrollment may occur. In the TROLL registry, 10% of the original study population was excluded due to lack of consent (10). No data was available for sensitivity analysis in these patients, but as for other epidemiological studies, one may speculate that individuals of higher social status and healthier lifestyles may be more likely to volunteer. This effect is known as response bias (357). In addition, obtaining written consent may be challenging in severe cases, particularly those who die before the scheduled follow-up. Therefore, to enhance the generalizability of the TROLL registry, particularly to severe cases, the ethics committee exempted deceased patients from the requirement of written consent. Although the lack of consent was limited to a small proportion of the study population, this may reduce the external validity for these less-represented groups.

### 5.1.3 Confounding

Traditionally, the term confounding is used when a non-causal association between a given exposure and an outcome is observed due to the influence of a third variable, i.e., a confounder (358). In epidemiology, a confounder is defined as a factor associated with the outcome and the exposure and is unevenly distributed between the compared exposure groups (Figure 8) (359, 360). Factors in the causal pathway between the exposure and the outcome should not be treated as a confounder. Instead, these factors are required to be handled as intermediate factors, often termed mediators (Figure 8) (359). In an RCT, potential confounders are expected to be evenly distributed between the compared groups due to the randomization process (360). Observational studies, however, have no inherent protection against confounders. Thus, the findings in observational studies may be vulnerable to confounding factors, especially

unmeasured or unknown confounders, i.e., residual confounding. Therefore, cohort studies must be analyzed with caution, and differences in the comparison groups must be considered.

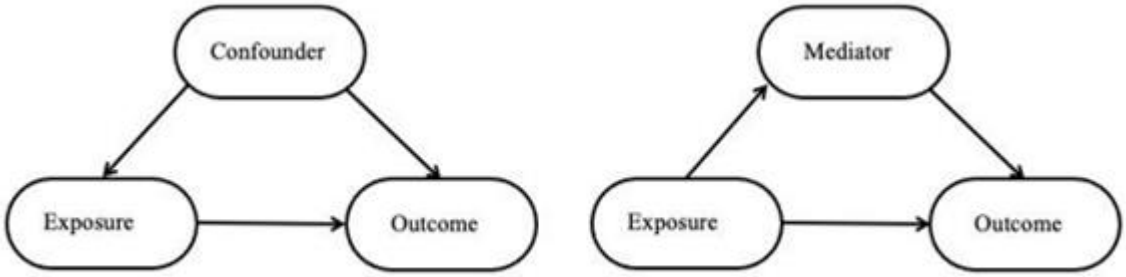


**Figure 8.** The concept of confounding and mediation.

Several approaches are applied to deal with confounders through the study design and analysis (361). Randomization, restriction of the study population, and matching are different methods to control for confounders commonly used when designing epidemiological studies. In the nested case-control study of paper II, controls were matched on age and sex, thus partly controlling for confounding for these factors. In addition, the matching may improve the precision of the study as the distribution of confounders among the cases and controls become more similar (362). For instance, as VTE mainly affects older individuals, an unmatched control group randomly sampled from the source population would have a much younger age distribution and yield a skewed basis of the subsequent adjustment. When age is then controlled for in the analysis, the young age stratum mainly consists of controls, whereas the old age stratum mainly consists of cases. Thus, matching on age may improve the precision of the study, as each age stratum has roughly the same number of cases and controls. However, as the matching makes cases and controls more similar, also in terms of the exposure, this technique may introduce confounding (362). Therefore, in paper II, logistic regression models were applied to control for the confounding by the matching process. Due to the limited number of matching factors (age and sex) and as the matching factors were not genuinely at the individual level, a pairwise analysis was not considered necessary, and unconditional logistic regression analyses were applied (362). Unconditional analyses of the study population yield a slightly increased precision, as pair-matched analyses will divide the population into multiple strata, resulting in lower statistical power (362).

In statistical analysis, confounders are usually handled with the help of stratification and/or regression models (361). Stratification divides the study population into homogeneous categories, i.e., strata, of the confounding variable. For example, patients with distal DVT may have a lower risk of recurrent VTE and a lower D-dimer level at the time of diagnosis. Thus,

the observed association between low D-dimer and low recurrence could potentially be confounded by a skewed distribution of distal and proximal DVTs in the D-dimer categories (338). Therefore, in paper III, we performed a sensitivity analysis separately for patients with distal DVTs and patients with proximal DVTs or PE. We observed that the association between low D-dimer and recurrence remained in the proximal DVT+PE category, indicating that DVT-localization did not confound the association. The main issue with stratification is the loss of statistical power as the study population is divided into smaller and smaller subsets in the presence of several confounding factors (359).

Regression modeling is another analytic method to control for confounding (363). Potential confounders are included in a multivariable regression model as covariates. Regression models estimate the effect of the confounder on the association, and the confounder is controlled by adjusting for its influence. By using multivariable regression models, an estimation based on the study population as a whole could be made, and in contrast to stratification, the study's statistical power is minimally affected (364). A potential limitation of regression models, as for most methods for adjusting for confounding, is the risk of overadjustment or unnecessary adjustment (365). By including mediators on the causal pathway as covariates in the model, i.e., overadjustment, the regression model may underestimate the true association between the exposure and the outcome. Adjusting for variables that do not affect the association, i.e., unnecessary adjustment, would not affect the casual relationship but may affect its precision, potentially demanding a greater statistical power to detect a true association. Last, regression models may only be applied to known and measured confounding factors and may therefore be vulnerable to residual confounders.

In the present thesis, several different regression models were applied. Obesity and inflammation may influence both the D-dimer levels (326, 366, 367) and the risk of VTE (190, 368). To adjust for these potential confounders in paper II, BMI and high-sensitivity CRP were included as covariates in the regression models. In paper III, the estimates were adjusted for age and sex, as these factors may be unequally distributed and potentially confound the association of interest. By including these factors in the regression models, we have attempted to control for these potential confounders. Another example is the duration of anticoagulant therapy and its effect on recurrence seen in paper III. In the study, treatment duration was unevenly distributed across levels of D-dimer, with the longest duration of therapy in patients with the highest D-dimer levels. As anticoagulants are highly effective in preventing recurrent VTE, this may confound the association between D-dimer levels and the risk of recurrence.

Therefore, in addition to age and sex, the duration of anticoagulant therapy was included in the regression model to control for its potential confounding effect.

### 5.1.4 Bias and misclassification

#### *5.1.4.1 Selection bias*

Bias, often defined as systematic errors, is an essential part of every epidemiological study (349). Bias is commonly grouped into selection bias and information bias, and sometimes, confounding is considered the third group of bias (349). Selection bias occurs due to systematic differences in characteristics between those who participate in a study and those who do not (369). Consequently, the chosen study population is not representative of the population intended to be investigated. This is a potential problem especially in RCTs, where rather strict enrolling criteria often is present. Thus, the study population may not represent the population intended to treat. Cohort studies may also be vulnerable to selection bias, as the participants who volunteer to enroll may differ from the non-responders, reducing the external validity as described in section 5.1.2 of this thesis.

Selection bias may affect the study's internal validity if a selection of exposed compared to non-exposed participants occur. In cohort studies, all participants are recruited from the same source population, and the risk of selection bias affecting the internal validity is usually not an issue. However, in case-control studies, the cases and controls may be recruited on different terms. In a well-planned study, both groups are recruited and represent the same source population. Unfortunately, this is not always the case. For example, if cases are recruited from hospitalized patients with the disease of interest, choosing the control group for the general population may introduce a selection bias. The cases would largely differ from the controls, and not only in the exposure of interest. In paper II, VTE cases were compared to non-VTE controls. However, due to the nested case-control study design, the cases and controls were both recruited from the same source population, the Tromsø study. The recruitment to a population-based cohort such as the Tromsø study may introduce differences in the subject participating compared to those not, i.e., the external validity. The comparison within the cohort, i.e., the internal validity, would not be affected. Therefore, in contrast to standard case-control studies, the nested case-control design we used in paper II minimizes the risk of low internal validity due to selection bias (351).

As for inclusion, the likelihood of a completed follow-up may differ between study participants, also introducing a risk of selection bias. Depending on the organization of the follow-up period, the time under observation may vary across subjects. In paper I, the patients were followed through their medical records, and a diagnosis of DVT during this three-month follow-up was considered an undetected event from their first visit. This follow-up depends on the patient recontacting the health service with persistent or worsening symptoms. In theory, one may speculate that specific groups of patients, such as patients with health anxiety, may be less likely to see a physician. In these groups, a DVT could be undetected due to loss of follow-up. However, the likelihood of such a case remains theoretical and probably has minimal impact on our findings. In Paper I, a complete follow-up also depended on referral to the original hospital, UNN. Migration or traveling may therefore reduce the degree of follow-up. However, as UNN is the sole provider of all VTE diagnostic procedures and VTE-related healthcare in the region, the risk of loss to follow-up is limited. To further ensure a high degree of complete follow-up, we excluded all study participants with a permanent address outside the hospital's catchment area who could not be followed for three months.

Competing risk may also introduce differences in time under observation between the participants (370). For instance, due to the high mortality rate among cancer patients, the association between cancer and VTE is shown to be overestimated due to competing risk by death (371). In regular survival analyses, the statistical estimations do not take competing risks into account, and death is usually recorded as the censoring event (370, 371). An essential assumption of the survival analysis is non-informative censoring, meaning that the future risk of VTE is unrelated to the censoring event. For instance, due to the high mortality risk among cancer patients, individuals with cancer may have a shorter follow-up time before the censoring event, death. Thus, the observation time in cancer patients is reduced, and the observed association may be overestimated (371). In Paper III, the exposure, elevated D-dimer, is known to be associated with an increased risk of all-cause mortality (324). As for cancer in the example above, the higher mortality risk among patients with elevated D-dimer could introduce a selection in the follow-up and yield a shorter follow-up time in these patients. This may potentially introduce an overestimation of the association. To evaluate the impact of competing risk by death in paper III, sensitivity analyses were conducted using cumulative incidence functions (370). In the paper, the estimated incidence of recurrence was slightly reduced across all quartiles of D-dimer, indicating the presence of competing risk by death. However, the effect

did not differ across the exposure of interest, implying that this would not affect the comparison between the groups.

#### *5.1.4.2 Information bias*

Information bias occurs when errors in the assessment of exposure or outcome data result in a different accuracy of information between the comparison groups (369). Misclassification, a subgroup of information bias, occurs in the presence of measurement errors or when a categorical variable is wrongly classified. This may result in a wrong classification regarding exposure or outcome status (372). For example, if an individual is recorded as a VTE patient when in fact there is no VTE present. Misclassification can be further categorized into differential misclassification and non-differential misclassification errors (349). Differential misclassification occurs when the error rate or the probability of being misclassified differs across different groups of study subjects. Non-differential misclassification arises when a variable is misclassified independent of the outcome or the exposure, i.e., all groups have the same error rate or probability of being misclassified.

In the present thesis, several sources for misclassifications are possible. In the Tromsø study, self-administered questionnaires were used to assemble baseline characteristics of the study participants. This approach is cheap and cost-effective to obtain a broad range of information. However, self-administered questionnaires have several limitations (373). The questions may be interpreted differently across participants and must be carefully planned to reduce the risk of misclassification (373). In paper II, questionnaires were used to obtain self-reported history of cancer or arterial cardiovascular disease. In contrast to other baseline characteristics, major life events, such as cancer, myocardial infarction, and stroke, are likely to be recalled and reported correctly (374), also in questionnaires. Therefore, misclassification due to self-administered questionnaires is probably limited in the study. In addition, the introduced misclassifications in cohort studies are generally non-differential, with an equal distribution of misclassification in participants with and without the exposure of interest and not related to the outcome occurring later in time (349).

The biomarker D-dimer is a central diagnostic marker and exposure in the present thesis. Despite the attempt to standardize the sampling and analysis of D-dimer, technical errors may occur. To account for small variations in the measurements, the study population in paper II and paper III were divided into quartiles based on their D-dimer values. Moreover, different

techniques and assays are applied to measure D-dimer levels. Regardless of the application of D-dimer through decades, no standardization of the measurement technique has been made (282). Therefore, several D-dimer assays, with different diagnostics properties, are commercially available (278). In the present thesis, the D-dimer assay, STA®-Liatest D-Di, was applied in papers I and III. The STA® Liatest® D-Di assay is recognized as a highly sensitive D-dimer assay, reducing the risk of misclassification (281, 297). As no standardization exists, our findings may not be directly transferable to other assays. In paper II, blood samples were drawn at inclusion and analyzed more than 20 years later. The long storage time may potentially affect the plasma levels of D-dimer and introduce a risk of misclassification. As the storage time affected both cases and controls in the same manner, the misclassification is likely non-differential. A non-differential misclassification of the exposure may dilute the observed difference between the groups, potentially biasing the estimates toward the null (375). In paper II, this may weaken the observed association between D-dimer and future risk of incident VTE, an effect known as regression dilution bias.

Classifying VTE events into provoked or unprovoked is an essential part of diagnosing and managing VTE patients. The presence of provoking factors is important to interpret the results in paper III. In the study, provoking status was defined based on recorded information on the index event, and patients were classified depending on the presence of defined provoking factors. Data on provoking factors were obtained by interview at the thrombosis clinic or through the patient's medical records. A lack of documentation of these factors, either at diagnosis or follow-up visits, may introduce a risk of misclassification and a provoked event could potentially be misclassified as unprovoked. However, due to the impact on treatment strategy, provoking factors are likely well documented in the medical records. Nevertheless, different interpretations of the medical history or lack of documentation may have occurred, and some degree of misclassification on the provoking status cannot be excluded.

### 5.1.5 Modifiable risk factors and regression dilution bias

Regression dilution bias may occur when exposure changes in an individual through the follow-up without repeating and correcting the measurement (376). Cohort studies are particularly prone to the bias as exposure variables often are based on single measurements at inclusion, and the outcome occurs after a long follow-up (376). Regression dilution bias may result in underestimating the true association due to temporary fluctuations or changes in the variable over time, and/or measurement errors. For example, the use of single baseline measurements to

assess risk factors for cardiovascular diseases has been shown to underestimate the true association (256, 377). In an attempt to handle this bias, repeated measures of central variables may be done (376). In Paper II, the exposure variable, intrinsic D-dimer, was assessed at inclusion in participants recruited from the general population. As D-dimer levels are affected by several conditions and situations, the levels may vary over time. Moreover, despite the high-sensitive D-dimer assay, measurement errors may occur. The association between intrinsic D-dimers and future VTE events may therefore be reduced and underestimated in the presence of regression dilution bias. To assess the extent of the bias in paper II, a sensitivity analysis was conducted considering the time elapsed between the blood sampling and the VTE event. By plotting the estimates as a function of time from the blood sampling to the VTE event, we showed that the ORs were higher with a shorter time between the sampling to the event, especially within the first three years. The sensitivity analysis illustrates the potential presence of regression dilution bias, and the overall analysis may therefore underestimate the true association.

### 5.1.6 Missing data

Missing data is a common problem and occurs in nearly all research and study designs, even in well-designed studies (378). Missing data is defined as the data value that is not stored for a variable in the observation of interest and can be divided into three categories: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR) (379-381). MCAR occurs when the missing data are not related to any observed or unobserved variables, i.e., it happens entirely at random (378). To accidentally skip a question when completing a questionnaire may be an example of MCAR. MAR is more likely to happen and occur when the missing data is related to a particular variable but not to the specific missing values expected to be obtained. For example, if women are more likely to avoid answering about their weight than men, missing data regarding weight is considered MAR. Missing data that do not fit the criteria for the two other categories are defined as MNAR. With MNAR the data are missing for a specific reason. For example, if a certain question in a questionnaire tends not to be answered by a particular group of subjects, this could be defined as MNAR. This could, for instance, be weekly alcohol consumption in alcoholics or data on weight in obese participants.

Missing data may cause various problems. Few missing values are often not a significant issue, but a large number of missing values could be a major threat to the study's integrity (380).

The absence of data will reduce the statistical power of the analysis, making the estimates more uncertain. Missing data could also introduce biases or reduce the representativeness of the sample (382), especially if the data are MNAR (379). With MNAR, the missing variables may introduce selection bias as the probability of missing is associated with specific groups of the study population, e.g., alcoholics in the example above.

There is no clear suitable way to solve problems regarding missing data, and as with misclassifications, the best strategy is to prevent missing data from occurring (380). However, there exist several different epidemiological solutions to handle missing data. A common approach is to exclude all cases without complete data, known as list-wise deletion or complete case analysis (379). If only a few observations are missing, the risk of introducing bias with list-wise deletion is minor. However, the approach may reduce the statistical power of the study or, in the absence of MCAR, cause selection bias. Alternatively, missing data could be handled by pair-wise deletion (383). In pair-wise deletion, individuals with missing data are not completely excluded from the analysis, only omitted in specific analyses including the missing value. Accordingly, the cases can be used when analyzing other variables with non-missing values. In pair-wise deletion, the results are only unbiased when data are missing at random. Further, the approach may introduce mathematical issues while computing estimates of some parameters (380, 383).

Imputation is a statistical method trying to predict the missing value (382). Different approaches for imputation are possible depending on the type of missing data and the remaining information in other variables. One option is mean substitution, where the mean value of a variable is used in place of the missing value. This approach is based on the assumption that the missing value is a randomly selected observation from a normally distributed variable. However, this is often not the case. An alternative method is to estimate the missing data based on other variables; an approach known as multiple imputations. By avoiding omitting cases, the sample size is preserved. However, by predicting the value from other variables, no new information is added (379).

In the present thesis, there were only a few missing values in the variables of interest. In paper III, 214 subjects (5.9%) had no D-dimer value recorded. Despite being a central part of the diagnostic work-up for VTE, D-dimers may not be measured in all settings, e.g., the most severe cases or hospitalized patients. According to the current guidelines, patients with a high pretest probability should be referred to radiological testing independent of the D-dimer levels

(110, 114, 300). However, as discussed in paper I of this thesis, this approach has poor adherence and is not correctly implemented in daily clinical practice (312-315). Furthermore, when comparing patients with and without information on D-dimer in paper III, no major differences were observed in variables indicating the VTE severity. In the same analysis, the proportion of events provoked by immobilization or hospitalization was higher in patients with missing D-dimer, indicating that the lack of D-dimer was particularly prevalent among already hospitalized patients. In the study, missing data was handled by excluding all cases with missing D-dimers, i.e., list-wise deletion. The limitation of the approach is the reduction of power, and the potential introduction of selection bias, as the data was not MCAR. As seen from the example above, hospitalized patients were overrepresented among cases without D-dimer. Thus, the list-wise deletion may reduce the generalizability of the findings to this patient group.

### 5.1.7 Sample size and study power

The size of the study population is of great importance and should be large enough to prevent the two types of errors in epidemiology, type I and type II errors (349). To reject a true null hypothesis, i.e., claiming there is an effect when it is not, is considered a type I error. A commonly accepted threshold for the probability of making a type I error is set to 5%, often visualized with a 95% confidence interval. A type II error is when one fails to reject the null hypothesis when it is false, i.e., claiming there are no differences between the groups when it in fact is. A type II error is related to the study power, and the power of a study is the probability that a type II error does not occur (349). By increasing the sample size, the power of the study increases, and the likelihood of a type II error decreases.

The study population in paper III consisted of over 2500 patients with an incident VTE and almost 400 recurrent events, a fairly extensive study compared to other surveys on the subject. Based on the effect size seen in previous studies, the study size and the outcome rate were likely to yield adequate power for our overall aim, reducing the risk of a type II error. However, several subanalyses were conducted, with considerably smaller subsets of the study population. For instance, in a sensitivity analysis restricted to patients with unprovoked proximal DVTs or unprovoked PE, the sample size was reduced to 1190 subjects. In this sensitivity analysis, the point estimates were in line with the overall analysis, but with considerably wider confidence intervals. The increased uncertainty was likely due to the smaller sample size and fewer outcome events, and the subsequent reduction in power may potentially introduce the risk of a type II error.

## 5.2 Discussion of the main results

### 5.2.1 D-dimer as a stand-alone test to rule out deep vein thrombosis

In paper I, our findings suggest that D-dimer as a stand-alone test is as safe as D-dimer combined with Wells score to rule out DVT. This approach had an estimated failure rate of 1.8% and necessitated 12% fewer ultrasound examinations than the combined strategy. Our results correspond with previous findings (319), although the failure rate for D-dimer as a stand-alone test was slightly higher in our study (1.8% versus 0.3%). However, the majority of the "false negative" patients in our study had a distal DVT (67%). In contrast to the study by Frønæs et al., patients with uncertain CUS findings in our study were further investigated with contrast venography. Using this highly sensitive diagnostic test, we would likely detect more distal DVTs than by CUS alone (384). Consequently, the different practices in objective testing and study designs could partly explain the distinction in failure rates between the two studies.

A proximal thrombus is considered a severe condition, while in contrast, the clinical significance of distal DVT is debated (114, 115). In the present study, the failure rate for D-dimer as a stand-alone test was lower when the analyses were restricted to proximal DVTs. In some centers, CUS is only performed in proximal veins and is normally repeated after one week to ensure no extension of a distal thrombus into the proximal venous segment (114). With this recognized approach (110, 114), distal DVTs are not detected and thus left untreated. As our study was a post hoc analysis of current clinical practice with whole-leg CUS, we do not know whether untreated distal DVTs would have progressed or not. The clinical significance of misdiagnosed distal DVT by the use of D-dimer as a stand-alone test in the present study is therefore unknown.

The failure rate, often considered the posttest probability in VTE diagnostics, is commonly used to validate diagnostic strategies for VTE (108, 110, 114). Based on the performance of venography, which is generally accepted as the reference standard within DVT diagnostics, a failure rate estimated at less than 2% is considered an acceptable degree of safety for a diagnostic pathway (107). In our study, the point estimate of the failure rate of D-dimer as a stand-alone test for the exclusion of all DVTs (including the distal DVTs) was 1.8%, but the upper limit of the 95% CI exceeded the recommended 2% limit. However, when restricting the outcome to proximal DVTs, the upper limit of the 95% CI was less than 2%. D-dimer as a stand-alone test may therefore be safe to exclude proximal DVTs but not distal DVTs. In a large individual patient-level meta-analysis of more than 10 000 patients with suspected DVT, the failure rate for D-dimer with a Wells score ≤1 point was 1.2% with a 95% CI ranging from

0.7% to 1.8% (385). Thus, the failure rates observed in our study for D-dimer as a stand-alone test to exclude proximal DVT corresponded well with those of current practice. This further supports that D-dimer as a stand-alone test may be safely used to exclude proximal DVT. Finally, the strategy compares satisfactorily to the failure rate observed for CUS, which is estimated to range between 0.6% and 2.0% (111, 386).

As anticoagulant therapy is known to interfere with the D-dimer level (387, 388), we performed sensitivity analyses with and without patients with ongoing anticoagulant treatment. As expected, the failure rates were higher when patients on anticoagulants were included. This indicates that D-dimer should not be used as a stand-alone test in patients already treated with anticoagulants.

Despite a central role in the current diagnostic work-up for suspected DVT, the Wells score has several limitations. The subjective assessment of some score items may introduce misclassification (311), and several studies have shown poor adherence and lack of correct implementation of the score in daily clinical practice (312-315). As many of today's clinics are organized to be as efficient as possible, standard blood samples, including D-dimer, are often obtained before the clinical evaluation is performed. Consequently, the D-dimer level is potentially available before calculating the Wells score, contrary to the intention behind the clinical decision rule (316-318). Due to these limitations of the current strategy, we suggest that D-dimer as a stand-alone test may be an alternative approach to simplify and optimize the efficiency of the diagnostic work-up and reduce the number of imaging tests. As shown in the paper, our findings imply that D-dimer as a stand-alone test may be safe for excluding proximal DVT in outpatients.

One of the main advantages of using D-dimer as a stand-alone test is the reduction of required CUS. Although CUS is a low-risk procedure, the examination is resource-demanding. As only a minority of patients with a suspected DVT have the disease, a reduction of CUS could increase the efficiency of the diagnostic work-up (307). Large campaigns, such as the 'Choosing Wisely' by The American Board of Internal Medicine Foundation (389) and the 'Gjør kloke valg' by the Norwegian Medical Association (390), have increased the focus on unnecessary medical tests and why they should be avoided. D-dimer as a stand-alone test may be an approach to reduce the amount of such medical tests.

In the paper, D-dimer as a stand-alone test was investigated in consecutive patients referred with suspected VTE. While this was a relatively comprehensive study, some subgroups of the patient population were less represented. For instance, only 5% of the study population had cancer. Therefore, our findings may not be applied to all groups of patients with suspected DVT. Further, the study was restricted to the diagnostic work-up among outpatients, and D-dimer's diagnostic performance among inpatients was therefore not assessed.

### 5.2.2 Intrinsic D-dimer and risk of incident VTE

In paper II, the risk of incident VTE increased across quartiles of plasma D-dimer. Compared with those in the lowest quartile, participants with D-dimer levels in the highest quartile had an almost 1.7-fold increased risk when adjusted for age and sex. The risk estimates were attenuated upon additional adjustment for BMI and CRP. The association between D-dimer and incident VTE has not been thoroughly investigated, especially in the general population. However, our findings are consistent with the previous studies (325-328), described in detail in section 1.6.4 of this thesis. In extension to earlier studies, our results suggest that the association between plasma D-dimer and risk of incident VTE could partly be attributed to BMI and inflammation.

The underlying mechanism behind the proposed association between D-dimer and incident VTE is not known. 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 (391, 392). Twin studies have found a wide range of heritability estimates for D-dimer levels, spanning from 23% to 65% in Northern Europeans (391-394), which could partly explain the association between intrinsic D-dimer and incident VTE. Further, data from a genome-wide association study revealed that D-dimer levels were partly explained by genetic variants located in hemostatic factor genes that encode key procoagulant factors (tissue factor, factor V, and fibrinogen) (330). However, these genetic variants accounted for only 1.8% of the total variance in the D-dimer phenotype (330), suggesting that environmental factors play an essential role in determining D-dimer levels.

An elevated D-dimer can be a marker of acquired factors related to thrombosis. Obesity, often assessed by an increased BMI, is a well-known risk factor for VTE (368). Folsom et al. showed in the ARIC study that BMI increased across quintiles of D-dimer (326), similar to what we observed in our analysis. In addition, while studying twins, BMI was found to have a small but significant effect on D-dimer concentration, explaining 2.7% of its variance (391).

When including BMI in 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 obesity. Upon additional adjustment for CRP, the risk estimates for overall VTE decreased further, and similar trends were observed for all VTE subgroups and in sensitivity analyses. As CRP is a sensitive downstream marker of inflammation, these results may indicate that D-dimer reflects underlying inflammatory conditions increasing the risk of VTE. Diseases associated with chronic low-grade inflammation, such as cancer, autoimmune diseases, and kidney disease, are all associated with higher D-dimer levels (366, 367, 395, 396) and increased risk of VTE (397-399). As an elevated D-dimer may reflect the sum of several underlying conditions that increase the risk of VTE, D-dimer could be useful as a global biomarker to identify those at a particularly high risk of incident VTE.

### 5.2.3 D-dimer at venous thrombosis diagnosis and risk of recurrence

In paper III, a low D-dimer measured at the time of VTE diagnosis was associated with a low risk of recurrence. Patients with D-dimer ≤1900 ng/mL had an 11% estimated five-year cumulative incidence of recurrent VTE, while the corresponding incidence of recurrent VTE was 18% in those with D-dimer >1900 ng/mL. The association between low D-dimer at the time of diagnosis and risk of recurrent VTE was consistent in subgroups of the index event, including proximal and distal DVT, PE, and unprovoked and provoked VTE. Our findings confirm those of Bjøri and colleagues, who previously evaluated the association between D-dimer measured at the time of diagnosis and the risk of recurrence in a study of 454 VTE patients (295). However, the cut-off level for the lowest D-dimer quartile was somewhat higher in our study (≤1900 ng/mL vs. ≤1500 ng/mL) (295), potentially due to a higher proportion of patients with PE in our study (56% vs. 44%). Accordingly, the cumulative incidence of recurrence at one and five years was slightly higher in our study (2.9% vs. 1.7% at one year, and 11.4% vs. 8.5% at five years).

Anticoagulant treatment efficiently prevents recurrences, though at the expense of increased risk of bleeding. In patients at high risk of recurrence, prolonged treatment is necessary, and the subsequent increased bleeding risk can be justified. However, in patients with a low risk of recurrence, prolonged anticoagulant treatment introduces an unnecessary risk of bleeding. Thus, identifying patients at low risk of recurrence is a high priority (400). However, identifying and validating such groups through randomized trials may be challenging due to the cost and sample size needed. Therefore, cohort studies are an alternative and more

applicable method to test and validate the safety of low-risk subgroups. To standardize the reporting and evaluation of recurrence risk in cohort studies, the Subcommittee on Control of Anticoagulation of the International Society of Thrombosis and Haemostasis has published its recommendation on assessing the risk of recurrence in cohort studies. In the statement, the committee argues that a recurrence rate below 5% at one year and 15% at five years is acceptable and justifies the termination of the anticoagulant (400). The one and five-year cumulative incidences of recurrence for the ≤1900 ng/mL D-dimer category were below these accepted thresholds. Even though the optimal D-dimer cut-off remains to be determined, our findings support the utility of D-dimer to identify patients at low risk of recurrence already at the time of diagnosis.

There is a consensus to treat VTE patients without contraindications with anticoagulation for at least three months (40, 117, 401) and to consider extended duration or indefinite treatment when the VTE is unprovoked. Choosing the course of anticoagulant therapy is particularly challenging when the VTE is unprovoked (117, 122), as the risk may not cease after the event. As one of several predictors of recurrence, D-dimer assessed after the initial treatment period has been proposed as a tool to guide decisions on whether to extend the treatment (343-345). Yet, the use of this approach is limited and not implemented in clinical guidelines (40, 117, 401). Our results indicate that low D-dimer levels at the diagnosis may identify patients with unprovoked VTE in whom anticoagulant treatment can be safely terminated after three or six months without needing further D-dimer assessment after the initial treatment phase.

In our study, we investigated the association between D-dimer and recurrence risk. However, several risk assessment models have been developed to increase the utility of D-dimer as a predictor of recurrence, such as the DASH (343), Vienna (344), and HERDOO2 (345) prediction models. All these models utilize D-dimer levels measured after discontinuation of the anticoagulant treatment in combination with clinical risk factors. Although the information on the clinical risk factors included in the Vienna and DASH models is available at the time of diagnosis, the risk assessment model cannot be applied until the initial phase of anticoagulation is completed several months later. Therefore, diagnostic D-dimer levels may potentially improve these models, as the prediction may be performed already at the time of diagnosis. A model that can be used at diagnosis may be beneficial for the patients and the health care system, as it can guide decisions on treatment duration of anticoagulants and possibly lower the need for follow-up consultations in patients with low recurrence risk.

However, whether diagnostic D-dimer may be utilized in these prediction models remains unsettled and should be further tested using a prediction framework.

# 6 Conclusion

- D-dimer as a stand-alone test may be safe for excluding proximal DVT in outpatients. The strategy has the potential to simplify and increase the efficiency of the diagnostic work-up for patients with suspected DVT. However, future prospective management studies are warranted to confirm our findings and further investigate the safety of D-dimer as a stand-alone test in outpatients.

- Higher plasma levels of D-dimer were associated with an increased risk of future incident VTE. The attenuation of risk estimates upon additional adjustment for BMI and CRP suggests that increased D-dimer partly reflects underlying conditions associated with obesity and an inflammatory state.

- D-dimer levels within the lowest quartile (≤1900 ng/mL) measured at the time of incident VTE diagnosis was associated with a low risk of recurrent VTE. These findings imply that a low D-dimer may be used to stratify VTE patients at low risk of recurrent VTE already at the time of diagnosis.

# 7 Final remarks and future perspectives

D-dimer has been used as a diagnostics marker of VTE for decades, and the present thesis shows its persistent relevance in modern medicine. Since its introduction, the biomarker has been applied in various settings with a wide range of uses. However, the unspecific nature of the biomarker and the wide range of applications could also represent a disadvantage and a challenge in the diagnostic work-up. Due to the high number of false positive tests in the diagnostic setting, D-dimer causes a considerable need for radiological testing. New approaches for optimization of the current strategy, such as D-dimer as a stand-alone test proposed in this thesis, are still not enough to sufficiently reduce the proportion of false positive tests. Fortunately, the field of clinical medicine is constantly developing, and as seen for other conditions, new advancements may change the diagnostic work-up remarkably. For example, in the diagnostics of myocardial infarction, the highly specific cardiac troponin has replaced its predecessor CK-MB becoming a cornerstone of the diagnosis (402). A similar development within biomarkers for VTE is desirable to simplify the diagnostic approach and reduce the need for objective radiological testing in the future. For instance, the field of proteomics is growing rapidly (403), and perhaps proteome-wide discovery analyses may identify novel diagnostic markers for VTE.

D-dimer has a wide range of use, not only as a diagnostic marker of VTE. In the present thesis, we have shown that D-dimer in the upper normal range may also represent an increased risk of future incident VTE in the general population. In theory, D-dimer could be applied to guide the use of primary prophylaxis in these patients. However, such an approach has several limitations. First, VTE is a complex, multifactorial disease, and the development of a VTE occurs as a combination of many simultaneous events and factors. Secondly, due to the considerably increased bleeding risk associated with today's anticoagulant therapy, the number of adverse events are likely to exceed the prophylactic utility in these patients. A D-dimer in the upper normal range is therefore not likely to be used to stratify the risk of VTE and guide thromboprophylaxis in the general population in the near future. However, with advancements in VTE therapy, for instance discovery of new drugs with less impact on bleeding risk, D-dimer may perhaps contribute to risk stratification and guide prevention of VTE in the future.

The major challenge with the treatment of VTE is the increased bleeding risk associated with anticoagulant therapy. Identification of VTE patients at high risk of recurrence that needs extended anticoagulant therapy, and likewise identification of patients at low risk in whom

anticoagulants can be safely discontinued, is therefore important. Our findings imply that a lower D-dimer may be used to stratify VTE patients at low risk of recurrent VTE already at the time of diagnosis. Currently suggested risk assessment models for stratification of patients at risk of recurrent VTE use D-dimer at follow-up combined with clinical risk factors, such as age and thrombus site (343-345). As D-dimer is often widely available at the time of diagnosis, risk assessment models including D-dimer at the time of diagnosis could guide the duration of anticoagulant therapy even before discharge from the hospital. Future studies should explore whether the inclusion of D-dimer at the time of diagnosis in risk assessment models can improve the identification of patients at low recurrence risk in whom short-term anticoagulation would be sufficient.

# 8 References


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# Paper I

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Full Length Article

# D-dimer as a stand-alone test to rule out deep vein thrombosis☆

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ABSTRACT

*Background:* Current guidelines recommend the use of clinical decision rules, such as Wells score, in combination with D-dimer to assess the need for objective imaging to rule out deep vein thrombosis (DVT). However, the clinical decision rule has limitations, and use of D-dimer as a stand-alone test has been suggested.
*Objective:* We aimed to assess the safety and efficiency of D-dimer as a stand-alone test to rule out DVT in outpatients referred with suspected DVT.
*Methods:* We collected data from consecutive outpatients referred to our hospital with suspected DVT in 2008–2018. D-dimer levels were analyzed using STA® Liatest® D-Di assay. D-dimer as a stand-alone test was theoretically applied in retrospect, and the number of misdiagnosed events were estimated as if such an approach had been initially used. All patients were followed for three months.
*Results:* Of 1765 included patients, 293 (16.6%) were diagnosed with DVT. A total of 491 patients (27.8%) had a negative D-dimer (< 500 ng/mL). Of these, nine were diagnosed with DVT, yielding a failure rate for D-dimer as a stand-alone test of 1.8% (95% CI 0.8%–3.5%). The majority of the misdiagnosed patients had distal DVT. In analyses restricted to proximal DVTs, the failure rate was 0.6% (95% CI 0.1%–1.8%). D-dimer as a stand-alone approach reduced the proportion of required ultrasounds from 81.8% to 72.2%.
*Conclusion:* D-dimer as a stand-alone test may be safe for excluding proximal DVT and reduce the proportion of required ultrasounds. Prospective management studies are needed to confirm our findings.

## 1. Introduction

D-dimer is a commonly used biomarker for coagulation activation and fibrinolysis. For patients with a suspected deep vein thrombosis (DVT), current guidelines recommend the use of pretest probability assessment and D-dimer test to evaluate whether DVT can be safely ruled out, or whether the patient should be referred to further diagnostic work-up with imaging techniques [1–3]. The pretest probability assessment is usually performed using Wells score [4], which is a well studied clinical prediction rule for DVT [5]. The original score was based on signs, symptoms and risk factors for DVT, and classified patients into three risk categories (low, moderate or high probability). A later modification of the Wells score additionally incorporated previous DVT as an item and classifies patients into two categories (unlikely or likely) based on the total score (< 2 or ≥ 2 points) [6]. If the modified Wells score is < 2 points, D-dimer should be measured, and DVT can be ruled out with a negative predictive value of 98–99% if the D-dimer level is < 500 ng/mL [7].

Despite the central role of Wells score in the diagnostic work-up of suspected DVT, the clinical prediction rule has several limitations. The subjective assessment of some of the score items may introduce misclassification [8] and several studies have shown a poor adherence and lack of correct implementation of the score in daily clinical practice [9–12]. As many of today's clinics are organized to be as efficient as possible, standard blood samples including D-dimer are often obtained before clinical evaluation. Consequently, D-dimer is potentially interpreted before calculating the Wells score, contrary to the intention behind the clinical decision rule [13–15].

Although D-dimer is typically increased in patients with acute venous thromboembolism (VTE), D-dimer levels may also be elevated in

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several other conditions, such as malignancy, infections, and pregnancy, thus decreasing its specificity [16]. Moreover, D-dimer increases with age [17]. Due to a decreased specificity among elderly patients with the fixed cut-off value (500 ng/mL), age-adjusted cut-off values for D-dimer testing has been suggested [17–20].

The use of D-dimer as a stand-alone test to rule out VTE has been proposed, mainly in PE patients [21–23]. A strategy that relies entirely on D-dimer to determine the need for objective imaging may simplify and optimize the efficiency of the diagnostic work-up and reduce the number of imaging tests. In a recent study, Frønæs et al. investigated the safety of D-dimer as a stand-alone test to rule out DVT in 913 outpatients referred with suspected DVT. Of 298 (33%) patients with a negative D-dimer, only one patient was diagnosed with DVT, yielding a failure rate of 0.3%. These findings indicated that fixed D-dimer as a stand-alone test could safely exclude DVT while requiring fewer compression ultrasounds (CUS) than the combined approach of D-Dimer and Wells score. In the present study, we aimed to validate the findings presented by Frønæs et al. in a larger population of unselected VTE patients. We, therefore, assessed the safety and efficiency of applying D-dimer as a stand-alone test in outpatients consecutively referred to our hospital with suspected DVT. Four diagnostic strategies for excluding DVT were investigated and compared: (i) fixed D-dimer as a stand-alone test, (ii) fixed D-dimer combined with modified Wells score, (iii) age-adjusted D-dimer as a stand-alone test, and (iv) age-adjusted D-dimer combined with modified Wells score.

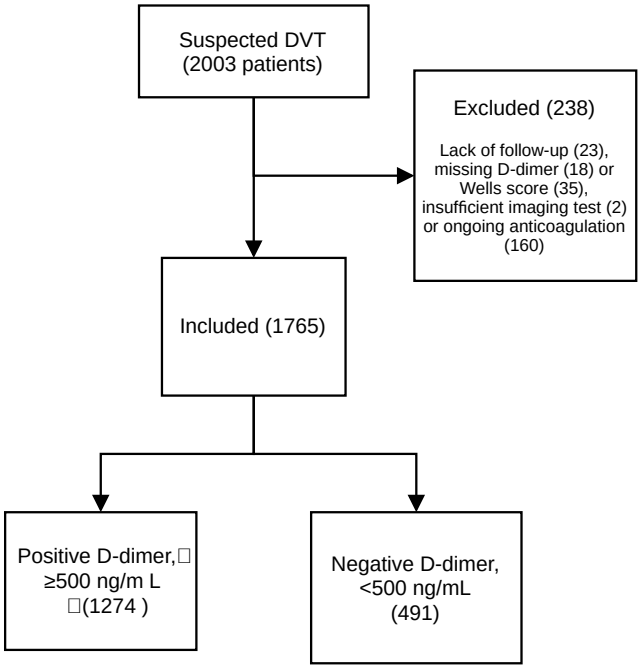


**Fig. 1.** Study population and design.

## 2. Methods

### 2.1. Study population

We collected data from consecutive outpatients referred with a suspected DVT to the Emergency Department of the University Hospital of North Norway (UNN), in Tromsø, Norway, between 2008 and 2018. The UNN is the sole provider of all VTE diagnostic procedures and VTE-related healthcare in the Tromsø region, serving a local population of 127,000 inhabitants. A total of 2003 patients was referred with a suspected DVT during the 10-year period. Patients with a permanent address outside the catchment area of the hospital ($n$ = 23), who could not be followed for three months, were excluded. All included patients were followed for three months after their visit to the Emergency Department by close review of their medical records. Our hospital is the only hospital providing diagnostic and therapeutic management of VTE within a radius of 250 km. Thus, the likelihood of a complete follow-up of patients living in this catchment area is high. Patients in whom the diagnostic work-up was incomplete, i.e. no D-dimer measurement ($n$ = 18), no assessment of Wells score ($n$ = 35), and patients with an insufficient imaging test ($n$ = 2), were excluded. In addition, patients with ongoing anticoagulation treatment were excluded ($n$ = 160) (Fig. 1). Consequently, 1765 patients were included in the analyses. The study was approved by the regional committee for health and research ethics.

### 2.2. Diagnostic procedure

All outpatients referred to the Emergency Department with a suspected DVT underwent evaluation by a physician using a modified, two-level Wells score and D-dimer test to guide the decision on further diagnostic testing. The Wells score was assessed in the Emergency Department using a standardized form. D-dimer levels were assayed with the STA®-Liatest D-Di from Stago (Diagnostica Stago, Asnieères, France). All blood samples were analyzed at the Department of Clinical Chemistry at the UNN.

According to existing clinical practice during the data collection period, all patients with either a Wells score ≥ 2 and/or a positive D-dimer (i.e., ≥500 ng/mL) at baseline, would be referred for further CUS. However, the physician's clinical judgment could also impact the decision, and some of the patients with negative fixed D-dimer and low clinical probability underwent objective testing, although this was not coherent with the guidelines.

Patients referred for further diagnostic testing underwent a whole-leg CUS, assessing all veins of the affected extremity for compressibility. Non-compressibility was the main criterion for DVT, but a confident gray-scale visualization of the thrombus was also considered diagnostic. In patients where a CUS was impractical (e.g., if leg casting or excessive subcutaneous tissue or fluid prevented adequate assessment of compressibility) or the result of the CUS was uncertain, venography was performed instead or as an additional test.

All patients were followed for three months. In patients where DVT was ruled out at baseline, a diagnosis of DVT occurring in the following three months was considered as an undetected and misclassified event from their first visit. Information on DVT during follow-up was obtained by thorough review of medical records, using the same criteria for objective verification as described above. DVT occurring after the initial three months follow-up period was considered as a new event (i.e., incident or recurrent DVT).

### 2.3. Definition of diagnostic strategies

Four diagnostic strategies for excluding DVT were investigated (Fig. S1). These included (i) fixed D-dimer as a stand-alone test; (ii) fixed D-dimer combined with modified Wells score; (iii) age-adjusted D-dimer as a stand-alone test; and (iv) age-adjusted D-dimer combined with modified Wells score. In the approaches combining a D-dimer with a modified Wells score, all patients with either a positive D-dimer or a Wells score ≥ 2 points (i.e., 'DVT likely'), were considered as referred for CUS. When using D-dimer as a stand-alone test, patients with a D-dimer below the chosen threshold were considered discharged without further testing. The fixed D-dimer cut-off was defined as positive when the D-dimer value was ≥500 ng/mL. For the two strategies that included age-adjusted D-dimer, a positive threshold was defined as ≥ age x 10 ng/mL for patients ≥50 years of age (i.e., the cut-off level for a 70-year-old would be ≥700 ng/mL). For patients < 50 years of age, a fixed threshold of ≥500 ng/mL was used.

In our analyses, which were a post hoc estimation of the diagnostic performance of these four different strategies, we theoretically applied

the different approaches and used the criteria that would have led to a referral for CUS in each diagnostic strategy. However, the estimations were based on the assumption that the chosen diagnostic criteria would be followed without exception. For instance, while estimating the performance of D-dimer as a stand-alone test, we assumed that all patients with a D-dimer value < 500 ng/mL would have been discharged without further testing and not treated with anticoagulation.

### 2.4. Statistical analyses

The failure rate expressed the safety of the strategy and was defined as the proportion of patients who did not meet the criteria for undergoing CUS defined by the chosen strategy (i.e., negative tests), but still was diagnosed with DVT. The amount of required CUS indirectly expressed the efficiency of the approach and was defined as the proportion of patients who met the criteria for undergoing CUS for the chosen strategy (i.e. positive tests).

Since there is an ongoing discussion about the clinical significance of distal DVT with regards to further evaluation and treatment [24,25], we performed a sub-analysis restricted to proximal DVTs. Previous studies have shown that ongoing anticoagulant treatment may influence D-dimer levels and thereby decrease the sensitivity of the test [26,27]. In our analyses, patients with ongoing anticoagulant treatment were excluded. The current strategies are based on the rationale that the post-test prevalence of DVT might be too high to safely rule out the disease based on a negative D-dimer in patients with a high pretest probability. Therefore, we additionally conducted a sensitivity analysis on the performance of D-dimer restricted to this subgroup of patients with a high pretest probability. Furthermore, as cancer is known to affect D-dimer levels, we performed a sensitivity analysis restricted to patients without cancer [16]. All statistical analyses were performed using Stata version 15.0 (Stata Corporation LP, College Station, TX, USA).

## 3. Results

Of the 1765 included patients with a suspected DVT, a total of 293 were diagnosed with DVT, yielding an overall prevalence of 16.6% (95% CI 14.9%–18.4%). Baseline characteristics are presented in Table 1. Age, sex and duration of symptoms were essentially similar between the two groups. The majority (81%) of the DVT patients had a Wells score of ≥2 points (i.e. 'DVT likely'), while the non-DVT patients had an approximately equal distribution between 'DVT unlikely' (< 2 points) and 'DVT likely' (52% and 48% respectively).

### 3.1. Fixed D-dimer as a stand-alone test

Of the 1765 included patients, 491 (27.8%) had a negative D-dimer and would have been sent home without further testing if D-dimer as a stand-alone test was the chosen diagnostic strategy (Fig. 2). Since this approach was not applied during the study period, 185 were referred for imaging testing despite a negative D-dimer. Of the patients referred for imaging testing, 134 had a Wells score of ≥2 points, while 51 were referred despite both a negative D-dimer and Wells score < 2 points. Of the 185 patients referred for CUS or venography despite a negative D-dimer, eight were diagnosed with DVT during their first visit to the Emergency Department. In addition, one patient was diagnosed with DVT during the three months follow-up. Of the nine DVTs in patients with a negative D-dimer, six were distal and three were proximal. Of the remaining 306 patients in whom DVT was ruled out due to a negative D-dimer and no CUS was performed, no patient was diagnosed with DVT during the follow-up period. Thus, a total of nine patients with a negative D-dimer were diagnosed with DVT, resulting in a failure rate of 1.8% (95% Cl 0.8–3.5%) for D-dimer as a stand-alone test (Table 2). D-dimer as a stand-alone test required 1274 referrals (72.2%, 95% CI 70.0%–74.3%) for CUS. When the modified Wells score was combined with fixed D-dimer, the number of undiagnosed DVTs were reduced to five patients, one proximal, yielding a failure rate of 1.6% (95% CI 0.5–3.6%). The combined strategy required 1443 referrals (81.8%, 95% CI 79.9%–83.5%) for CUS (Table 2).

In the sub-analysis restricted to proximal DVT, three of the 491 patients with a negative D-dimer were diagnosed with DVT (Fig. S2), yielding a failure rate of 0.6% (95% CI 0.1–1.8%) for D-dimer as a stand-alone test (Table 3). In the combined strategy, one patient was diagnosed with a proximal thrombi, and the corresponding failure rate for this strategy was 0.3% (95% 0.0–1.7%).

**Table 1**
Demographics and patients characteristics.

| | All | DVT | No DVT |
|---|---|---|---|
| | *n* = 1765 | *n* = 293 | *n* = 1472 |
| Age, years, median (IQR) | 63 (28) | 65 (29) | 62 (27) |
| Symptoms duration, days, median (IQR) | 5 (12) | 5 (11) | 5 (12) |
| Female sex, n (%) | 969 (55) | 141 (48) | 828 (56) |
| Modified Wells score DVT likely, n (%) | 948 (54) | 237 (81) | 711 (48) |
| Modified Wells score DVT unlikely, n (%) | 817 (46) | 56 (19) | 761 (52) |
| Previous DVT, n (%) | 252 (15) | 73 (27) | 179 (13) |
| Active cancer within past 6 months, n (%) | 77 (4) | 24 (8) | 53 (4) |

Abbreviations: IQR, interquartile range; DVT, deep vein thrombosis.

### 3.2. Age-adjusted D-dimer as a stand-alone test

Age-adjusted D-dimer as a stand-alone test would have resulted in 13 missed DVTs, corresponding to a failure rate of 2.0% (95% CI 1.1%–3.4%) (Table 2). Of these 13 patients, nine were diagnosed with a distal thrombus, yielding a failure rate of 0.6% (95% CI 0.2–1.6%) in the sub-analysis restricted to proximal DVT (Table 3). The modified Wells score in combination with the age-adjusted D-dimer test yielded five undiagnosed DVTs (failure rate 1.2%, 95% CI 0.4–2.9%). One of these undiagnosed DVTs was a proximal thrombosis yielding a failure rate of 0.2% (95% CI 0.0–1.4%) in the analysis restricted to proximal DVT (Table 3).

Sensitivity analyses restricted to patients with high pretest probability and non-cancer patients.

In our study population, 169 patients with a high pretest probability had a negative D-dimer. Of these 169 patients, four patients were diagnosed with DVT, yielding a failure rate of 2.4% (95% CI 0.6–6.0%) in this particular subgroup. Two of these four patients were diagnosed with proximal DVT, yielding a failure rate of 1.2% (95% CI 0.1–4.2%) when restricted to proximal DVTs.

Only one cancer patient with a D-dimer level < 500 ng/mL was diagnosed with DVT, and the analyses restricted to patients without cancer showed similar results as the main analysis for all approaches, including both fixed and age-adjusted D-dimer (Table S1).

## 4. Discussion

In the present study, we investigated the safety and efficiency of D-dimer as a stand-alone test for ruling out DVT. With a fixed cut-off value of 500 ng/mL, D-dimer as a stand-alone test performed similarly as D-dimer combined with Wells score (failure rate 1.8%, versus 1.6%), but necessitated fewer CUS (72.2% versus 81.8%). The majority of the misclassified patients (i.e., false negative test) had a distal DVT (6/9). Thus, when we restricted our analysis to proximal DVT, the failure rate for D-dimer as a stand-alone test was 0.6% and the proportion of required CUS was reduced from 81.8% for the combined strategy to 72.2% for the stand-alone test. Our findings suggest that D-dimer as a stand-alone test is as safe as D-dimer combined with Wells score to rule out DVT and necessitates 12% fewer ultrasound examinations.

Frønæs et al. previously evaluated the safety of D-dimer as a stand-

**Table 2**
Diagnostic performance of the different strategies (n = 1765).

| | Fixed D-dimer | | Age-adjusted D-dimer | |
|---|---|---|---|---|
| | | with Wells score ≥ 2 | | with Wells score ≥ 2 |
| Sensitivity | | | | |
| TP/(TP + FN) | 284/293 | 288/293 | 280/293 | 288/293 |
| Estimate,% | 96.9% | 98.3% | 95.6% | 98.3% |
| 95% CI | 94.2–98.6 | 96.1–99.4 | 92.5–97.6 | 96.1–99.4 |
| Specificity | | | | |
| TN/(TN + FP) | 482/1472 | 317/1472 | 635/1472 | 397/1472 |
| Estimate, % | 32.7% | 21.5% | 43.1% | 27.0% |
| 95% CI | 30.3–35.2 | 19.5–23.7 | 40.6–45.7 | 24.7–29.3 |
| Negative predictive value | | | | |
| TN/(TN + FN) | 482/491 | 317/322 | 635/648 | 397/402 |
| Estimate, % | 98.2% | 98.4% | 98.0% | 98.8% |
| 95% CI | 96.5–99.2 | 96.4–99.5 | 96.6–98.9 | 97.1–99.6 |
| Positive predictive value | | | | |
| TP/(TP + FP) | 284/1274 | 288/1443 | 280/1117 | 288/1363 |
| Estimate, % | 22.3% | 20.0% | 25.1% | 21.1% |
| 95% CI | 20.0–24.7 | 17.9–22.1 | 22.5–27.7 | 19.0–23.4 |
| Failure rate | | | | |
| FN/(FN + TN) | 9/491 | 5/322 | 13/648 | 5/402 |
| Estimate, % | 1.8% | 1.6% | 2.0% | 1.2% |
| 95% CI | 0.8–3.5 | 0.5–3.6 | 1.1–3.4 | 0.4–2.9 |
| Required CUS | | | | |
| (TP + FP)/(TP + FN + FP + TN) | 1274/1765 | 1443/1765 | 1117/1765 | 1363/1765 |
| Estimate, % | 72.2% | 81.8% | 63.3% | 77.2% |
| 95% CI | 70.0–74.3 | 79.9–83.5 | 61.0–65.5 | 75.2–79.2 |

Abbreviations: CI, confidence interval; TP, true positive; TN, true negative; FP, false positive; FN, false negative; CUS, compression ultrasound.

alone test in patients with suspected DVT [21]. Among the 913 patients included in their study, 33% had a negative D-dimer (< 500 ng/mL), while the prevalence of diagnosed DVT was 18.9%. D-dimer as a stand-alone test yielded a failure rate of 0.3% while the proportion of required CUS was reduced from 76.9% (D-dimer combined with Wells score) to 67.4% (D-dimer as a stand-alone test) [21]. Our study included twice as many patients, and there was no selection of participants in the emergency department. A total of 28% of the study population had a negative D-dimer, while the prevalence of diagnosed DVT in our study was 16.5%. The failure rate for D-dimer as a stand-alone test was higher in our study (1.8% versus 0.3%), but the majority of the "false negative" patients in our study had a distal DVT (67%). In contrast to the study by Frønæs et al., patients with uncertain CUS findings in our study were referred for contrast venography for further testing. By using this highly sensitive diagnostic test, we would likely detect more distal DVTs than by CUS alone [28]. Consequently, the different practice in objective testing, in addition to different study designs, could partly explain the higher failure rate in our study.

**Table 3**
Diagnostic performance of the different strategies for exclusion of proximal DVT (n = 1765).

| | Fixed D-dimer | | Age-adjusted D-dimer | |
|---|---|---|---|---|
| | | with Wells score ≥ 2 | | with Wells score ≥ 2 |
| Sensitivity | | | | |
| TP/(TP + FN) | 185/188 | 187/188 | 184/188 | 187/188 |
| Estimate, % | 98.4% | 99.5% | 97.9% | 99.5% |
| 95% CI | 95.4–99.7 | 97.1–100.0 | 94.6–99.4 | 97.1–100.0 |
| Specificity | | | | |
| TN/(TN + FP) | 488/1577 | 321/1577 | 644/1577 | 401/1577 |
| Estimate, % | 30.9% | 20.4% | 40.8% | 25.4% |
| 95% CI | 28.7–33.3 | 18.4–22.4 | 38.4–43.3 | 23.3–27.7 |
| Negative predictive value | | | | |
| TN/(TN + FN) | 488/491 | 321/322 | 644/648 | 401/402 |
| Estimate, % | 99.4% | 99.7% | 99.4% | 99.8% |
| 95% CI | 98.2–99.9 | 98.3–100.0 | 98.4–99.8 | 98.6–100.0 |
| Positive predictive value | | | | |
| TP/(TP + FP) | 185/1274 | 187/1443 | 184/1117 | 187/1363 |
| Estimate, % | 14.5% | 13.0% | 16.5% | 13.7% |
| 95% CI | 12.6–16.6 | 11.3–14.8 | 14.3–18.8 | 11.9–15.7 |
| Failure rate | | | | |
| FN/(FN + TN) | 3/491 | 1/322 | 4/648 | 1/402 |
| Estimate, % | 0.6% | 0.3% | 0.6% | 0.2% |
| 95% CI | 0.1–1.8 | 0.0–1.7 | 0.2–1.6 | 0.0–1.4 |
| Required CUS | | | | |
| (TP + FP)/(TP + FN + FP + TN) | 1274/1765 | 1443/1765 | 1117/1765 | 1363/1765 |
| Estimate, % | 72.2% | 81.8% | 63.3% | 77.2% |
| 95% CI | 70.0–74.3 | 79.9–83.5 | 61.0–65.5 | 75.2–79.2 |

Abbreviations: CI, confidence interval; TP, true positive; TN, true negative; FP, false positive; FN, false negative; CUS, compression ultrasound.

In the present study, the failure rate was lower when we restricted our analyses to proximal DVT. While proximal thrombosis is considered a serious condition with a potentially severe outcome, the clinical significance of distal DVT is debated [1,25]. In some centers, CUS is only performed in proximal veins and normally repeated after one week in those with an initial negative test to ensure that there is no proximal thrombus extension. With this approach, distal DVTs are not detected and thus left untreated. As our study was a post hoc analysis of current clinical practice, we do not know whether the distal DVTs detected in our study would have progressed or not if they were left untreated. Therefore, our findings should be confirmed in a prospective management study using D-dimer as a stand-alone test.

The failure rate, often considered as the posttest probability in VTE diagnostics, is commonly used to validate diagnostic strategies for VTE [1,3,29]. Based on the performance of venography, which is generally accepted as the reference standard within DVT diagnostics, a failure rate estimate of < 2% is considered an acceptable degree of safety for a diagnostic pathway [30]. In our study, the failure rate of D-dimer as a stand-alone test for exclusion of all DVTs (including the distal DVTs) was 1.8%, but the upper limit of the 95% CI exceeded the recommended 2% limit. However, when restricting the outcome to proximal DVTs, the upper limit of 95% CI was < 2%. D-dimer as a stand-alone test may therefore be safe to exclude proximal DVTs, but not distal DVTs. In a large individual patient level meta-analysis of > 10,000 patients with suspected DVT, the failure rate for D-dimer in combination with a Wells score ≤ 1 point was 1.2% with a 95% CI ranging from 0.7% to 1.8% [31]. Thus, the failure rates observed in our study for D-dimer as a stand-alone test to exclude proximal DVT corresponded well with those of current practice. This further supports that D-dimer as a stand-alone test may be safely used to exclude proximal DVT. Finally, the strategy compares satisfactorily to the failure rate observed for CUS, which is estimated to range between 0.6% and 2.0% [32,33].

In the subanalysis restricted to patients with a high pretest probability, the failure rate for proximal DVT was to 1.2%. Due to limited statistical power of such a small subanalysis ($n$ = 169), the point estimate and the following 95% CI should be interpreted with caution. Nevertheless, the point estimate for the failure rate of proximal DVT was below the suggested 2% cut-off even when restricted to those with a high pretest probability.

An age-adjusted cut-off value has been suggested to increase the usefulness of D-dimer among elderly [34]. In our analysis, age-adjusted D-dimer as a stand-alone test would have led to a further reduction in required CUS of 8.9% percentage points compared to fixed D-dimer as a stand-alone test. However, this approach misdiagnosed additional four DVT patients, yielding a failure rate of 2.0%. While this failure rate is on the 2% threshold, the fixed D-dimer as a stand-alone test provided a slightly higher degree of safety, and due to its simplicity, one may therefore argue that the fixed D-dimer is a more favorable strategy.

Large campaigns, such as the 'Choosing Wisely' by The American Board of Internal Medicine Foundation, have in recent years increased the focus on unnecessary medical tests and why they should be avoided [35]. One of the main advantages of using D-dimer as a stand-alone test is the reduction of required CUS. Although CUS is a low-risk procedure, the examination is resource demanding. As only a minority of patients with a suspected DVT have the disease, a reduction of CUS could increase the efficiency of the diagnostic work-up [7].

The main strength of our study is the inclusion of consecutive patients with suspected DVT within a confined geographical area for ten subsequent years, yielding a relatively large and unselected study population. Furthermore, the same diagnostic work-up, including the same high sensitive D-dimer assay, was used during the entire study period. Moreover, all diagnostic work-up of VTE in the region is carried out at the UNN, which enhances the likelihood of a complete 3-month follow-up for those living within the catchment area of this hospital. Our study has some limitations that need to be addressed. First, as our study retrospectively applied the diagnostics strategies, the results for the diagnostics strategies are only theoretical estimates of the diagnostic performances. Consequently, clinical aspects in the implementation of D-dimer as a stand-alone test are not revealed, and our findings must be further validated in a prospective management study. Furthermore, the study only investigates the diagnostic work-up among outpatients, while D-dimer's performance among inpatients is likely to differ [36,37].

Our findings suggest that D-dimer as a stand-alone test may be safe for excluding proximal DVT in outpatients. This strategy has the potential to simplify and increase the efficiency of the diagnostic work-up for patients with suspected DVT. Since our study was conducted as a post hoc analysis, future prospective management studies are warranted to confirm our findings and investigate the safety of D-dimer as a stand-alone test in outpatients.

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## Declaration of competing interest

The authors state that they have no conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2020.04.026.

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# Paper II

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Full Length Article

# Elevated plasma D-dimer levels are associated with risk of future 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.

## 1. 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

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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.

## 2. Methods

### 2.1. 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.

### 2.2. 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 h or longer in the past 14 days), or other factors described as provoking in the medical record by a physician (e.g. intravascular catheter).

### 2.3. 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

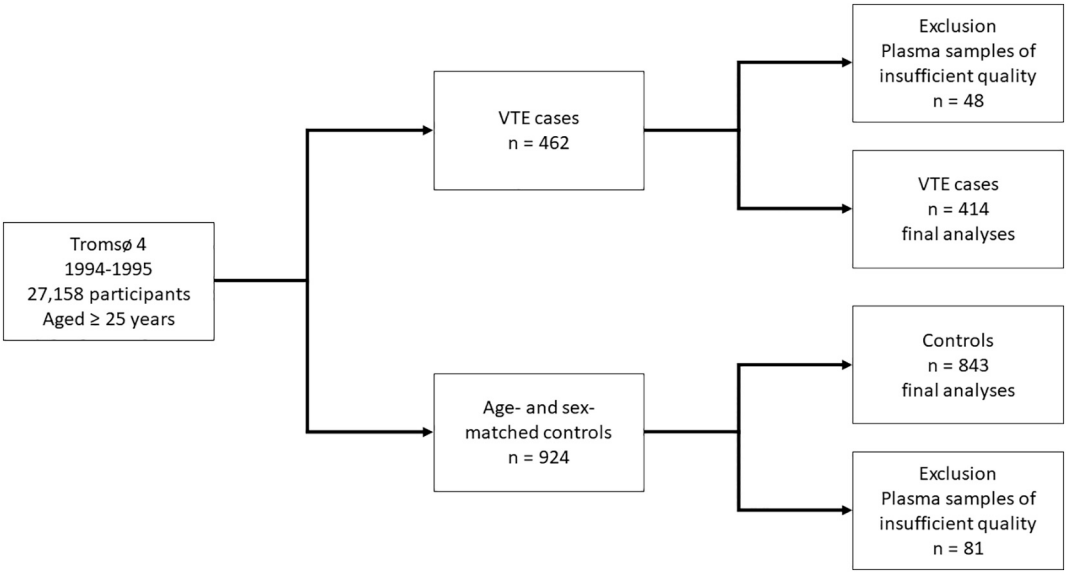


**Fig. 1.** Flowchart of the study population. The flowchart shows the nested case-control study derived from the fourth survey (Tromsø 4) of the Tromsø Study, conducted in 1994–1995. Venous thromboembolism (VTE).

obtained from a self-administered questionnaire [23,24].

### 2.4. 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 min at room temperature. The supernatant was then transferred into cryovials (Greiner Bio-One, Nürtingen, Germany) in 1-mL aliquots and stored at −80 °C.

### 2.5. 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 min and then subjected to centrifugation at 13,500*g* for 2 min 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].

### 2.6. 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.

## 3. 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.

## 4. 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

**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 | Quartile 2 | Quartile 3 | Quartile 4 |
| | <94 | 94–119 | 119–152 | ≥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.

| D-dimer (ng/mL) | Controls | Cases | OR (95% CI) | | |
|---|---|---|---|---|---|
| | | | 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.

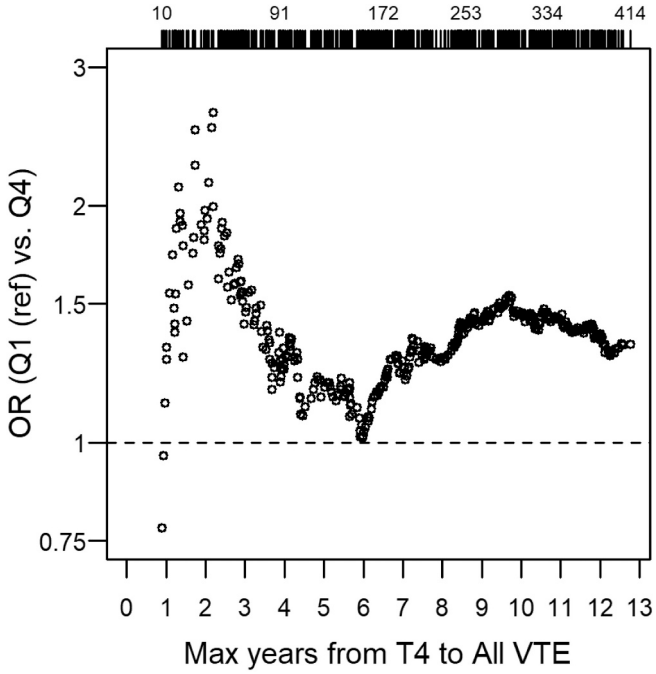


**Fig. 2.** Plots of estimated odds ratios (ORs) for overall venous thromboembolism (VTE) as a function of time between blood sampling in Tromsø 4 (T4, 1994–95) and VTE events. Participants with D-dimer plasma levels in the highest quartile (Q4) were compared with those with D-dimer levels in the lowest 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.

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 study derived from 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.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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*Author contributions*

E-S Hansen analyzed data, interpreted the results, and drafted the manuscript. F. B. 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2021.10.020.

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# Paper III

# Low D-dimer levels at diagnosis of venous thromboembolism are associated with reduced risk of recurrence: data from the TROLL registry

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## Essentials

- Can D-dimer levels at incident venous thromboembolism (VTE) predict recurrence risk?
- We explored the association in a cohort of 2585 patients with first symptomatic VTE.
- Low D-dimer measured at incident VTE diagnosis was associated with a low risk of recurrence.
- This implies that low D-dimer may be used to stratify VTE patients already at diagnosis.

## Summary

**Background**: Venous thromboembolism (VTE) is a frequent disease with a high risk of recurrence. It has been suggested that D-dimer level at the time of VTE diagnosis can be used to identify patients at low risk of recurrence.

**Objectives**: To investigate the impact of D-dimer measured at the time of VTE diagnosis on the risk of recurrence in a large cohort of patients with a first-time VTE.

**Methods**: The study included 2585 patients with first symptomatic non-cancer associated VTE from The Venous Thrombosis Registry in Østfold Hospital (TROLL) study (2005-2020). All recurrent events during follow-up were recorded and cumulative incidences of recurrence were estimated according to D-dimer levels ≤1900 ng/mL (≤25$^{th}$ percentile) and >1900 ng/mL.

**Results**: During a median follow-up of 3.3 years, 395 patients experienced a recurrent VTE. The one- and five-year cumulative incidence of recurrence was 2.9% (95% CI, 1.8-4.6) and 11.4% (95% CI, 8.7-14.8) in those with D-dimer ≤1900 ng/mL, and 5.0% (95% CI, 4.0-6.1) and 18.3% (95% CI, 16.2-20.6) in those with D-dimer>1900 ng/mL, respectively. In patients with unprovoked VTE, the five-year cumulative incidence was 14.3% (95% CI, 10.3-19.7) in the ≤1900 ng/mL category, and 20.2% (95% CI, 17.3-23.5 95%) in the >1900 ng/mL category.

**Conclusions**: D-dimer levels within the lowest quartile, measured at the time of VTE diagnosis, were associated with lower recurrence risk. Our findings imply that D-dimer levels measured at the time of diagnosis may be used to identify VTE patients at low risk of recurrent VTE.


Keywords: D-dimer; epidemiology; prediction; recurrence; venous thromboembolism.

# Introduction

Venous thromboembolism (VTE) is a frequent disease, causing considerable morbidity and mortality [1]. The risk of recurrence after a first VTE is high, and up to 30-40% of VTE patients experience a recurrent event within ten years [2]. Anticoagulant treatment efficiently prevents recurrences, though at the expense of increased risk of bleeding [3, 4]. In patients at high risk of recurrence, prolonged treatment is necessary, and the subsequent increased bleeding risk can be justified. However, in patients with a low risk of recurrence, prolonged anticoagulant treatment introduces an unnecessary risk of bleeding. Therefore, identifying patients at low risk for recurrent VTE, in whom short-term anticoagulation will be sufficient, is desirable [3, 5-7].

D-dimer is a global biomarker of coagulation activation and fibrinolysis[8]. Several studies have shown the utility of D-dimer to stratify patients according to the risk of recurrent VTE, and thereby provide guidance for the duration of anticoagulant therapy [9-12]. However, current strategies are based on D-dimer levels measured during or after discontinuation of the anticoagulant treatment [13-15], an approach requiring additional blood samples and revisits to the clinics. In addition, discontinuation of anticoagulant treatment leads to a rebound increase in thrombosis risk [16, 17]. Therefore, this approach may expose the patient to a higher risk of recurrence after discontinuing anticoagulation. As D-dimer is commonly used in the diagnostic work-up of patients with suspected VTE [18], D-dimer assessment is widely available at the time of diagnosis. Thus, using the D-dimer level measured at the time of diagnosis to assess recurrence risk is potentially less resource-demanding and may guide the decision on treatment duration already at the initiation of anticoagulant therapy.

In a study of 454 patients with first-time VTE, Bjøri and colleagues showed that D-dimer measured at the time of diagnosis could identify patients at low risk of recurrence [19]. Patients with a D-dimer level >1500 ng/mL had an estimated five-year cumulative incidence of 23%, while the corresponding cumulative incidence was only 9% among patients with D-dimer levels ≤1500 ng/mL.

These findings are promising but need further investigation and confirmation in other, larger study populations. Therefore, the present study aimed to investigate the impact of D-dimer measured at the time of VTE diagnosis on the risk of recurrence in a large cohort of patients with a first-time VTE.

## Methods

*Study population*

The study population comprised patients enrolled in the Venous Thrombosis Registry in Østfold Hospital (TROLL) registry between January 1, 2005, and April 30, 2020. Østfold Hospital, located in Østfold county, Norway, serves a local population of approximately 317 000 inhabitants. Inclusion required objectively confirmed lower limb deep vein thrombosis (DVT) or pulmonary embolism (PE). Patients diagnosed with both DVT and PE were categorized as PE. The details of the TROLL registry have been described previously [20]. The study has been approved by the Regional Committees for Health and Research Ethics South-East, Norway. All patients have given their informed written consent to participate. In addition, the ethical committee has exempted deceased patients from the requirement for written consent.

Patients with a permanent address outside the hospital's catchment area (n=46) were not included to increase the likelihood of a complete follow-up. A total of 3586 patients with first-time symptomatic VTE were eligible for the study. All cancer patients (n=787) were excluded since D-dimer may often be elevated in cancer patients independently of VTE [8]. Furthermore, patients with no D-dimer measurement (n=214) were excluded. Consequently, 2585 patients were included in the analyses and followed from the date of the first VTE to the end of the follow-up, April 30, 2020.

*D-dimer*

The D-dimer levels were assessed at the time of VTE diagnosis by the immuno-turbidometric method of STA®-Liatest® D-Di Plus (Stago Diagnostics, Asnieres, France). For the diagnostic purpose (used in routine clinical practice), a positive D-dimer was defined as levels ≥500 ng/mL and levels were

reported as a continuous variable up to >20 000 ng/mL (higher levels were truncated at this cut-off)., In this study, we divided the population into quartiles based on the D-dimer levels (quartile 1, ≤1900 ng/mL; quartile 2, 2000-3500 ng/mL; quartile 3, 3600-8200 ng/mL; quartile 4, >8200 ng/mL). Since the previous study by Bjøri et al. had shown a threshold effect at the lowest quartile, we merged the upper three quartiles yielding two final categories (≤1900 ng/mL and >1900 ng/mL).

*Assessment of VTE*

In- and outpatients diagnosed with VTE (i.e., distal DVT, proximal DVT, or PE) were referred to and followed up at the hospital's thrombosis clinic. In addition, the hospital discharge diagnosis registry was searched to identify patients with VTE who had not been referred to the thrombosis clinic (i.e., patients who died during hospitalization or who were not able to come to the thrombosis clinic). All recurrent events during follow-up were verified and recorded by the thrombosis clinic. In addition, an extensive review of the medical records in search of recurrent events was conducted in all patients after the end of the follow-up. Recurrent events included distal DVT, proximal DVT and/or PE (fatal and non-fatal). For fatal PE, the diagnosis was determined by imaging performed shortly before death or autopsy. Individuals without recurrence who died of unknown or uncertain causes during follow-up were not considered as recurrent events.

The VTE event was categorized as provoked or unprovoked, determined by the presence of known provoking factors at the time of diagnosis. An event was defined as provoked by the presence of one or more of the following factors: recent surgery or trauma, immobilization due to medical conditions, paralysis of the lower extremities or long-distance travel (>4 hours) within the previous 12 weeks, or any other factor explicitly described as being provoking in the medical records.

*Statistics*

For all included patients, person-time was counted from the date of the first VTE event to the first occurring date of recurrent VTE, the date of death, or the end of the study period, whichever came first. Patients who died during the follow-up were censored at the time of death.

All statistical analyses were performed using Stata version 17.0 (Stata Corporation LP, College Station, TX, USA). Crude incidence rates (IR) of VTE were calculated and expressed as the number of events per 100 person-years at risk. Hazard ratios (HR) were estimated across categories D-dimer by Cox proportional hazards regression models. The highest category was set as the reference, and the HR was expressed with a 95% confidence interval (CI). Two different Cox models were used to estimate the HR. The first model was adjusted for age and sex, while the second model was additionally adjusted for the duration of anticoagulant treatment. The proportional hazard assumption was tested for all models using Shoenfeld residuals. Furthermore, 1-Kaplan-Meier plots were estimated to visualize cumulative incidences of VTE over time.

Patients with distal DVTs may have a lower risk of recurrent VTE and a lower D-dimer level at the time of diagnosis[21]. Therefore, sensitivity analyses were performed to assess the potential confounding by such low-risk groups. Accordingly, we conducted the analyses restricted to patients with unprovoked proximal DVT or PE. Anticoagulant therapy has a strong impact on the risk of recurrent VTE. In addition to adjusting for the duration of anticoagulant treatment in the second Cox model, we performed sensitivity analyses with follow-up restricted to the time after discontinuation of anticoagulant therapy (i.e., follow-up started on the date of discontinuation of anticoagulant therapy). We also performed analyses restricted to patients treated with anticoagulant therapy for three and six months, respectively, starting on the date of discontinuation of therapy. D-dimer is associated with an increased risk of mortality[22]. To evaluate the potential impact of competing risk by death, sensitivity analyses were conducted using cumulative incidence functions[23]. The analyses were performed and visualized for overall VTE using Stata's stcrreg and the cif curve commands.

## Results

Characteristics of the VTE patients according to categories of D-dimer (≤1900 ng/mL and >1900 ng/mL) are presented in Table 1. As expected, the median age was highest in the highest category of D-dimer. In addition, the proportion of unprovoked events was slightly higher in the upper category. Patients with unprovoked index events had a larger proportion of patients with a long duration of anticoagulation treatment (>12 months) compared to patients with a provoked VTE (Table S1). Characteristics of patients excluded due to missing D-dimer values are presented in Table S2. Compared to patients with recorded D-dimers, patients with missing D-dimers had a higher proportion of events provoked by surgery and immobilization/hospitalization. No other major differences were observed when comparing patients with and without missing D-dimer levels.

During a median follow-up of 3.3 years, 395 of the 2585 patients experienced a recurrent VTE, yielding an overall IR of 3.57 (95% CI, 3.23-3.94) per 100 person-years. The one- and five-year cumulative incidence of recurrence was 2.9% (95% CI, 1.8-4.6) and 11.4% (95% CI, 8.7-14.8) in those with D-dimer ≤1900 ng/mL, and 5.0% (95% CI, 4.0-6.1) and 18.3% (95% CI, 16.2-20.6) in those with D-dimer >1900 ng/mL (Fig 1). The overall trend in the estimated cumulative incidence remained essentially similar when considering competing risk by death, although a slight reduction in the estimates was observed in both categories of D-dimer (Figure S1).

The cumulative incidence of recurrent VTE differed across provoking status at the incident event, as shown in Figure 2. In patients with unprovoked VTE, the five-year cumulative incidence of recurrence was 14.3% (95% CI, 10.3-19.7) in the ≤1900 ng/mL category and 20.2% (95% CI, 17.3-23.5 95%) in the >1900 ng/mL category. Among patients with a provoked VTE, the five-year cumulative incidence of recurrence in the ≤1900 ng/mL category was 8.5% (95% CI, 5.4-13.3), and 16.0% (95% CI, 13.2-19.3) in the >1900 ng/mL category (Figure 2B). Analyses stratified by DVT and PE yielded similar cumulative incidences as observed in the analysis of overall VTE (Figure S2).

In the sensitivity analysis restricted to patients with either an unprovoked proximal DVT or an unprovoked PE, the cumulative incidence in the ≤1900 ng/mL category was 3.6% (95% CI, 1.8-7.1) at one year and 14.6% (95% CI, 9.9-21.3) at five years (Figure S3), while it was 4.3% (95% CI, 3.1-6.0) at one year and 19.8% (95% CI, 16.8-23.3) at five years in the >1900 ng/mL category.

In analyses with follow-up restricted to the time after discontinuation of anticoagulant treatment, the association between low D-dimer and low risk of recurrent VTE persisted, with a cumulative incidence in the ≤1900 ng/mL category of 4.8% (95% CI, 3.1-7.2) at one year and 15.0% (95% CI, 11.5-19.5) at five years (Figure S4A, Table S3). In analyses restricted to patients initially treated for three months, the five-year cumulative incidence after discontinuation of anticoagulants was 11.1% (95% CI, 6.7-18.3) in the ≤1900 ng/mL category and 16.0% (95% CI, 11.2-22.6) in the >1900 ng/mL category (Figure 3A). Correspondingly, in patients initially treated for six months, the five-year cumulative incidence was 10.0% (95% CI, 5.3-18.6.2) in the ≤1900 ng/mL category and 24.7% (95% CI, 20.5-29.6) in the >1900 ng/mL category (Figure 3B). In patients with unprovoked VTE who had been initially treated for six months, the five-year cumulative incidence of recurrence after discontinuation of anticoagulants was 15.9% (95% CI, 8.1-29.0) in the ≤1900 ng/mL category and 29.2 (95% CI, 23.3-36.2 in the >1900 ng/ml category (Figure 3C).

HRs of recurrent VTE according to categories of D-dimer are presented in Table 2. After adjustment for age and sex, patients with a D-dimer ≤1900 ng/mL had a 39% lower risk of recurrence (HR 0.61, 95% CI 0.47-0.79) compared with patients with a D-dimer >1900 ng/mL. The relative risk reduction was particularly prominent in patients with a provoked index event (HR 0.49, 95% CI 0.33-0.74) and somewhat more pronounced in DVT patients (HR 0.58, 95% CI 0.41-0.82) than in PE patients (HR 0.63, 95% CI 0.43-0.93), though the CIs overlapped. Including the duration of anticoagulant treatment in the adjustment model strengthened the relationship between D-dimer ≤1900 ng/mL and a lower risk of recurrent VTE (Table 2).

## Discussion

In the present cohort of patients with a first VTE, those with a low D-dimer (≤1900 ng/mL) at the time of diagnosis had a 38% lower risk of VTE recurrence. The estimated five-year cumulative incidence of recurrent VTE was 11% in those with D-dimer ≤1900 ng/mL and 18% in those with D-dimer >1900 ng/mL. The association between low D-dimer at the time of diagnosis and reduced risk of recurrent VTE was consistent in subgroups of the index event, including DVT, PE, and unprovoked and provoked VTE.

Our findings confirm those of Bjøri and colleagues, who previously evaluated the association between D-dimer measured at the time of diagnosis and the risk of recurrence in a study of 454 VTE patients [19]. In their study, the D-dimer cut-off for the lowest quartile was ≤1500 ng/mL, and the estimated five-year cumulative incidence of recurrence was 9% and 23% among patients below and above this cut-off, respectively [19]. Overall, patients in the lowest quartile of D-dimer levels had a 53% lower relative risk of recurrent VTE. In the present study, we included six-fold as many patients and confirmed the association between low D-dimer levels measured at VTE diagnosis and recurrence risk. The cut-off level for the lowest D-dimer quartile was somewhat higher in our study (≤1900 ng/mL) than in the Bjøri et al. study (≤1500 ng/mL) [19], potentially due to a higher proportion of patients with PE in our study (56% vs. 44%). Accordingly, the cumulative incidence of recurrence at one and five years was slightly higher in our study (2.9% vs. 1.7% at one year and 11.4% vs. 8.5% at five years).

In the evaluation of recurrence risk in cohort studies, the Subcommittee on Control of Anticoagulation of the International Society of Thrombosis and Haemostasis recommends a recurrence rate below 5% at one year and 15% at five years to justify termination of anticoagulant treatment [24]. The one and five-year cumulative incidences of recurrence for the ≤1900 ng/mL D-dimer category were below these accepted thresholds. Even though the optimal D-dimer cut-off

remains to be determined, our findings support the utility of D-dimer to identify patients at low risk of recurrence already at the time of diagnosis.

There is a general consensus to treat VTE patients without contraindications with anticoagulation for at least three months [25-27] and to consider extended duration or indefinite treatment when the VTE is unprovoked [25, 28]. D-dimer assessment after the initial treatment period has been proposed as a tool to guide decisions on whether or not to extend the length of treatment, particularly after a first unprovoked VTE [13-15]. Yet, the use of this approach is limited and not implemented in clinical guidelines [25-27]. Our results indicate that low D-dimer levels at the time of diagnosis may facilitate identification of patients with unprovoked VTE in whom anticoagulant treatment can be safely terminated after six months without requiring or the need for further D-dimer assessment after the initial treatment phase. However, whether D-dimer alone is sufficient to guide treatment duration after unprovoked VTE is uncertain, as the confidence interval surrounding the 5-year recurrence estimate in the subgroup of patients with unprovoked VTE who had discontinued treatment after six months was wide (likely due to a limited number of patients in this subgroup). Moreover, since the VTE patients in our cohort were managed according to regular clinical practice (i.e., D-dimer levels were not used to determine treatment duration), the utility of our findings need to be further explored in a outcome study. In addition, whether diagnostic D-dimer could be useful in combination with other predictive factors for VTE recurrence, particularly for assessment of low risk among patients with unprovoked VTE, should be further explored.

Several risk assessment models for VTE recurrence, such as the Vienna [13], DASH [14], and HERDOO2 [15] prediction models, utilize D-dimer levels measured after discontinuation of the anticoagulant treatment in combination with clinical risk factors. Although the information on the clinical risk factors included in the Vienna and DASH models is available at the time of diagnosis, the risk assessment model cannot be applied until the initial phase of anticoagulation is completed several months later. Whether the D-dimer level at the time of diagnosis could be utilized in these

models remains unsettled and should be further tested in a prediction framework. A model that can be used already at the time of diagnosis may be beneficial for the patients and the health care system, as it can inform prognosis, guide decisions on treatment duration, and lower the need for follow-up consultations in patients with low recurrence risk.

The main strength of our study is the recruitment of VTE patients from a general population for 15 subsequent years, yielding a large study population. Furthermore, the same diagnostic work-up, including the same highly sensitive D-dimer assay, was used during the entire study period. In addition, we only included patients residing within the hospital's catchment area and applied a comprehensive case validation through the outpatient clinic and extensive review of the patients' medical records, which enhanced the likelihood of a complete follow-up. Our study has some limitations that need to be addressed. The study was based on a longitudinal cohort with different treatment strategies applied during the study period, independent of D-dimer at the time of diagnosis. This likely affects the natural history of the disease and the observed recurrence risk in our study population. Nevertheless, adjustment for the duration of anticoagulation strengthened our risk estimates, and the association between low D-dimer and lower risk of recurrence persisted in analyses where follow-up was restricted to the time after discontinuation of anticoagulant treatment. Secondly, 5.9% of the eligible patients were excluded due to missing D-dimer values. While D-dimer is often part of the diagnostic work-up for VTE, D-dimers are not measured in all settings, such as hospitalized patients [29, 30]. When comparing patients with and without information on D-dimer in the present study, no major differences were observed in variables indicating the VTE-severity (i.e., type of VTE, proportion treated with unfractionated heparin or thrombolysis, or proportion of death during follow-up). However, the proportion of events provoked by immobilization or hospitalization was higher in patients with missing D-dimer, indicating that lack of D-dimer was particularly prevalent among already hospitalized patients. Thus, our findings may be less generalizable to this particular patient group. Lastly, in some patients with expected prolonged diagnostic work-up (e.g., outside regular working hours, pending radiological testing), empiric

treatment with low-molecular-weight heparin may have been initiated by primary care physicians before referral to the hospital. This could potentially have introduced misclassification of D-dimer levels in these patients. However, the extent of this approach is limited and probably of negligible impact on our findings.

In conclusion, D-dimer levels within the lowest quartile (≤1900 ng/mL) measured at the time of incident VTE diagnosis was associated with a low risk of recurrent VTE. These findings imply that a low D-dimer may be used to stratify VTE patients at low risk of recurrent VTE already at the time of diagnosis. Future studies should explore whether the inclusion of diagnostic D-dimer in risk assessment models can improve the identification of patients at low recurrence risk in whom short-term anticoagulation would be sufficient.

## Addendum

F. B. Rinde contributed to the data collection, data analysis, and writing the manuscript. C. T. Jørgensen contributed to the data analysis, data interpretation, and revision of content. H. H. Pettersen and W. Ghanima contributed to the data collection and revision of content. J.-B. Hansen contributed to the conception and design of the study, data interpretation, and revision of content. S. K. Brækkan contributed to the conception and design of the study, interpretation, and writing the manuscript.

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## Conflict of interest.

C. T. J. reports lecture honoraria from Bayer. H. H. P. reports fees from Sanofi and Novartis. W. G. reports fees for participation in an advisory board from Amgen, Novartis, Pfizer, Principia Biopharma Inc- a Sanofi Company, Sanofi, SOBI, Grifols, UCB, Argenx, Cellphire; lecture honoraria from Amgen, Novartis, Pfizer, Bristol Myers Squibb, SOBI, Grifols, Sanofi, and Bayer; and research grants from Bayer, BMS/Pfizer, and UCB.

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