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Venous thromboembolism and cancer

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List of papers

I. Joint effects of cancer and variants in the factor 5 gene on the risk of venous thromboembolism.
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II. Impact of time since diagnosis and mortality rate on cancer-associated venous thromboembolism in a general population – the Scandinavian Thrombosis and Cancer (STAC) cohort.
Blix K, Gran OV, Severinsen MT, Cannegieter S, Jensvoll H, Overvad K, Hammerstrøm J, Tjønneland A, Næss IA, Brækkan SK, Rosendaal FR, Kristensen SR, Hansen JB
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III. Occult cancer-related first venous thromboembolism is associated with an increased risk of recurrent venous thromboembolism.
Gran OV, Brækkan SK, Paulsen B, Skille H, Rosendaal FR, Hansen JB.
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IV. D-dimer measured at first venous thromboembolism is associated with future risk of cancer.
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Summary

Venous thromboembolism (VTE) is a collective term for deep vein thrombosis and pulmonary embolism. VTE is a common cause of mortality and morbidity in patients with cancer, and may also be the first sign of an underlying malignancy. The first aim of this thesis was to investigate the risk of incident and recurrent VTE in active cancer compared to a cancer-free reference population and to explore the influence of genetic risk factors on cancer-related VTE. Secondly, we aimed to investigate the association between plasma D-dimer levels at VTE and the subsequent risk of cancer.

All four papers in this thesis utilize the Tromsø Study. The first survey of the Tromsø Study was conducted in 1974 and, thus far, seven surveys have been completed. Our study populations were recruited from the Tromsø 4 and 6 surveys for Paper I, and Tromsø 1 to 6 surveys for Papers III and IV. Participants were followed from 1994, when the VTE registry was established in Tromsø, throughout 2012. Paper II was based on the Scandinavian Thrombosis and Cancer (STAC) cohort, which comprises individual data from the Tromsø 4 survey, the second Nord-Trøndelag Health Study (HUNT2) and the Danish Diet, Cancer and Health (DCH) Study. Validated VTE events and cancer diagnoses were registered from inclusion (1993 to 1997) to the end of follow-up (2007 to 2012).

We found a joint effect between two single nucleotide polymorphisms (SNPs) in the F5 gene (F5 rs6025 (Factor V Leiden) and F5 rs4524) and active cancer on the risk of VTE. The incidence of cancer-related VTE increased considerably in the six months following a cancer diagnosis, and especially so in patients with risk alleles at these SNPs.

Further, cancer-related factors consistently demonstrated a strong influence on incident and recurrent VTE. In traditional analysis, the risk of VTE was highest in the first 6 months after cancer diagnosis and the risk declined markedly thereafter. However, when mortality was taken into account, the risk of VTE was equal in the 6 months before and 6 months after a cancer diagnosis, which suggests that cancer itself is a major contributor to the VTE risk. The risk of VTE by cancer sites was greatly influenced by mortality and the time since cancer diagnosis. We found that patients with an occult cancer-related incident VTE had a higher rate of VTE recurrence than those with overt cancer and those without cancer. Patients with occult cancer-related incident VTE who experienced a VTE recurrence more often had prothrombotic and advanced cancers at the time of cancer diagnosis. The majority of VTE recurrences were not related to cancer treatment as they occurred prior to cancer diagnosis.

Finally, we found that plasma D-dimer levels above 5000 ng/ml at incident VTE were associated with an increased risk of subsequent cancer at one and two years. Patients with higher D-dimer levels at incident VTE had more advanced cancers at the time of diagnosis and mortality was greater among these patients.

Sammendrag

Venøs tromboembolisme (VTE) er fellesbetegnelsen for dyp venetrombose og lungeemboli. VTE er en vanlig årsak til morbiditet og mortalitet blant kreftpasienter, og kan i tillegg være et tidlig tegn på underliggende malignitet. Formålet med denne avhandlingen var å undersøke risikoen for førstegangs- og residiverende VTE ved aktiv kreftsykdom sammenlignet med en kreftfri referansepopulasjon, og å undersøke hvordan genetiske risikofaktorer påvirker VTE. Videre ville vi undersøke assosiasjonen mellom plasma D-dimer ved VTE diagnose og påfølgende risiko for kreft.

Alle fire artikler i denne avhandlingen benytter data fra Tromsøundersøkelsen. Den første Tromsøundersøkelsen ble gjennomført i 1974 og så langt har 7 undersøkelser blitt utført. Studiepopulasjonen i artikkel I ble rekruttert fra Tromsø 4 og 6. For artikkel III og IV benyttet vi undersøkelsene 1 til 6. Deltakere ble fulgt fra 1994, da VTE-registeret ble etablert, ut desember 2012. Artikkel II er basert på «the Scandinavian Thrombosis and Cancer (STAC)» kohorten, som inkluderer individuelle data fra Tromsø 4, den andre Helseundersøkelsen i Nord-Trøndelag (HUNT2) og den danske «Diet, Cancer and Health» (DCH) studien. Validerte VTE- og kreftdiagnoser ble registrert fra inklusjonsperioden (1993 -1997), til og med oppfølgingsperioden (2007-2012).

To genetiske varianter (single nucleotide polymorphism, SNP) i FV genet (F5 rs6025 (Faktor V Leiden) og F5 rs4524) i kombinasjon med aktiv kreftsykdom, hadde en synergistisk effekt på risikoen for VTE. Insidensen av kreftrelatert VTE økte betydelig i de første 6 månedene etter en kreftdiagnose, og spesielt blant pasienter med risikoalleler av disse SNPene. I tillegg hadde kreftrelaterte faktorer som type kreft og metastasegrad en sterk innvirf2010kning på risikoen for førstegangs- og residiverende VTE. Ved bruk av tradisjonelle analysemetoder fant vi at risikoen for VTE var høyest de første 6 månedene etter kreftdiagnosen. Men, når vi tok høyde for mortaliteten blant disse pasientene, var risikoen for VTE den samme i perioden 6 måneder før og 6 måneder etter kreftdiagnosen, hvilket antyder at kreftsykdommen alene har stor innvirkning på VTE-risikoen. Videre var risikoen for VTE ved ulike krefttyper sterkt påvirket av mortalitet og tid siden kreftdiagnosen. Vi oppdaget at VTE pasienter med okkult kreft hadde høyere forekomst av residiverende VTE enn både de med diagnostisert kreftsykdom og kreftfrie pasienter. Pasienter med okkult kreft som fikk residiverende VTE hadde oftere protrombotisk og avansert kreftsykdom når kreftdiagnosen ble stilt. De fleste VTE residivene var ikke relatert til kreftbehandling da de inntraff før kreftsykdommen ble oppdaget.

Til slutt fant vi at plasma D-dimer nivå over 5000 ng/ml ved førstegangs VTE var assosiert med økt risiko for kreft både ett og to år etter VTE en VTE hendelse. Pasienter med høyt D-dimer nivå ved førstegangs VTE hadde i større grad avansert kreftsykdom ved diagnosetidspunktet, og mortaliteten var høyere blant disse pasientene.

Abbreviations

ACCP	American College of Chest Physicians
ANC	Awareness of Neutropenia in Chemotherapy
AP	attributable proportion due to interaction
APC	activated protein C
BMI	body mass index
CATS	Vienna Cancer and Thrombosis Study
CCR	California Cancer Registry
CI	confidence interval
CRN	Cancer Registry of Norway
CRP	C-reactive protein
CT	computed tomography
CTPH	chronic thromboembolic pulmonary hypertension
CVC	central venous catheter
DCH	Diet, Cancer and Health Study
DOAC	direct oral anticoagulant
DVT	deep vein thrombosis
EPCR	endothelial protein C receptor
F	factor
FDA	Food and Drug Administration
FGD	fluorodeoxyglucose
FVL	factor V Leiden
GWAS	genome-wide association study
HR	hazard ratio
HUNT	Health Survey in Nord-Trøndelag (Helseundersøkelsen i Nord-Trøndelag)
ICD	International Classification of Diseases
IR	incidence rate

KM	Kaplan-Meier
LMWH	low molecular weight heparin
MEGA	Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis
MP	microparticle
OR	odds ratio
PAR	population attributable risk
PE	pulmonary embolism
PET	positron emission tomography
PT	prothrombin
PTS	post-thrombotic syndrome
RCT	randomized controlled trial
RR	relative risk
RERI	relative excess risk due to interaction
RIETE	Registro Informatizado de la Enfermedad Trombo Embolica
SHR	subdistribution hazard ratio
SIR	standardized incidence rates
SNP	single nucleotide polymorphism
STAC	Scandinavian Thrombosis and Cancer
SVT	superficial vein thrombosis
TF	tissue factor
TFPI	tissue factor pathway inhibitor
UFH	unfractionated heparin
VKA	vitamin K antagonist
VTE	venous thromboembolism
vWF	Von Willebrand factor
WHO	World Health Organization

1. Introduction

Venous thromboembolism (VTE) is a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT is the formation of a blood clot in the deep veins which may obstruct venous blood flow. DVT most commonly occurs in the large veins of the leg or thigh, but can also occur in other parts of the body. Classical symptoms and signs of DVT are pain, swelling and redness of the affected extremity. Traditionally, PE is thought to be a complication of DVT. PE primarily occurs when all, or parts, of the deep venous clot breaks free (embolizes). The clot then travels via the blood stream, through the right side of the heart and into the lungs, where it becomes lodged and prevents the flow of blood. However, the origin of PE remains undetected in up to 50% of patients with PE.[1,2] PE is traditionally characterized by dyspnea, tachypnea, pleuritic-type chest pain, coughing and, in severe cases, circulatory collapse and death. DVTs account for approximately two-thirds of all VTE events, while PEs make up about one-third, although the two conditions often exist concurrently.[3]

Historically, the French doctor, Armand Trousseau, is credited with originally describing the relationship between VTE and cancer in 1865.[4] However, it was actually another French physician, Jean-Baptiste Bouillard, who first described an association between thrombosis and cancer in 1823 – nearly half a century earlier than Trousseau.[5] Nevertheless, the spontaneous formation of a venous thrombus in association with an underlying cancer has been termed Trousseau's syndrome. Trousseau's syndrome is especially well-known in the field of medicine because only two years after Armand Trousseau described the condition, he diagnosed himself with a VTE secondary to gastric cancer, before succumbing to the illness shortly after.[4] Since the time of Bouillard and Trousseau, several publications have confirmed the two-way relationship between malignancy and VTE.[6,7] Today, cancer is established as one the strongest risk factors for VTE, and it is associated with a four- to seven-fold higher risk of VTE, when compared to cancer-free subjects.[8] Approximately 15% of cancer patients will develop a symptomatic VTE during the course of their disease and up to 50% have a VTE at autopsy.[9] Clinical consequences of VTE are typically more common and more severe in cancer patients,

and the risk of death following a VTE is higher in cancer patients than in cancer-free subjects.[10] With the use of prophylactic anticoagulants, VTE is a potentially preventable disease. Current guidelines, however, do not recommend blanket thromboprophylaxis to all cancer patients, as the risk of bleeding on anticoagulants is high in cancer patients.[11] Thus, it is important to identify cancer patients who are at high risk of VTE, to determine who would most benefit from targeted thromboprophylaxis.

VTE can be the first manifestation of cancer, and up to 10% of patients with an unprovoked VTE are diagnosed with cancer within the first year.[12] The risk of cancer is highest in the first year following a VTE event, but persists for several years thereafter.[6,13] In recent years, there has been an ongoing debate regarding to what extent it is pertinent to screen for cancer in patients with an unprovoked VTE, as it is not apparent if earlier detection of cancer favors a patient's prognosis. Thus, the understanding of both risk factors for VTE in cancer and cancer in VTE are fundamental for further understanding of the association between cancer and VTE.

There is a great need to further explore established risk factors and to identify novel risk factors for cancer-related VTE. Risk factors for cancer-related incident and recurrent VTE, as well as the two-way relationship between cancer and VTE will be the topics of the present thesis.

1.1 Epidemiology

1.1.1 Venous thromboembolism in the general population

Although VTE is the third most common cardiovascular disease worldwide, it is relatively neglected compared to myocardial infarction and stroke.[14] Venous thromboembolism occurs in 1 to 2 per 1000 individuals annually in developed countries, and based on European data, the estimated annual burden of symptomatic VTE in 25 European countries exceeds 1.6 million.[15] Furthermore, it is estimated that there are approximately 540 000 VTE-related fatalities in Europe each year, which is double the combined deaths due to AIDS, breast cancer, prostate cancer and transport accidents.[15,16] Since the 1980s, the incidence of VTE is increasing, owing primarily to the substantial increase of the incidence of PE.[17] In the Tromsø Study, the age-adjusted incidence rates (IR) of PE per 100 000 person-years increased from 45 (95% confidence interval (CI) 23–67) in 1996/1997 to 113 (95% CI 82–144) in 2010/2011.[18]

VTE events can be classified into provoked and unprovoked, depending on the presence of risk factors at the time of the VTE event.[19] Provoked events can occur in the presence of transient or persistent clinical risk factors, and transient risk factors are further classified into major and minor.[20] Provoked VTE events can occur in the presence of major transient risk factors (i.e. surgery with general anesthesia >30 min, Cesarean section) in the 3 months before an event, or by minor *transient risk factors* (i.e. surgery with general anesthesia <30 min, estrogen therapy, pregnancy) in the 2 months before a VTE diagnosis.[20] VTE events may also be provoked by *persistent risk factors*, such as active cancer or any other on-going non-malignant condition associated with at least a 2-fold risk of recurrent VTE after stopping anticoagulant therapy (i.e. inflammatory bowel disease).[20] Unprovoked events are VTEs that occur in the absence of a provoking risk factor, transient or persistent. VTE in the presence of provoking factors is generally associated with lower recurrence rates and does not normally warrant prolonged treatment with anticoagulants.[19,21] However, persistent provoking risk factors (i.e. active

cancer) are associated with higher VTE recurrence rates than unprovoked VTEs.[20] Population-based studies estimate that about 50 to 60% of VTEs are associated with provoking factors.[22-24]

Short-term complications of VTE include thrombus extension or further embolization, VTE recurrence in the weeks to months following the initial event, and death. VTE may also lead to serious long-term complications such as post-thrombotic syndrome (PTS), chronic thromboembolic pulmonary hypertension (CTPH) and late recurrence.[25] PTS is the most common complication of DVT, affecting between 20 to 50% of patients with lower limb DVTs.[26,27] PTS typically causes chronic pain, swelling and skin changes in the affected leg. In severe cases, 10% may also develop venous leg ulcers, which can be very difficult to treat.[26,28] Risk factors for PTS include female sex, obesity, proximal DVT location, recurrent DVTs and varicose veins, whereas cancer, surgery, plaster casts or inherited thrombophilias (i.e. FVL, PT 20210A) have not been found to influence the risk of PTS.[26,29,30] PTS adds significantly to the cost of VTE treatment, places greater demands on the healthcare system and impairs patient mobility and quality of life.[27,31] Furthermore, a Norwegian study found that DVT was associated with a 60% increased risk of disability pension, while no significant association was seen for PE, and the most common and debilitating complication of DVT is PTS.[32] CTPH is caused by chronic obstruction of major pulmonary arteries, causing the right side of the heart to work harder than normal due to the abnormally high blood pressure in the arteries of the lungs. CTPH complicates approximately 2 to 4% of acute PE events and is characterized by dyspnea, chest discomfort and signs of right-sided heart failure.[33,34]

VTE is a major cause of morbidity and mortality. A recent Norwegian study including 710 subjects with an incident VTE, reported an overall cumulative mortality rate of 8.6% (95% CI 6.7-11.0) at 30 days and 24.2% (95% CI 21.2-27.6) at one year.[35] These results are in line with a large Canadian study of 67 354-definite and 35 123-probable VTEs that reported 30-day and 1-year case-fatality rates of 10.6% (95% CI 10.4-10.8) and 23.0% (95% CI 22.8-23.3), respectively.[36] Mortality rates among subjects with unprovoked VTE events are lower than in subjects with provoked events, which is likely

explained by a higher age and the presence of additional comorbidities among those with provoked VTE.[35] Furthermore, mortality rates are also higher among those with cancer.[35,37] Almost 25% of all PE cases are thought to present as sudden death.[38] Distinguishing sudden, fatal PE events is, however, a challenge as autopsies are not often performed in these patients, and therefore, these deaths are frequently mistakenly attributed to cardiac causes.[39]

1.1.2 Recurrent venous thromboembolism in the general population

VTE is a chronic disease that frequently recurs. Anticoagulation is thought to treat the acute thrombotic event, although it does not cure the underlying pro-thrombotic predisposition.[40] The risk of VTE recurrence after an incident VTE is 5 to 7% per year, and the risk of VTE after an incident VTE is more than 50 times higher than in patients without a previous VTE event.[3] The incidence of recurrent VTE is reported to be 7 to 10% at 6 months, 11 to 18% after 1 year, and 30 to 40% after 10 years.[40-42] Another study reported the two-year incidence of recurrent VTE to be 7.7% overall, and 14.2% in patients with cancer.[24] VTE events tend to recur as the same clinical type as the initial event. Meaning that a patient with a DVT is more likely to suffer from a recurrent DVT, and a patient with a PE is more likely to suffer from a recurrent PE.[40,43]

Independent clinical predictors of VTE recurrence include male sex, increasing age and body mass index, active cancer, neurologic disease with extremity paresis, and neurologic surgery.[40] The risk of recurrence is lower in patients with events provoked by transient risk factors.[21] Several risk prediction models exist for risk stratification for recurrent VTE such as the Vienna prediction model, the DASH score and the Rodger score.[44-46] This risk prediction models include a combination of patient characteristics (i.e. sex, age, obesity, hormone use), VTE characteristics (i.e. DVT location, presence of redness or edema), and laboratory parameters (i.e. D-dimer levels) as predictive variables. Furthermore, recurrent VTE is associated with a substantially higher likelihood of post-thrombotic syndrome and chronic pulmonary hypertension.[47]

1.1.3 Venous thromboembolism in cancer patients

VTE is a frequent and severe complication of cancer, and several studies have confirmed that 20 to 30% of all incident VTE events in the general population are cancer-related.[7,22,48] Active cancer is associated with a 4 to 7-fold increased risk of VTE, when compared to cancer-free subjects.[49-51] The absolute risk of cancer-related VTE has been reported between 1 to 12%.[52-54] The wide range of reported cumulative incidences is due to the heterogeneity of these studies, and may be a result of differences in the study populations, follow-up duration, definitions of active cancer and the VTE identification and validation criteria.

The incidence of VTE in cancer is increasing.[55,56] A large cohort study with registry data on over 660 000 subjects found that the overall incidence of cancer-related VTE increased from 10 (95% CI 8-14) per 1000 person years in 1997 to 19 (95% CI 18-21) per 1000 person years in 2006, while the same trend was not observed in cancer-free subjects.[55] Improvements in the clinical and public awareness of cancer-related VTE and more aggressive cancer treatments (i.e. anti-angiogenic drugs, and surgery in later stages of cancer) were attributed as the main contributors to the increase in the incidence rates. Furthermore, increased utilization of improved non-invasive diagnostic imaging for the detection and staging of cancers, may identify incidental VTE events.

Clinical consequences of VTE such as recurrence, PTS, CTPH, as well as treatment-related bleeding complications are typically more common and more severe in cancer patients than in patients without. The American College of Chest Physicians (ACCP) guidelines recommend patients with cancer-related VTE extended anticoagulation (no scheduled stop date) over 3 months of therapy for patients at a low (Grade 1B) and high (Grade 2B) bleeding risk.[19] However, treatment failure despite adequate anticoagulation occurs frequently in patients with cancer and subsequently, cancer patients are at a 2 to 9-fold higher risk of VTE recurrence compared to cancer-free subjects.[40,57-59] In a cohort study of 477 Olmsted county residents with cancer-related incident VTE, the cumulative incidence of VTE recurrence in cancer-related VTE was 18.0% at three months and 26.7% at one year.[60] This study also

reported that cancer patients are at an increased risk of anticoagulant-associated bleeding and the cumulative incidence of major bleeding while on anticoagulation was 2.5% at three months and 4.7% at one year.[60] Survival was significantly worse in cancer patients with recurrent VTE and with bleeding on anticoagulation. Furthermore, this study also found that tumor site (brain, lung, ovarian and pancreatic cancers, and myeloproliferative and myelodysplastic disorders), stage (stage IV, cancer stage progression) and leg paresis were independent predictors of VTE recurrence.

Cancer patients with VTE have reduced survival compared to cancer patients without VTE. In a Danish population-based study, the one-year survival rate was 12% in patients with cancer-related VTE, compared to 36% in cancer patients without a VTE.[61] A study utilizing the California Cancer Registry (CCR) between 1993 and 1995, found that after adjustment for age, race, and cancer stage, VTE was a significant predictor of decreased one-year survival at all cancer sites (hazard ratios (HRs) 1.6-4.2).[52] Increased mortality was present in localized, regional and metastatic-stage cancers, however, metastatic disease was the strongest predictor of death. The majority of VTE-associated deaths occurred in the first 90 days following the VTE event.[52] This suggests that the cause of death may be due to the VTE event (i.e. massive pulmonary embolism) or its treatment (anticoagulant-related bleeding), or it may simply reflect the extent of cancer in these patients and/or the presence of comorbid conditions.

Studies have suggested that the use of prophylactic anticoagulants in cancer patients may improve quality of life and survival. Low molecular weight heparins (LMWH) are the anticoagulants of choice for primary prophylaxis and treatment of VTE in cancer patients.[19,62,63] The effect of thromboprophylaxis in ambulatory cancer patients receiving chemotherapy has been summarized in a recent Cochrane review from 2016 which included results from 26 randomized control trials.[64] During a median follow-up of 10 months, thromboprophylaxis with LMWH was found to be associated with a reduced risk of symptomatic VTE across different cancer types (relative risk (RR) 0.54, 95% CI 0.38-0.75), with a reduction of 33 (95% CI 18-44) per 1000 VTE events. This was, however, associated with

an increase in major bleeding events (RR 1.44, 95% CI 0.98-2.11). Current guidelines do not recommend blanket thromboprophylaxis to all cancer patients.[19] Therefore, it is crucial to be able to identify cancer-patients who are at high risk of VTE, in order to provide targeted thromboprophylaxis to the patients with a favorable benefit to harm ratio. Current risk prediction models for VTE among cancer patients, are based mainly on cancer localization, patient-related factors and laboratory parameters.[65,66] A validation study reported, however, that these risk prediction models may be of limited clinical value, as they have a low potential to identify high-risk patients, as only 12% of the entire cancer cohort study population was considered high risk. Furthermore, these risk prediction models may have an insufficient capacity to predict VTE in high-risk subjects, as only 7% of the high-risk patients developed VTE over 2.5 years in the validation study.[65]

1.2 Pathophysiology

1.2.1 General pathophysiology of venous thromboembolism

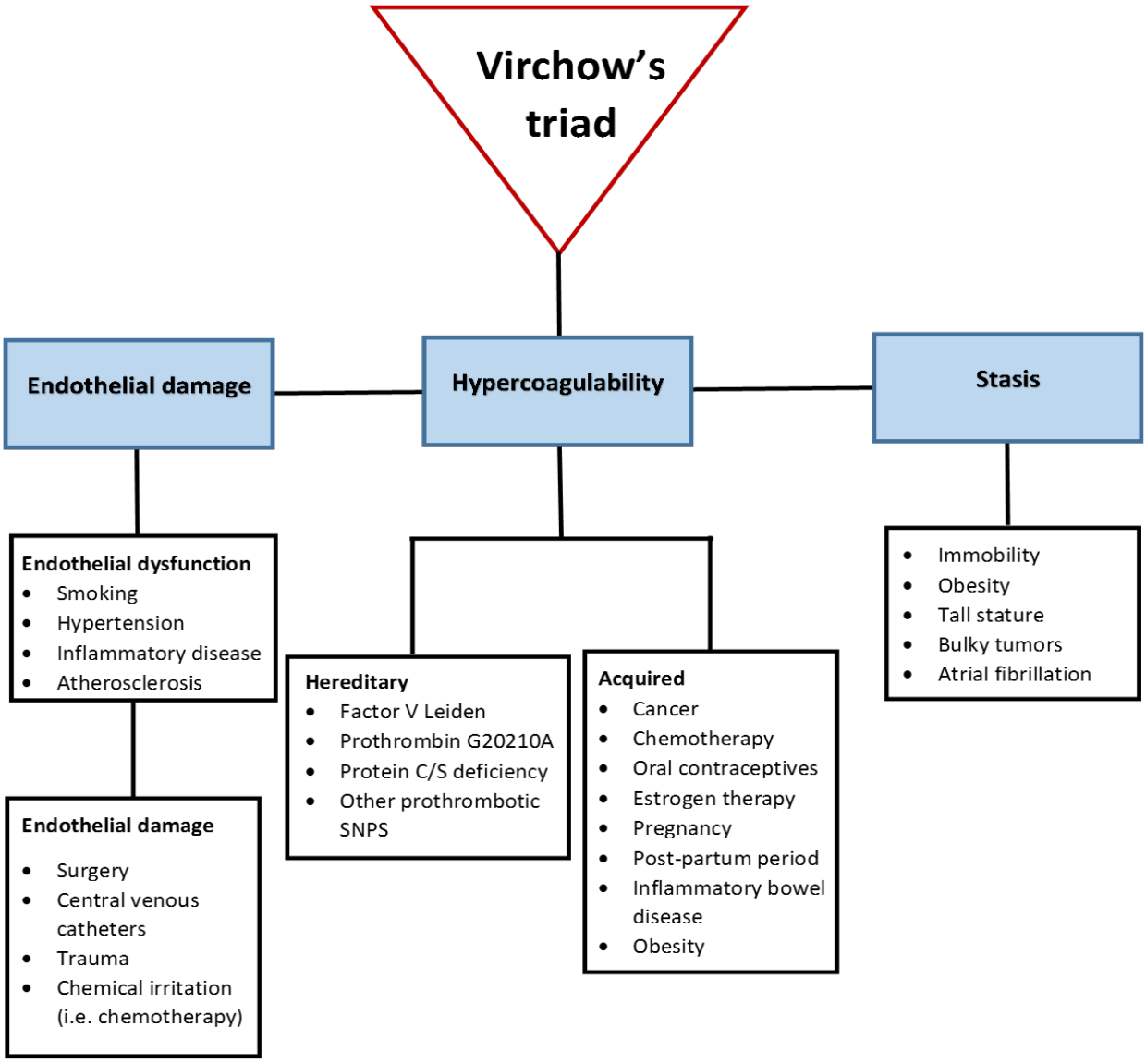
In 1856, the German scientist Rudolph Virchow proposed a triad of causes for thrombosis, theorizing that stasis, hypercoagulability, or vessel wall damage could lead to thrombosis (Figure 1). Although advances in research have provided us with more sophisticated tools to expand the triad, it still represents a cornerstone in our understanding of the pathophysiological mechanisms of VTE.

A healthy and undamaged endothelium expresses various anticoagulants, such as tissue factor pathway inhibitor (TFPI), thrombomodulin, endothelial protein C receptor (EPCR), and heparin-like proteoglycans.[67] ***Endothelial damage*** results in expression of tissue factor (TF) and adhesion molecules, which in turn results in activation of the coagulation cascade and adhesion of white blood cells to the endothelial surface.[68] Together, factor (F) VIIa and TF, activate the extrinsic coagulation pathway via activation of FIX and FX. Activated FX and FVa, convert prothrombin (FII) to thrombin (FIIa), and ultimately lead to fibrin deposition and clot formation. A relationship between arterial thrombosis and blood vessel injury is well-established. Rupture of an atherosclerotic plaque exposes subendothelial TF, collagen and von Willebrand factor (vWF).[69] The role of endothelial damage in the development of VTE, however, is less obvious. Sevitt reported the histological findings from 50 thrombi recovered from autopsies and did not find evidence of endothelial damage for most thrombi. He concluded that vessel wall injury may not contribute significantly to DVT, except for when associated with acute insults (i.e. surgery, trauma).[70] However, hypoxia has been suggested as a more subtle form of endothelial injury, as it can promote endothelial activation and permeability.[71] Endothelial dysfunction can result in a prothrombotic state by alterations in the balance between clot formation and breakdown due to decreased synthesis of nitric oxide and prostaglandin I2 and increased entothelin-1.[72]

Hypercoagulability, or an abnormally increased tendency toward clotting, plays an important part in the pathogenesis of VTE. Activated factors are concentrated in areas of reduced flow, such as

the valve pockets.[73] Hypercoagulable states may be inherited or acquired. A number of genetic defects influence an individual's risk of VTE, such as single nucleotide polymorphisms (i.e. Factor V Leiden and prothrombin G20210A) and deficiencies in natural anticoagulants (i.e. protein C/S).[73] Acquired hypercoagulable states include obesity, pregnancy, oral contraceptives, as well as cancer and chemotherapy. Hyperestrogenemia, caused by pregnancy, oral contraceptives, or hormone replacement therapy, results in increased hepatic synthesis of procoagulant proteins and decreased synthesis of anticoagulants.[74] In addition, release of tissue factor from damaged tissues or tumor cells, can result in activation of the coagulation cascade.[75]

Figure 1. Virchow's Triad



Venous thrombi most often develop in the valvular sinuses of the venous valves.[76,77] An experimental study on dogs demonstrated that, in the absence of pulsatile flow, prolonged stasis resulted in severe hypoxia in the recesses of the venous sinuses.[77] Immobility can result in prolonged blood ***stasis*** and further potentiate the hypoxia in these regions.[77] As blood travels against gravity in the veins, some is caught in a secondary vortex of the valve sinuses (Figure 2). This leads to localized hypoxia in this region and promotes prothrombotic and proinflammatory processes in endothelial cells, as well as the recruitment and activation of leukocytes and platelets. Endothelial cells mobilize P-selectin and vWF on their surface, which recruit platelets and leukocytes that express TF, which then activates the coagulation cascade.

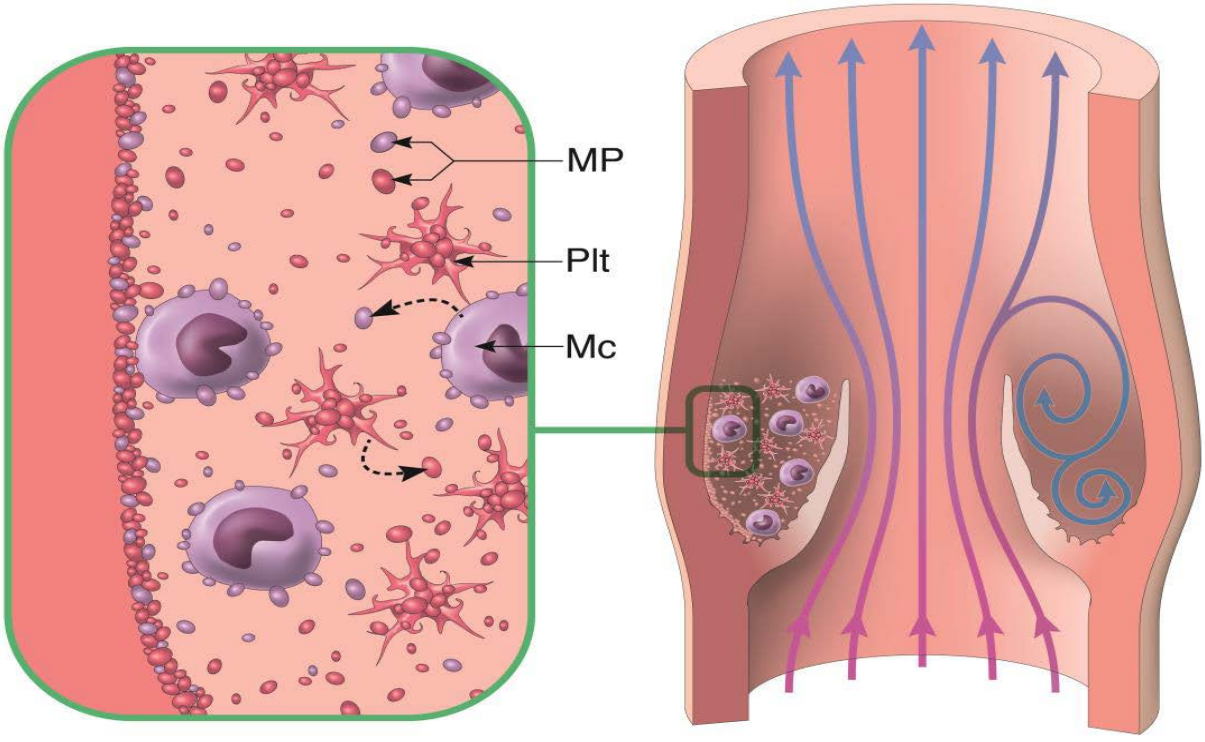


Figure 2. The pathophysiology of DVT in the venous valves: when blood is trapped in a vortex of the valve pockets, it becomes desaturated. This hypoxia promotes prothrombotic processes in endothelial cells, leukocytes, especially monocytes (Mc), and platelets (Plt). Activated platelets and leukocytes bud-off procoagulant (TF positive) microparticles (MP), which can activate the coagulation cascade. These procoagulant microparticles have been suggested as important triggers of VTE.

1.2.2 Pathophysiology of cancer-related venous thromboembolism

Virchow's triad of endothelial damage, hypercoagulability and stasis are important features of cancer and they play an important role in the pathophysiology of VTE in cancer patients. Coagulation activation and tumor growth and progression are closely related and cancer represents a ***hypercoagulable state***. Cancer cells can activate coagulation via several mechanisms including procoagulant and proaggregating activities, as well as proinflammatory processes (i.e. tumor necrosis factor alpha).[78,79] Changes in hemostasis that promote thrombosis among cancer patients include platelet activation, FX activation by TF, reduced hepatic synthesis of anticoagulants and reduced clearance of activated factors.[78] Furthermore, it has been demonstrated that cancer patients have increased markers of coagulation activation such as elevated plasma D-dimer levels.[80] TF is normally not expressed in healthy vascular cells, although tumor cells express high levels of TF.[78] Compared to non-cancer patients, cancer patients have significantly higher levels of TF and FVIIa, indicating activation of the extrinsic pathway of the coagulation cascade.[81] The intrinsic pathway, however, is not involved in cancer-related thrombosis to the same extent, as levels of factor XIIa are only marginally increased in cancer patients.[82] Cytotoxic drugs can also release procoagulants and cytokines from lysed tumor cells.[83]

Tumor invasion may result in ***vessel wall injury***. Cancer treatment including surgery, central venous catheters, chemotherapy and radiotherapy can all result in endothelial damage.[84] The endothelium can be directly damaged as a result of trauma from surgery or central venous catheter insertion. Chemotherapy and radiotherapy can also cause endothelial cell injury.[83,85]

Venous stasis may occur in cancer patients as a result of direct compression of nearby blood vessels by bulky tumors.[86] Cancer patients are often immobilized as a result of surgery, treatment, or other complications of cancer, such as advancing stage, which can further lead to venous stasis.[86]

1.3 Risk factors

A risk factor is defined as any attribute, characteristic, or exposure of an individual that increases the likelihood of developing a disease or injury. The impact of a risk factor depends on its prevalence and its associated relative risk.[87] Venous thromboembolism is a multicausal disease, meaning that several factors must be present for an event to occur.[87] Several inherited and acquired risk factors for VTE have previously been described. The complex interplay between risk factors for VTE may be explained by the thrombosis potential model (Figure 3).[87] In this model, the thrombosis risk depends on an accumulation of risk factors that adds to an individual's thrombosis potential, resulting in a VTE only when the joint effects of these risk factors outweighs natural anticoagulant properties and exceeds the thrombosis threshold. In order to prevent VTE, and therefore, improve survival, patient suffering, and reduce healthcare costs, we must identify those persons who are at high risk of developing a VTE event.

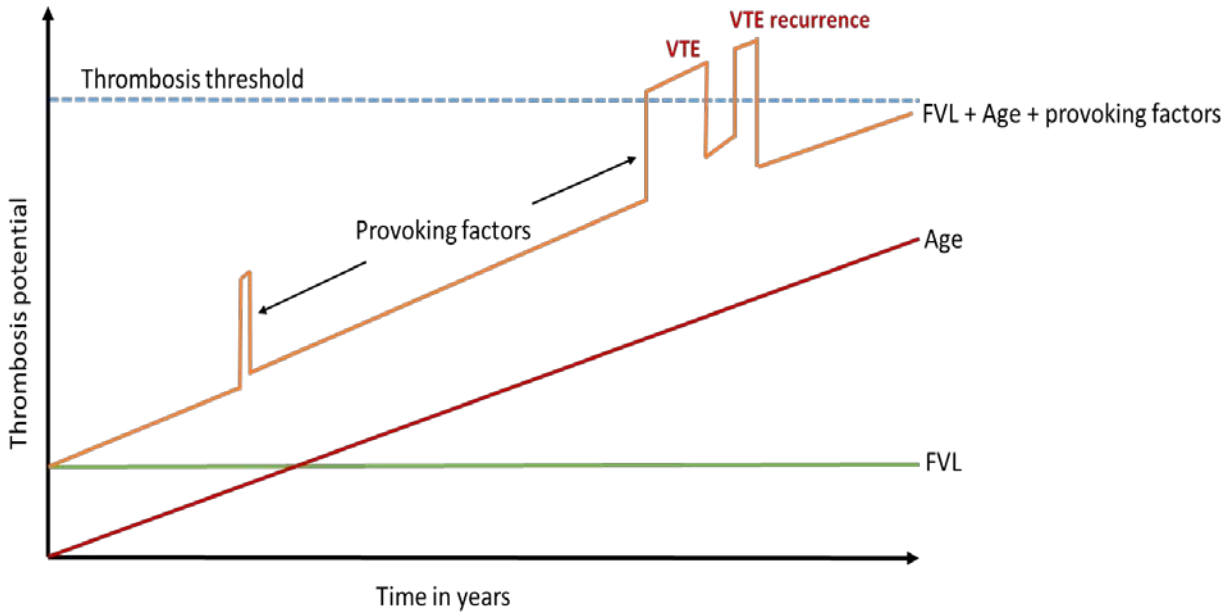


Figure 3. The thrombosis potential model. The green line represents intrinsic factors that are stable over time such as Factor V Leiden (FVL), and the red line represents the effect of a risk factor that increases over time, like age. The orange line demonstrates the joint effects of FVL and age in combination with provoking factors. Provoking factors early in life may not be enough to reach the thrombosis threshold. However, a provoking factor later in life may exceed the thrombosis threshold and results in a VTE. If the thrombosis potential remains increased following a VTE event, a provoking factor may exceed the thrombosis threshold again and result in a recurrent VTE.

1.3.1. Risk factors for venous thromboembolism

VTE is strongly heritable. Family and twin studies propose that genetic factors account for approximately 60% of the VTE risk, [88] and a family history of VTE is associated with a 2 to 3-fold higher risk of VTE.[89,90] ***Inherited thrombophilias*** are generally caused by two main mechanisms: gain-of-function mutations and loss-of-function mutations.[91] Susceptibility genes associated with VTE can be visualized in Table 1. In ***gain-of-function*** mutations, there is gain of function of procoagulant factors. Gain of function mutations can result in the increased synthesis of a normal protein (i.e. prothrombin G20210A), impaired breakdown or down-regulation of a normal protein (i.e. Factor V Leiden), or rarely, can result in the synthesis of a functionally hyperactive protein (i.e. factor IX Padua). Factor V Leiden (FVL) is a missense (arginine to glutamine) single nucleotide polymorphism (SNP).[91] FVL heterozygosity is present in approximately 5 to 8% of white Americans and Europeans.[92,93] FVL is thought to be prothrombotic by its resistance to activated protein C (APC) and also by the abnormal breakdown of FVIII by APC.[94] Heterozygous carriers have a 2 to 5-fold higher risk of VTE, while the risk is 10- to 80-fold higher in homozygous carriers.[92] The prothrombin (PT) 20210A mutation on the prothrombin (F2) gene is characterized by high plasma levels of prothrombin.[95] Similar to FVL, PT20210A is also prothrombotic by its APC resistant properties.[96] The variant is present in about 1 to 2% of the population and is associated with a 1.5 to 3.0-fold higher risk of VTE.[97,98] A non-O blood type is present in 60 to 70% of the population and it is associated with a 1.5 to 2.0-fold higher risk of VTE.[97,99] The association between VTE and non-O blood groups is thought to be mediated in part by higher levels of vWF and FVIII, however, the association of non-O blood type and VTE remains even after adjustment for both factors.[100]

In ***loss-of-function*** mutations, there is loss of function of an endogenous anticoagulant. Loss-of-function mutations are generally rarer than gain-of-function mutations, and tend to be associated with higher risk estimates for VTE. Inherited thrombophilias that results from loss-of-function mutations include deficiencies in antithrombin, protein C and protein S.[100] Antithrombin deficiency can

be a result of several gene variations.[101] Antithrombin is a strong inhibitor of the coagulation cascade. Antithrombin deficiencies are associated with a 10 to 50-fold increased risk of VTE.[98] They are, however, rare and the prevalence ranges from 5 to 17 per 10 000 individuals in the general population.[98,102] Deficiencies of protein C and protein S are also rare, occurring in less than 1% of the general population and are associated with an approximately 10-fold increased risk of VTE.[100]

Table 1. Known susceptibility genes for VTE[100]

Gene	Site	Phenotype	Frequency	VTE OR
Genes associated with VTE identified before GWAS				
F2	rs1799963	VTE	0.02	2.5
F5	rs6025	VTE	0.05	3
FGG	rs2066865	VTE	0.25	1.47
ABO	rs8176719	VTE	0.3	1.5
PROC	multiple	VTE	rare	~10
PROS1	multiple	VTE	rare	~10
SERPINC1	multiple	VTE	rare	~10
HIVEP1	rs169713	VTE	0.21	1.2
Novel SNPs associated with VTE identified by GWAS				
VWF	rs1063856	Increase vWF	0.37	1.15
TC2N	rs1884841	Increase vWF	0.44	1.27
STXBP5	rs1039084	Increase vWF	0.46	1.11
GP6	rs1613662	Increased platelet function	0.82	1.15
F11	rs2289252	Increased FXI	0.41	1.35
F11	rs2036914	Increased FXI	0.52	1.35
C4BPB/C4BPA	rs3813948	Increased C4BP	0.08	1.18
KNG1	rs710446	Increased aPTT	0.45	1.2
SERPINC1	rs2227589	Decreased antithrombin	0.1	1.29

In the last decades, major advances have been made in understanding the role of genetic factors in the risk of VTE. Several novel SNPs associated with VTE have been identified through genome-wide association studies (GWAS). VTE-associated SNPs identified via GWAS approaches can be visualized in Table 1. GWAS consists of testing the association of a huge number of SNPs with a phenotype in studies with large sample sizes where participants are classified first by their clinical manifestation(s) and not by their genotype.[103] VTE-associated SNPs have been predominately located in or near genes encoding for proteins in the coagulation or fibrinolytic pathways and have the

potential to alter the function and plasma levels of proteins.[100] Bezemer and colleagues were the first to conduct a large-scale association genetic study on VTE.[104] Their study genotyped nearly 20 000 SNPs in known prothrombotic genes and identified two new susceptibility loci in GP6 and F11. The majority of these SNPs identified through GWAS have only a modest effect (odds ratios (ORs) ranging from 1.10 to 1.35) on the VTE risk and alone may have limited clinical utility. However, combinations of these SNPs may improve the predictive ability of risk prediction models for VTE.[105] A VTE risk prediction model incorporating established VTE-related SNPs was proposed by de Haan and colleagues.[106] They reported that a 31-SNP and 5-SNP risk score composed of the five most strongly associated SNPs performed similarly. Combining non-genetic and genetic risk scores then further improved the model. However, the authors conclude that in order for these genetic risk scores to become useful in the clinical setting, high risk persons need to be identified in whom genetic profiling will be cost effective.[106]

Classic ***acquired risk factors*** for VTE include advancing age, cancer, surgery, prolonged immobility, trauma, pregnancy, puerperium, and the use of oral contraceptives.[107,108] ***Advancing age*** is a well-established risk factor for VTE, and several studies have demonstrated that the risk of VTE increases exponentially with age.[3,22] The incidence of VTE increases from 0.6 to 0.7 per 100 000 per year in children to about 1 per 100 in the elderly.[23,109] Accordingly, the risk of VTE is 50 to 80-fold higher in the older population.[23] The increased risk of VTE by age may be attributed to age-related degeneration of the vein walls or venous valves.[77] Furthermore, increased levels of procoagulants (i.e. fibrinogen, FV, FVII, FVIII, FIX, vWF) and inhibitors of fibrinolysis (i.e. PAI-1, TAFI) are observed with increasing age.[110]

Obesity is another important risk factor for VTE. According to 2014 estimates from the World Health Organization (WHO), worldwide obesity rates have more than doubled since 1980.[111] A meta-analysis reported that obesity, defined as a body mass index (BMI) ≥30 kg/m^2, is associated with a 2.3-fold higher risk of VTE, and risk increases with increasing BMI.[112] Previous work using the Tromsø Study

data reported that increasing BMI, waist and hip circumference, as well as weight gain were associated with an increased VTE risk.[113,114] Obesity-driven chronic inflammation and impaired fibrinolysis may be major effector mechanisms on the association between obesity and VTE.[115] Furthermore, excess body fat may physically result in impaired venous return.[116]

Hospitalization is potent risk factor for VTE. One study found that the age- and sex-adjusted incidence of VTE was 960 (95% CI 795-1125) per 10 000 person-years for hospitalized patients, while it was only 7.1 (95% CI 6.5-7.6) per 10 000 person-years among community residents.[117] Furthermore, Heit and colleagues reported that 61% (95% CI 57-66%) of all confirmed cases of VTE could be attributed to institutionalization (i.e. hospital, nursing home) within the preceding three months.[118] In this study, the number of VTE cases among medical and surgical patients was roughly equal (22 vs 24%, respectively), however, there are far more medical than surgical admissions. Several VTE risk factors can be present during hospitalization, such as surgery, immobilization and acute medical conditions. Recent major ***surgery*** has long been considered a strong risk factor for VTE.[50] Surgical procedures associated with a high VTE risk include neurosurgery, major orthopedic surgeries of the leg, cancer surgeries of the thorax, abdomen or pelvis, and renal transplantation.[119] Major surgery is associated with a 4 to 22-fold increased risk of VTE.[50,120] In the LETS study, non-surgical hospital admissions within the previous year were associated with an 11-fold (OR 11.1, 95% CI 4.7-25.9) increased risk of VTE.[121] ***Immobilization*** is an important risk factor for VTE and immobility was recorded in up to 25% of patients with a hospital-related VTE.[122] Immobilization, defined as confinement to bed or chair, was found to be associated with a 6-fold (95% CI 2.3-13.7) increased risk of DVT.[123] Several ***acute medical conditions*** such as myocardial infarction, stroke, infections, congestive heart failure, and respiratory disease are recognized as risk factors for VTE.[19,124-126] Hospitalization for an acute medical condition is associated with an 8-fold increased risk of VTE.[127] Thromboprophylaxis guidelines are in place for hospitalized patients, however, studies have shown that these guidelines are often underutilized.[19,128]

1.3.2 Patient-related risk factors for venous thromboembolism in cancer

Several risk factors for VTE have been identified among cancer patients, and its risk stratification can be broadly done in patient-, cancer-, and treatment-related factors. Several acquired risk factors for VTE in the general population, as described above, are also risk factors for VTE in cancer patients. These include advancing age, obesity, immobility, comorbid conditions and inherited thrombophilias.

Increasing age is a strong risk factor for VTE and cancer is more prevalent with advancing age, thus, it would be reasonable to assume that cancer contributes to the increased risk of VTE in the elderly. However, studies investigating the impact of age on the risk of cancer-related VTE have been inconclusive. A study using the California Cancer Registry (CCR) did not find an association between increasing age and overall cancer-related VTE, however, advancing age had a modest effect on VTE in patients with breast and ovarian cancer and non-Hodgkin's lymphoma.[52] Khorana and colleagues did not find an association between age and cancer-related VTE, and age is not included in the Khorana risk prediction model which assesses the risk of VTE in ambulatory cancer patients.[65] A Danish population-based cohort study, however, found that the incidence rates of cancer-related VTE increased with increasing age.[51] Furthermore, an Italian prospective study on the risk of VTE following cancer surgery, found that age over 60 years was associated with a 2.6-fold higher risk of VTE when compared to those under the age of 60.[129] In the Tromsø Study, the relative risk for VTE by cancer was higher in younger subjects than in those over 70 years.[130] However, the difference between the age-specific population attributable risks (PAR) for cancer-related VTE was minimal between the young (<50 years, PAR 14%) and the elderly (>70 years, PAR 18%) subjects. These findings indicate that cancer may not explain a substantial proportion of the VTE events in the elderly.

The effect of anthropometric measures on the risk of cancer-related VTE has not been widely studied. ***Obesity***, defined as a BMI ≥35 kg/m^2, is one of the five predictive variables used in the Khorana risk model.[65] In the above study, obesity was associated with an OR of 2.1 in multivariable analysis.[65]

Studies investigating the risk of VTE in patients with ovarian cancer had similar findings,[131,132] while other studies in prostate and ovarian cancer did not find an association between obesity and VTE.[133,134] Furthermore, in the European population included in the CATS cohort, a BMI ≥35 kg/m^2 was an infrequent observation and was not found to be associated with VTE among cancer patients.[135]

Although ***immobilization*** is a strong risk factor for VTE in the general population, it has not been directly studied in cancer patients. In the Tromsø Study, immobilization defined as bedrest over three days, wheelchair use and long-haul travel over four hours in the last 14 days, was the most frequent provoking factor for VTE in both non-cancer and cancer subjects, present in 19% and 23% of subjects, respectively.[130] In a prospective observational study, bedrest lasting longer than three days was associated with a 4.5-fold (95% CI 2.45-7.78) increased risk of cancer-related VTE in surgical patients.[129] Currently, primary thromboprophylaxis for prevention of VTE is only recommended in high risk patients, which is often based on primary cancer site and pre-treatment biomarker levels.[65] Society guidelines, however, suggest that cancer patients with additional risk factors, such as immobilization, should also be considered candidates for thromboprophylaxis.[19,136,137]

Several ***comorbid conditions*** have been found to be associated with an increased risk of cancer-related VTE, especially if several are present simultaneously. Comorbid conditions that have been found to be associated with cancer-related VTE in hospitalized patients include arterial thromboembolism (OR 1.45, 95% CI 1.39-1.52), pulmonary disease (OR 1.37, 95% CI 1.34-1.40), and renal disease (1.53, 95% CI 1.49-1.58), as well as infections (OR 1.77, 95% CI 1.73-1.81) and anemia (OR 1.35, 95% CI 1.32-1.39).[56] The number of medical comorbidities has also been shown to be an independent risk factor for cancer-related VTE in several studies.[138-141] Using the CCR study in patients with colorectal cancer, the presence of three of more comorbid conditions was associated with a 2-fold (95% CI 1.7-2.3) increased risk of VTE.[139]

In recent years, major advances have been made in the understanding of genetic predispositions in VTE, however the effect of ***inherited thrombophilias*** on the risk of cancer-related

VTE has not been widely studied. The majority of genetic studies in VTE have excluded cancer patients. The limited number of studies on established genetic risk factors (i.e. FVL, PT 20210A) that have been performed on VTE in cancer have had inconsistent results, likely because of the small patient populations in the majority of these studies.[142-146] In a case-control study using data from the Multiple Environmental and Genetic Assessment (MEGA) Study, FVL carriers with cancer were at a 12-fold (95% CI 1.6-88.1) higher risk of VTE when compared to cancer-free non-carriers.[142] Using the CATS cohort, Pabinger and colleagues reported a 2-fold (HR 2.0, 95% CI 1.0-4.0) higher risk of VTE among cancer patients with the FVL mutation compared to cancer patients without.[147] Small studies have investigated the effect of the PT 20210A mutation on the risk of cancer-related VTE, however, the results have been conflicting, most likely because of the lack of power due to the rarity of this mutation.[142,143,148] Furthermore, small studies have reported an association between the non-O blood group (ABO rs8176719) mutation and cancer-related VTE at specific cancer sites (i.e. pancreatic, glioma).[149-151]

1.3.3 Cancer-related risk factors for venous thromboembolism in cancer

Several cancer-related risk factors have been identified including cancer type, stage, tumor grade, histological type, and time since cancer diagnosis. Although the incidence of VTE by ***cancer type*** varies in different studies, pancreatic, brain, lung, and ovarian cancers are consistently reported as the highest risk cancer types.[51,55,152,153] Lymphomas, myeloma, kidney, and gastrointestinal cancers are associated with moderate risks for VTE, while breast and prostate cancers are associated with a relatively low risk.[51,52,56] The ***histological subtype*** of a cancer can influence the VTE risk.[140,154] For instance, squamous cell lung cancer is associated with a lower incidence of VTE than adenocarcinoma of the lung, at 4.8 vs. 9.9 per 100 person years in the first half year following a cancer diagnosis.[154]

Cancer stage is strongly correlated with the risk of VTE.[51,142,152] In a study using the CCR data, metastatic disease at cancer diagnosis was the strongest predictor of VTE.[52] The risk of VTE increased

across progressive cancer stages (local, region and distant) at all 12 cancer sites. Another study reported the relative risk of VTE for stage I, II, III and IV cancers to be 2.9, 2.9, 7.5 and 17.1, respectively.[51] Furthermore, in the MEGA study distant metastasis was associated with a 19.8-fold (95% CI 2.6-149.1) higher risk of VTE when compared to cancer patients without distant spread.[142] Some studies have, however, reported that the risk of VTE correlates with the rate of growth and spread of a cancer rather than the extent of spread (stage).[139,140] Patients with cancers that rapidly progress from local-stage disease to widespread metastatic disease are more likely to develop a VTE, whereas patients with more slow-growing cancers, like breast and prostate cancer, have a significantly lower risk of VTE.[52,155] In a CCR study of 13 031 women with ovarian cancer, 15% of the women with metastatic cancer at diagnosis died within three months and 15% died in the four to 12 months following the cancer diagnosis. The incidence of VTE was 27% and 10.7%, respectively.[140] Thus, this study suggests that fast growing cancers, evidenced by early mortality, are associated with a higher risk of VTE.

A relationship between VTE and ***time since cancer diagnosis*** has been observed and the risk of VTE in cancer is not constant over the course of disease. Blom and colleagues found that the risk of VTE was greatest in the first three months following a cancer diagnosis.[142] When compared to cancer-free subjects, the risk in the first three months was 54-fold (95% 8.6-334.3) higher, and fell to 14.3-fold (95% CI 5.8-35.2) in four to 12 months after a cancer diagnosis. The risk continued to decline over time, although a 2-fold (95% CI 0.9-5.8) increased risk still remained in the 10 to 15 years following a cancer diagnosis. The high VTE risk in the first three months following a cancer diagnosis is thought to be related to cancer treatment (i.e. surgery, chemotherapy, central venous catheters) which is initiated at this time, and complications (i.e. infections, transfusions, immobilization) that often accompany cancer treatment, as well as tumor burden itself.

1.3.4 Treatment-related risk factors for venous thromboembolism in cancer

Cancer treatment modalities are thought to substantially affect the incidence of VTE. ***Surgery*** is a well-established risk factor for VTE both in cancer and cancer-free subjects. In a cohort of patients undergoing major surgery, cancer patients had a 2 to 4-fold higher incidence of VTE in the post-operative period when compared to patients without cancer.[119] Another study reported that cancer patients undergoing surgery of the abdomen and pelvis were at the highest risk of VTE.[156] Blom and colleagues, however, found no association between surgery and VTE in cancer patients (adjusted RR 1.0, 95% CI 0.8-1.2).[157] Studies on patients with breast, colon and ovarian cancer reported a protective effect of major surgery on the risk of VTE, even after multivariable adjustment for age, sex, race, cancer stage and concomitant comorbid conditions.[138-140] This may be because patients that are suitable for surgery have a better performance status, and/or may have non-advanced, and therefore operable cancer. Furthermore, surgical removal of the tumor may decrease the cancer burden and, thus, reduce the VTE risk.

Chemotherapy is a well-established risk factor for VTE. The annual incidence of VTE in patients receiving chemotherapy ranges from 11 to 29%.[158] A nested case-control study of 625 cancer patients with VTE and 625 matched cancer patients without VTE demonstrated in a multivariable model that cancer (without chemotherapy) was associated with a 4.0-fold (OR 4.1, 95% CI 1.93-8.52) increased risk of VTE, while adding chemotherapy resulted in a 6.5-fold (OR 6.5, 95% CI 2.11-20.23) increased risk.[50] In a case-crossover study, Rogers and coworkers found that the adjusted incidence rate ratio (IRR) for having chemotherapy in the three months before a hospitalization for VTE was 6.0-fold (IRR 5.7, 95% CI 2.11-15.43) higher when compared to the 18 months leading up to the VTE.[159] The risk of VTE varies by the chemotherapeutic agent used. Immunomodularly chemotherapeutics such as thalidomide and lenalidomide, are associated with an especially high VTE risk, in particular when they are used in combination with high-dose dexamethasone in the treatment of multiple myeloma.[160] As the risk of VTE in patients with multiple myeloma is already high, thromboprophylaxis is recommended

to myeloma patients on this treatment.[160] In a randomized controlled trial on 704 node-positive primary operable breast cancer patients, the cumulative incidence of VTE was 2.6% in the tamoxifen only treatment group, while it was 13.6% in tamoxifen combined with additional chemotherapy group.[161] The use of bevacizumab was also reported to be associated with a relative risk of 1.3 (95% CI 1.1-1.6) in a meta-analysis of 15 trials with patients with solid tumors.[162]

The impact of ***radiotherapy*** on the risk of cancer-related VTE has not be as extensively studied. Using the CATS cohort, a study of 821 cancer patients found that 47.3% had received radiotherapy, and the risk of VTE associated with radiotherapy was associated with a 2.3-fold (95% CI 1.2-4.4) increased risk of VTE.[163]

Complications of cancer and its treatment, such as central venous catheters, acute infections and blood transfusions are also associated with an increased risk of VTE. ***Central venous catheters*** (CVCs) are often used in cancer patients for the administration of chemotherapy, medications, hydration, and blood products. CVCs are traditionally associated with an increased incidence of upper extremity DVT. The incidence rates of catheter-related VTE among cancer patients ranges from 0.3% to 28.3%.[164] A retrospective cohort study of 400 cancer patients with newly implanted ports found that 8.5% (95% CI 6.0-11.7%) developed a symptomatic VTE.[165] Of the 34 VTE events, 16 were DVTs, 16 were PEs and 2 were both. Anemia occurs frequently in cancer, and patients are often treated with ***blood transfusions*** and erythropoiesis-stimulating agents. Khorana and colleagues found that of 70 542 cancer patients, 15% received at least one red blood cell (RBC) transfusion and 3% at least one platelet transfusion.[166] In multivariable analysis, RBC transfusions (OR 1.6, 95% CI 1.53-1.67) and platelet transfusions (OR 1.2, 95% CI 1.11-1.29) were independently associated with VTE. In a meta-analysis, ***erythropoiesis-stimulating agents*** were found to be associated with a 1.67-fold (95% CL 1.35-2.06) increased risk of thromboembolic events in 35 trials of 6769 patients.[167]

1.3.5 Biomarkers of venous thromboembolism in cancer

A biomarker, short for a biological marker, is a naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process or disease can be identified. The Food and Drug Administration (FDA) defines a biomarker as "*a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".*[168] The measurement of biomarkers has several applications, including evaluating the risk of a disease, in the diagnostic workup of a disease, measuring the progress of disease, and evaluating the most effective treatment of a disease. An ideal biomarker has a high sensitivity and specificity, is safe and easy to measure, and is consistent across sex and ethnic groups. In the last decade, knowledge on cancer-related biomarkers has increased and is utilized in VTE risk prediction models in cancer patients.

Blood count parameters are of interest as biomarkers in cancer as they are measured at regular intervals in cancer patients. The Awareness of Neutropenia in Chemotherapy (ANC) study group reported an association between pre-chemotherapy platelet and leukocyte counts and hemoglobin levels on the risk of cancer-related VTE.[65,169] They found that ***leukocytosis*** (>11 x 10^9/L) was associated with a 2.2-fold (95% CI 1.2-4.0) increased risk, ***thrombocytosis*** (>350 x 10^9/L) with a 1.8-fold (95% CI 1.1-3.2) increased risk and low ***hemoglobin*** levels (<100 g/L) with a 2.4-fold (95% CI 1.4-4.2) increased risk of chemotherapy-associated VTE. These blood parameters are used as part of the Khorana risk prediction model to identify high risk cancer patients suitable for thromboprophylaxis. Furthermore, in the Tromsø Study pre-cancer diagnosis platelet and leukocyte counts were associated with VTE in cancer patients, but not in cancer-free subjects.[170,171] These studies found that leukocyte counts above the 80th percentile (≥8.6 x 10^9 cells/L) were associated with a 2.4-fold (95% CI 1.44-3.87) increased risk of VTE in cancer.[170] Pre-cancer platelet counts above the 80th percentile (≥295 x 10^9/L) were associated with a 2.0-fold (HR 1.98, 95% CI 1.21-3.23) higher risk of VTE when compared to platelet counts below the 40th percentile (<235 x 10^9/L).[171]

Various markers of platelet and coagulation activation have also been described as biomarkers for cancer-related VTE. ***P-selectin*** mediates adhesion and migration of leukocytes on activated endothelial cells, mediates platelet-leukocyte interactions and supports fibrin.[172] P-selectin levels can reflect a prothrombotic state.[173] In the CATS cohort, elevated levels of soluble P-selectin were independently associated with cancer-related VTE (HR 2.6, 95% CI 1.4-4.9), even after adjustment for age, sex, surgery, chemotherapy, and radiotherapy.[174] In this study, the cumulative probability of VTE at six months was 11.9% in patients with soluble P-selectin levels above the 75th percentile and 3.7% in those below (P = .002). However, P-selectin assays are not routinely performed in cancer patients which may limit its practical use.

D-dimer is a degradation product of cross-linked fibrin and is a global indicator of coagulation activation and fibrinolysis. D-dimer is a sensitive, although non-specific marker for VTE that is often measured in the diagnostic workup of VTE.[175] D-dimer levels are also increased in malignancy.[176] An association between D-dimer and cancer-related VTE has been demonstrated in several studies.[177-179] Again, in the CATS cohort, D-dimer levels over the 75th percentile of the entire study population was associated with a 2.3-fold (95% CI 1.4-4.0) increased risk of VTE.[180] Ay and colleagues incorporated soluble P-selectin and D-dimer into the Khorana risk prediction score to identify high risk cancer patients that require thromboprophylaxis.[66] Incorporation of these two additional parameters to the risk prediction model improved its accuracy to identify high risk cancer patients.

Prothrombin fragment 1+2 (F1+2) is released when prothrombin is cleaved to thrombin by activated FX. Increased levels (>75th percentile) of prothrombin F1+2 have been found to be associated with a 2.0-fold (95% CI 1.2-3.6) higher risk of cancer-associated VTE.[163] The risk was even further increased when both prothrombin F1+2 and D-dimer levels were elevated (HR 3.6, 95% CI 1.4-9.5). A joint effect on the risk of VTE is observed when both of these biomarkers are elevated, likely because both are associated with a prothrombotic state.

In addition, tissue factor-bearing microparticles (TF+ MPs), factor VIII and C-reactive protein have been found to be potential suitable biomarkers for cancer-related VTE.[181-184]

1.3.6 Risk factors for recurrent venous thromboembolism

VTE is a chronic disease that frequently recurs**.** The cumulative incidence of VTE recurrence is 5.2% at 30 days, 12.9% at one year and 22.8% at five years.[40] In the Tromsø Study, the one-year cumulative incidence rate was 7.3% (95% CI 5.4-9.7) overall, 7.4% (95% CI 4.6-11.8) in provoked VTE and 16.3% (95% CI 9.9-25.9) in cancer-related VTE.[35] Patients with a first symptomatic DVT have a higher risk of recurrent VTE than patients whose first event is a PE.[35,185,186]

Independent predictors for VTE recurrence include advancing age, male sex, obesity, and active cancer.[26,40,60,186,187] A cohort study of patients in Olmsted County found that in multivariable adjusted analysis, the risk of VTE recurrence was increased by 17% per increase in decade of age, 24% per 10 point increase of body mass index and 29% in men than in women.[40] In another study also using data from Olmsted County from 1988 to 2000, hospitalization, pregnancy, central venous catheters, active cancer, and respiratory infections were associated with an increased risk of VTE recurrence, after adjusting for interim exposures and treatment.[188] Several risk factors for incident VTE, however, have not been found to be associated with VTE recurrence or have been found to be associated with a decreased risk of VTE recurrence. Recent surgery, fractures and trauma, although risk factors for incident VTE, are not associated with recurrent VTE.[40,189] Pregnancy/puerperium, oral contraceptive use, and hormone replacement therapy have been found to be associated with a reduced risk of VTE recurrence.[50,190]

Although the risk of an incident VTE is increased in the presence of FVL and prothrombin (PT) 20210A mutations, their role in recurrent VTE is disputed. The Leiden Thrombophilia Study found no association between thrombophilias (i.e. FVL, PT 20210A) and recurrent VTE,[191] while other studies

found a significant association.[41,192] Several SNPs were tested as predictors for recurrent VTE in the MEGA Study, and a 31-SNP and 5-SNP genetic risk score was useful in prediction of recurrent VTE.[193] Using the 5-SNP risk score (F5 rs6025, F2 rs1799963, ABO rs8176719, FGG rs2066865, and F11 rs2036914), the six-year cumulative incidence of recurrent VTE was substantially higher in patients with ≥5 risk alleles (20.3%, 95% CI 16.5-24.1) versus those with ≤1 risk alleles (9.54%, 95% CI 6.7-12.1). However, currently, the presence of thrombophilias is not a major determinant regarding the optimal type or duration of anticoagulation.[19] Instead, these genetic markers are often used in conjunction with other risk factors for risk stratification for recurrent VTE.

A 2014 Cochrane review evaluated VTE recurrence in 11 studies including 3716 participants.[194] They reported a consistent risk reduction (RR 0.20, 95% CL 0.11-0.38) in recurrent VTE during prolonged treatment with vitamin K antagonists (VKAs), independent of the period elapsed since initial VTE. During the entire study period, a substantial increase in bleeding was observed in patients receiving prolonged anticoagulation (RR 2.60, 95% CI 1.51-4.49), while no reduction in mortality was seen (RR 0.89, 9 % CI 0.66-1.21). Over time, the absolute VTE recurrence risk decreases, while the major bleeding risk remains. Therefore, the benefit-harm ratio of VKAs declines progressively from the incident VTE event. In a systematic review, no differences in VTE recurrence rates were observed between VKAs and direct oral anticoagulants (DOACs).[195]

1.3.7 Risk factors for recurrent venous thromboembolism in cancer

Active cancer is associated with a 2 to 9-fold higher risk of VTE recurrence. [26,59,186] Prandoni and co-workers found a one-year cumulative incidence of 20.7% in cancer patients receiving conventional anticoagulants versus 6.8% in cancer-free patients on anticoagulants.[59] In the Tromsø Study, the cumulative incidence of cancer-related recurrent VTE is 2.7% (95% CI 1.0-7.0) at 30 days, 8.2% (95% CI 4.3-15.7) at six months, 16.3% (95% CI 9.9-25.9) at one year and 22.0 (95% CI 16.2-41.0) at two years.[35] Similar to non-cancer patients, the risk of recurrence is greater after a DVT as the first

VTE event. A study using the RIETE Registry data, reported a 2.0-fold (OR, 1.9, 95% CI 1.2-3.2) increased risk recurrence for PE and 2.4-fold (95% CI 2.0-3.2) for DVT.[196]

The risk of recurrent VTE is not homogeneous among patients with cancer-associated VTE. Predictors of VTE recurrence among patients with cancer are uncertain, [197] although female sex, younger age, number of previous VTEs, cancer type and stage, and treatment modality have been associated with VTE recurrence in previous studies.[197,198] The risk of recurrence has been reported to be present across all cancers but is highest in brain, pancreatic, lung, gastrointestinal, ovarian, and hematological cancers.[188]

Current guidelines recommend long-term treatment with LMWH to cancer patients with a VTE as long as there is evidence of ongoing cancer.[19] The Ottawa prognostic score is a clinical risk prediction tool developed to differentiate between cancer patients at low and high risk of recurrent VTE.[199] The model includes four independent predictors: sex (+ 1 point for female sex), primary tumor site (+1 for lung cancer, -1 for breast cancer), cancer stage (-2 for TNM stage 1) and history of prior VTE (+1 for previous VTE) and patients score on a scale from -3 to 3 points. They found that patients with a score of 0 or less had a low risk (≤4.5%) of VTE recurrence, while patients with a score >1 were at a high risk (19%). However, thus far, validation studies involving the Ottawa prognostic score have had mixed results.[200-202]

1.4 Risk of cancer after venous thromboembolism

1.4.1 VTE as a first sign of cancer

VTE can be the first manifestation of an underlying cancer and several studies have confirmed that the risk of cancer is increased after a VTE.[6,61,203,204] A large, Scottish population-based registry study of almost 60 000 participants with a VTE reported the excess risk (via standardized incidence ratios, SIRs) of cancer in relation to time since VTE diagnosis.[205] The overall SIR for developing cancer following a VTE during the 19-year follow-up was 1.28 (95% CI 1.25-1.33), compared to what was expected based on the incidence of first cancers in the country. There was an especially high excess risk of cancer (SIR 4.2, 95% CI 3.9-4.5) during the 1 to 6 months after a VTE event. The risk was increased across all cancer types, but it was highest for ovarian cancer and Hodgkin's and non-Hodgkin's lymphomas. A Swedish study also reported similar findings in terms for excess risk (SIR 3.2, 95% CI 3.1-3.4) and for cancer sites associated with a higher risk (liver, pancreatic, ovarian, brain, and Hodgkin's lymphoma).[6] A systematic review reported that among patients with an incident VTE event, 4.1% (95% CI 3.6-4.6%) were diagnosed with cancer within 30 days of the VTE, and 6.3% (95% CI 5.6-6.9%) within one year.[206]

The risk of cancer following an incident VTE event does not differ according to the anatomical location of the VTE. Using the Scandinavian Thrombosis and Cancer (STAC) cohort the one-year risk of cancer was essentially similar for an incident event of DVT (HR 4.12, 95% CI 3.12-5.43) and PE (HR 3.97, 95% CI 2.80-5.61).[207] The risk estimates also remained equal after the first year. A large, French, multicenter, prospective observational study reported an overall incidence of cancer of 1.4% (95% CI 0.9-2.1) for distal DVT and 1.5% (95% CI 0.8-2.4) for proximal DVT.[208] Furthermore, a Danish study found that patients with superficial vein thrombosis (SVT) also have an increased risk of cancer.[209] The SIRs for SVT were comparable to those for DVT and PE (2.46, 2.75, and 3.27, respectively).

Patients with an unprovoked VTE are thought to be at a higher risk of developing cancer than those with a provoked VTE.[206,210] In a systematic review, Carrier and coworkers reported a one-year incidence of cancer following VTE at 10% (95% CI 8.6-11.3%) for unprovoked VTE and a 2.6% (95% CI

1.6-3.6%) for provoked VTE.[206] The definitions of provoked VTE were provided from the original studies and varied slightly across studies. In the STAC cohort, the risk of cancer did not vary as greatly in provoked and unprovoked VTE events.[207] In the first year following a VTE event, the risk of cancer was 4.47-fold (95% CI 3.43-5.83) increased in unprovoked VTE and 3.52-fold (95% CI 2.39-5.17) increased in provoked events in a multivariable model using age as a time-scale adjusted for sex, height, BMI, alcohol units per week, smoking, self-reported diabetes, higher education, and hard physical activity. The difference was even smaller after the first year for unprovoked (HR 1.26, 95% CI 1.04-1.54) and provoked (HR 1.36, 95% CI 1.05-1.74) VTE events. These results suggest that VTE may be the first sign of an occult cancer regardless of whether it was provoked or not. This is in accordance with the multicausal nature of VTE (thrombosis potential hypothesis), where several risk factors must be present for an event to occur.

1.4.2 Screening for cancer in patients with venous thromboembolism

The risk of cancer is increased following a VTE, and several studies have investigated predictors of cancer in VTE patients. In a study using data from the RIETE Registry, several biomarkers were investigated as predictors for cancer within three months of a VTE event, including patient-related factors (i.e. age, body weight), risk factors for VTE (i.e. immobilization, surgery, prior VTE), VTE characteristics (i.e. DVT, PE), laboratory parameters (i.e. hemoglobin, platelet count, D-dimer), and treatment (i.e. LMWH, UFH).[13] Age (60 to 75 years), unprovoked VTE, bilateral thrombosis and anemia were independent predictors of cancer in VTE patients, while no significant association was found between platelet counts, D-dimer levels, surgery and anticoagulant treatment. Two small retrospective studies, however, did find an association between D-dimer levels at VTE and an increased risk of cancer.[211,212]

During the last years there has been an ongoing debate regarding screening for cancer in patients with incident VTE and if any, to which extent it should be done. Some studies have

demonstrated that limited screening (i.e. patient history, physical examination, routine blood work, chest x-ray) for cancer at VTE is adequate for the detection of most cancers, while other studies have suggested that additional extensive screening measures (i.e. ultrasound, CT, measurement of tumor biomarkers) are needed to better detect underlying cancers.[206,213-216] In a Canadian randomized controlled trial limited cancer screening (routine blood work, chest x-ray, and screening for breast, cervical, and prostate cancer) was compared to limited screening in combination with an abdominal and pelvic CT scan.[217] There was no significant difference between the number of cancers or the average time to diagnosis, nor in cancer-related mortality between the limited and extensive screening groups. A study from the Netherlands on 630 patients reported that the number of cancers and cancer-related deaths was similar among patients who had undergone limited screening and those who had undergone extensive screening. A French multicenter study compared fluorodeoxyglucose (FDG) PET/CT cancer screening to limited screening, and found that extensive screening was not significantly associated with higher rates of cancer diagnosis after unprovoked VTE.[218] Even though extensive screening may detect cancer earlier, there is no evidence of improved prognosis. Thus, current guidelines recommend limited screening for patients with unprovoked VTE and extensive evaluation only in those patients with suspicion of underlying cancer.

2. Aims of the thesis

The aims of this thesis were:

- To assess the joint effect of two single nucleotide polymorphisms, F5 rs6026 (Factor V Leiden) and F5 rs4524 and active cancer, on the risk of venous thromboembolism in a case-cohort study with subjects recruited from the general population.
- To assess the overall and time-specific risk of VTE in cancer patients recruited from three large Scandinavian population-based cohorts accounting for the differential mortality between cancers.
- To assess the risk of VTE recurrence and mortality in VTE patients with overt and occult cancer in a large population-based cohort study.
- To investigate the association between plasma D-dimer levels measured at incident VTE diagnosis and the risk of cancer within the subsequent two years in a cohort of VTE patients recruited from the general population.

3. Methods

3.1 Study populations

3.1.1 The Tromsø Study

The Tromsø Study is a single center, population-based cohort study with repeated health surveys of the inhabitants of the municipality of Tromsø. The first survey was carried out in 1974 with an emphasis on cardiovascular disease, however, the focus of the Tromsø Study has expanded over time and now includes a broad spectrum of diseases. Thus far, seven surveys have been conducted, in 1974, 1979 to 1980, 1986 to 1987, 1994 to 1995, 2000 to 2001, 2007 to 2008, and most recently the seventh survey in 2015 to 2016. Paper I uses the Tromsø Study 4 to 6 surveys, while Paper II uses the Scandinavian Thrombosis and Cancer (STAC) cohort, which includes the Tromsø 4 Study. Papers III and IV use information from the first six surveys of the Tromsø Study. Tromsø 1 was conducted in 1974, and men aged 20 to 49 were invited and 6595 participated. Tromsø 2 invited men and women between the ages of 20 and 54 and 16 621 subjects participated. Tromsø 3 was conducted from 1986 to 1987 and 21 826 people between the ages of 12 and 67 participated. The Tromsø 4 Study was conducted from 1994 to 1995 and a total of 27 158 subjects aged 25 to 97 years participated. Tromsø 5 was conducted from 2001 to 2002 and included 8130 subjects aged 30 to 89 years. Finally, Tromsø 6 was conducted from 2007 to 2008, and included 12 984 subjects aged 30 to 87 years. The participation rates for all surveys were high, with 83%, 74%, 75%, 77%, 79% and 66% of the invited population participating in Tromsø 1 to 6, respectively. Participants were followed up from January 1, 1994, when VTE registration started, until December 31, 2012 in Papers I and II, and from the date of their incident VTE event until December 31, 2012 in Papers III and IV.

3.1.2 The Scandinavian Thrombosis and Cancer Cohort

The Scandinavian Thrombosis and Cancer (STAC) cohort is a large, population-based cohort comprising data from three large Scandinavian cohorts, the Tromsø 4 Study, the second Nord-Trøndelag Health Study (HUNT 2) and the Danish Diet, Cancer and Health (DCH) study. The STAC cohort was used in Paper II. The Tromsø 4 survey has already been described in the above section. The HUNT 2 Study was carried out from 1995 to 1997 in the Nord-Trøndelag County in Norway. All residents of this county above the age of 20 were invited to take part in the survey, and 65 237, or 69% of the eligible population, participated. The DCH Study was conducted from 1993 to 1997, and inhabitants aged 50 to 64 years living in the urban areas of Copenhagen and Aarhus, without a previous cancer were invited to participate. In total, 57 054, or 35% of the eligible population, attended the study. Study participants were followed up from the day of inclusion in the individual cohorts (1993 to 1997) until the end of follow-up (2007 to 2012). VTE events were identified until December 31, 2012 in Tromsø 4, December 31, 2007 in HUNT 2 and April 30, 2008 in DCH. Cancer diagnoses were registered until December 31, 2012 in Tromsø 4 and DCH and until December 31, 2008 in HUNT 2. Participants with a pre-baseline diagnosis of cancer or VTE were excluded from all cohorts before merging. Ultimately, the STAC cohort consisted of 144 952 individuals aged 19 to 101 years, without a previous cancer diagnosis.

3.2 Baseline measurements

Baseline data at study inclusion for the Tromsø and STAC cohorts was collected by self-administered questionnaires, non-fasting blood samples and physical examination performed by trained personnel. Body weight and height were measured in subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated by the weight in kilograms (kg) divided by height in meters (m) squared (kg/m^2). Information regarding history of cardiovascular disease (myocardial infarction, angina or stroke), diabetes mellitus, smoking status (never/former/current) and level of physical activity was obtained by using self-reported questionnaires. Blood samples were collected from an antecubital vein and analyzed at the Department of Clinical Chemistry at the University Hospital of North Norway.

DNA was isolated from whole blood and stored at -70°C at the national CONOR biobank, located at the HUNT Biobank in Levanger, Norway. For the purpose of Paper I, two SNPs in the F5 gene (rs6025, rs4524) were genotyped. Genotyping was performed by using the Sequenom platform, which uses single-base extension followed by mass spectrometry to measure the molecular mass of the extended primers. Samples were genotyped using the Sequenom iPlex Gold Assay according to the recommended protocol, using an initial input of 10-20 ng DNA, and were analyzed using the MassARRAY Analyzer 4. Only genotypes with a high quality score of "A. Conservative" or "B. Moderate" were used. When multiple attempts were made to genotype an individual, one of the highest quality genotypes across all attempts was chosen for each SNP.

Papers III and IV included subjects with an incident VTE, and participants were followed up from the date of the incident VTE. Trained personnel reviewed the medical records for each VTE case and extracted information, which included information on clinical risk factors and laboratory markers using standardized forms. Information regarding comorbid conditions, clinical risk factors, and provoking factors in the eight weeks preceding the VTE event were extracted by review of medical records. D-dimer levels were measured as part of the diagnostic assessment of patients with suspected

VTE. The blood samples were analyzed at the Department of Clinical Chemistry at the University Hospital of North Norway, using the NycoCard D-Dimer (Nycomed Pharma, Oslo, Norway) assay from 1994 to 1998, and the STA®-Liatest® D-Di FM from Stago (Diagnostica Stago, Ansieres, France) from 1998 to 2012. In our study population, the majority (92%) of VTE events occurred during the period that the STA®-Liatest® D-Di FM assay was used. We performed a sensitivity analysis that was restricted only to these patients, and the risk estimates remained unchanged, which indicates that the two measurement methods are comparable.

3.3 Outcome measures

3.3.1 Identification and validation of venous thromboembolic events

Only first lifetime, symptomatic VTE events were included in the Tromsø Study and STAC cohorts. Each potential VTE case was reviewed and validated by trained personnel by assessment of each patient's medical records. VTE events were classified as a DVT or a PE, and if DVT and PE occurred simultaneously, it was recorded as a PE.

In the Tromsø Study, VTE events were recorded from January 1, 1994 until December 31, 2012. Three registries at the University Hospital of North Norway were used to identify VTE events during the follow-up: the hospital discharge diagnosis registry, the autopsy registry, and the radiological procedure registry. The University Hospital of North Norway is the sole hospital in the municipality, and it provides all hospital-based inpatient and outpatient medical care and relevant treatment in the region. Trained personnel reviewed the medical journals for each potential VTE case, and were blinded to the patient's baseline variables. Relevant International Classification of Diseases, revision 9 (ICD-9) codes for the period 1994 to 1998 were 325, 415.1, 452, 453, 671.3, 671.4, and 671.9, and ICD-10 codes for the period 1999 to 2012 were I26, I80, I81, I82, I67.6, O22.3, O22.5, O87.1, and O87.3.[23] For the subjects that were derived from either the hospital discharge diagnosis registry or the radiological procedure registry, the following four criteria were required for a VTE event to be recorded; (1) the presence of signs and symptoms accordant with either a DVT, PE, or both; (2) objective confirmation by a diagnostic procedure (i.e. compression ultrasound, ventilation-perfusion scan, CT scan, pulmonary angiography, or autopsy); (3) a diagnosis of a DVT or PE noted by a physician in the patient's medical records; and (4) initiation of therapy for the VTE (i.e. anticoagulant medication, thrombolysis, vascular surgery). For those subjects extracted from the autopsy registry, a VTE was only recorded when the autopsy report indicated VTE as the cause of death or as a significant affliction associated with the cause of death.

In the HUNT 2 Study, VTE events were recorded from January 1, 1995 until December 31, 2007. VTE events were identified and validated by trained personnel by searching the hospital discharge diagnosis registries and radiological procedure registries from one tertiary-care hospital (St Olav's University Hospital in Trondheim, Norway) and two local hospitals (Nord-Trøndelag Hospital Trust hospitals in Levanger and Namsos, Norway). Relevant discharge codes used to identify potential cases of VTE before the validation process were ICD-9 codes 415.x, 451.x, 452, 453.x, 325, 362.3, 433, 557.0, 634–638 (with decimals 6 and 7), 639.6, 639.8, 639.9, 671.x, 673.x, 674, and 997.2, and ICD-10 codes I26.x, I80.x, I81, I82.x, I 63.6, I67.6, K55, H34.8, O08.x, O22.x, O87.x, and O88.x.[22] A VTE event was only recorded if it was symptomatic, confirmed by an objective diagnostic test (i.e. ultrasound, venography, ventilation-perfusion scan or CT), and required treatment.

In the DCH Study, VTE events were recorded from December 1, 1993 until April 30, 2008. First lifetime VTE events were identified by linkage to the Danish National Patient Registry and the Danish National Death Registry by the use of participants' civil registration numbers. Based on the available hospital discharge information for each participant, those with a discharge diagnosis code for VTE were registered (ICD-8: 450.99, 451.00, 451.08, 451.09, 451.99 and ICD-10: I26, I80.1–I80.9).[219] Information was obtained regarding symptoms, laboratory blood testing, and diagnostics from the review of patient medical records. A VTE diagnosis was considered to be verified when typical clinical symptoms (i.e. unilateral leg swelling, leg pain and redness, dyspnea, chest pain) were combined with confirmatory diagnostic tests (i.e. ultrasound, venography, echocardiography, ventilation-perfusion scan, or CT scan).

3.3.2 Identification and validation of cancer

First lifetime cancer diagnoses during follow-up were identified by linkage to the Cancer Registry of Norway (CRN) (Papers I to IV) and the Danish Cancer Registry (Paper II) by the use of participants' unique national civil registration numbers, which are assigned to all people residing in the

Nordic countries. Cancer registration has been mandatory by law in both Norway and Denmark since 1952 and 1987, respectively. Both registries receive information from general practitioners, hospital doctors, pathological laboratories, and death certificates.[220,221] These cancer registries are also linked to the Norwegian National Cause of Death Registry and The Danish Register of Causes of Death in their respective countries, as well as the patient discharge diagnosis registries. Reminders are sent to physicians when cancer cases which have not been officially reported to the CRN are reported via another source (i.e. pathologist, death certificate). Evaluations of data quality have found both cancer registries to be valid and complete, with an estimated 98.8% completeness in Norwegian Cancer Registry and 95% to 98% completeness in the Danish Cancer Registry, and with 94% and 93% of diagnoses microscopically verified, respectively.[220,221] The registries provide information regarding date of cancer diagnosis, primary site of the disease (ICD10 codes C00-96), tumor histology (ICO-3), cancer stage (localized, regional, distant, or unknown stage), and initial planned treatment. Subjects with non-melanoma skin cancers (ICD 191.0–191.9) were classified as cancer-free.

3.3.3 Definition of active cancer

Proximity to cancer diagnosis is a strong predictor of VTE risk. Studies have found that nearly half of the cancer-related VTEs occur in the 2.5 year period starting at six months prior to a cancer diagnosis until two years following a cancer diagnosis.[55,222,223] This observation is in accordance with evidence suggesting that VTE risk is closely related to the rate of cancer growth, rather than the extent of cancer.[139]

In Paper I, we investigated the effect of prothrombotic SNPs on the risk of VTE in *active cancer*. For the purpose of our study, the active cancer period included an occult cancer period from six months before a cancer diagnosis and an overt cancer period which extended until two years following a cancer diagnosis. If a VTE event occurred during this two and a half year timeframe, then it was labeled as an active cancer-related VTE. Extending the cancer observational period increases the chance of dilution,

as VTE events that may not necessarily be caused by cancer may be included. Following the active cancer period, subjects with cancer were classified as having a previous cancer, since the risk of a VTE remains minimally increased for several years after the active cancer period.

In Paper III, we investigated the risk of VTE recurrence in subjects who experience an incident VTE during occult and overt cancer, compared to those who were cancer-free at first VTE. In this study, active cancer was defined as the time ranging from one year before a cancer diagnosis until two years after. An incident VTE event was labeled as being related to overt cancer if it occurred within two years following a cancer diagnosis. Accordingly, an occult cancer-related VTE was defined as a VTE occurring within one year before a cancer diagnosis. The definition of occult cancer as part of active cancer in Paper I differed from the definition of occult cancer in Paper III as the presence of an occult cancer in Paper III was obtained from the patient's medical notes and predefined as 'one year prior to a VTE event'. This information was, however, not available for the sub-cohort subjects in Paper I. Increasing the duration of the occult cancer period from six months to 12 months could lead to a dilution of the association of occult cancer and VTE recurrence in Paper III.

4. Main results

4.1 Paper I

JOINT EFFECTS OF CANCER AND VARIANTS IN THE FACTOR 5 GENE ON THE RISK OF VENOUS THROMBOEMBOLISM

Venous thromboembolism (VTE) is a frequent complication in cancer. Two single nucleotide polymorphisms (SNPs) in the factor 5 (F5) gene (rs6025 (FVL), rs4524) have previously been found to be associated with an increased risk of VTE in the general population. The effect of these SNPs, however, has not been widely investigated on the risk of VTE in cancer patients. Therefore, in a case-cohort study, we assessed the joint effect of active cancer and these two F5 variants on VTE. Cases with a first VTE (n=609) and a randomly selected age-weighted sub-cohort (n=1961) were sampled from the general population in Tromsø, Norway. A VTE event was classified as cancer-related if it occurred in the period six months before to two years after a cancer diagnosis. Active cancer was associated with an 8.9-fold higher risk of VTE (95% CI 7.2-10.9). The risk of a cancer-related VTE was 16.7-fold (95% CI 9.9-28.0) higher in subjects heterozygous for rs6025 compared with non-carriers of the FVL allele without active cancer. In subjects with active cancer the risk of VTE was 15.9-fold higher (95% CI 9.1-27.9) in those with one risk allele at rs4524, and 21.1-fold (95% CI 12.4-35.8) higher in those with two risk alleles, when compared to non-carriers without active cancer. A synergistic interaction, on an additive scale, was observed between active cancer and FVL (Relative excess risk due to interaction (RERI) 7.0; 95% CI 0.5-14.4) and F5 rs4524 (RERI 15.0; 95% CI 7.5 -29.2). The incidence of VTE during the initial six months following a cancer diagnosis was particularly steep in subjects with risk alleles at these sites. This implies that the combination of cancer and F5 variants yields a synergistic increase on the risk of VTE.

4.2 Paper II

IMPACT OF TIME SINCE DIAGNOSIS AND MORTALITY RATE ON CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM IN A GENERAL POPULATION – THE SCANDINAVIAN THROMBOSIS AND CANCER (STAC) COHORT

VTE occurs frequently in cancer, and previous studies suggest that aggressive cancers are associated with the highest risk of VTE. High rates of early mortality among cancer patients with VTE may result in an over-estimation of the VTE risk, especially so in patients with cancers associated with high mortality. Competing risk for death analysis should be taken into account when the rate of death can differ greatly between two study groups. Therefore, we estimated the risk of VTE by cancer sites, accounting for the differential mortality between cancers. We used the STAC cohort which included 144952 participants followed from 1993 to 1997 until 2008 to 2012. Incidence rates, cause-specific hazard ratios (HR) and sub-distribution hazard ratios (SHR) were assessed for overall cancer and by cancer sites according to time-intervals since cancer diagnosis. During follow-up, 14272 subjects developed cancer, and 567 experienced cancer-related VTE events. In cause-specific analyses, the risk of VTE was highest the first 6 months following a cancer diagnosis (HR 17.5, 95% CI 15.1-20.3), and declined rapidly thereafter. However, when mortality was taken into account, the risk was the same in the period 6 months before (SHR 4.8, 95% CI 3.6-6.4) and 6 months after (SHR 4.6, 95% CI 3.9-5.4) a cancer diagnosis. The range of the 2-year cumulative VTE incidences was substantially narrowed at all cancer sites after competing risk by death was taken into account (from 1 to 10% to 1 to 4%). The risk of VTE by cancer sites was influenced by the mortality rate and the time since cancer diagnosis. Our findings suggest that the cancer itself is a major contributor to VTE risk, and competing risk by death should be taken into account when exploring VTE risk in cancer.

4.3 Paper III

OCCULT CANCER-RELATED FIRST VENOUS THROMBOEMBOLISM IS ASSOCIATED WITH AN INCREASED RISK OF RECURRENT VENOUS THROMBOEMBOLISM

Although VTE is associated with a high recurrence rate, the absolute recurrence rates in cancer-related VTE, particularly in occult cancer, are not well established. We aimed to investigate the risk of VTE recurrence in patients with occult and overt cancer-related VTE. Incident VTE events among participants of the Tromsø Study 1 to 6 surveys occurring in the period from 1994 to 2012 were included. Occult cancer was defined as cancer diagnosed within a year following a VTE, whereas overt cancer was defined as cancer diagnosed within the two years before a VTE. Among 733 patients with incident VTE, 110 had overt cancer and 40 had occult cancer. There were 95 recurrent VTE events during a median of 3.2 years of follow-up. The one-year cumulative incidence of VTE recurrence was 38.6% in occult cancer, 15.5% in overt cancer, and 3.8% in non-cancer subjects. The one-year risk of recurrence was 12-fold (HR 12.4, 95% CI 5.9-26.3) higher in occult cancer, and 4-fold (HR 4.3, 95% CI 2.0-9.2) higher in overt cancer, when compared with non-cancer subjects. The risk estimates for VTE recurrence were lowered when competing risk by death were taken into account, especially in the occult cancer-related VTEs, where early mortality was high. Occult cancers associated with VTE recurrence were typically located at pro-thrombotic sites (i.e. lung and gastrointestinal) and presented at advanced stages. The majority (69%) of recurrences in occult cancer occurred before or within five days of a cancer diagnosis, and were therefore not related to cancer treatment. Patients with an occult cancer-related VTE were more often diagnosed with late-stage cancers at cancer sites typically associated with VTE. In conclusion, our findings suggest that individuals with an incident VTE event during an occult cancer period had a substantially higher rate of VTE recurrence than patients with overt cancer and cancer-free patients. Patients with occult cancer-related incident VTE who experienced a VTE recurrence had a preponderance of prothrombotic and advanced cancers at diagnosis, suggesting that the recurrence risk can be attributed to tumor-related factors, such as tumor type and stage.

4.4 Paper IV

D-DIMER MEASURED AT FIRST VENOUS THROMBOEMBOLISM IS ASSOCIATED WITH FUTURE RISK OF CANCER

VTE can be the first sign of an underlying cancer. The risk of cancer is highest in the first year following a VTE but remains increased for several years. D-dimer is a fibrin degradation product, and it is used as part of the assessment of a suspected VTE, where low D-dimer levels are used to exclude VTE. D-dimer levels are also elevated in cancer. The link between markedly increased D-dimer plasma levels at first VTE and the risk of cancer has not been widely studied. Therefore, in a cohort of VTE cases (n=422) recruited from the Tromsø Study, we aimed to investigate the association between plasma D-dimer levels measured at incident VTE and the risk of cancer within the subsequent two years. D-dimer levels were divided into tertiles based on the D-dimer distribution: tertile 1: <2000 ng/ml, tertile 2: 2000–5000 ng/ml and tertile 3: >5000 ng/ml. The cumulative incidence of cancer at two years was 4.3% in tertile 1, 6.9% in tertile 2 and 15.5% in tertile 3. The one-year risk of cancer was 1.6-fold (95% CI 0.5–5.0) higher in subjects in tertile 2, and 3.3-fold (95% CI 1.2–9.1) higher in subjects in tertile 3, when compared to the lowest D-dimer tertile. The risk persisted when extending the follow-up period to two years. The most common cancer sites were lung, prostate, colorectal and hematological cancers. Subjects in the highest D-dimer tertile who developed cancer within one year, typically had a more advanced cancer at the time of diagnosis, and at the time of cancer diagnosis 80% of subjects in tertile 3 had some degree of metastases, while only 20% of patients in tertile 1 did. The risk of death during the first year after VTE increased across the tertiles of D-dimer, from 1.9-fold (95% CI 0.6–6.4) in tertile 2 to 5.7-fold (95% CI 2.0–16.5) in tertile 3, when compared to tertile 1. In conclusion, plasma D-dimer levels >5000 ng/ml at incident VTE are associated with a higher one- and two-year risk of cancer. High D-dimer levels are also associated with more advanced cancers with poor prognoses in these patients. As D-dimer is routinely measured in the assessment of suspected VTE, it may be a useful surrogate marker for the presence of an underlying cancer.

5. General discussion

5.1 Methodological considerations

5.1.1 Study design

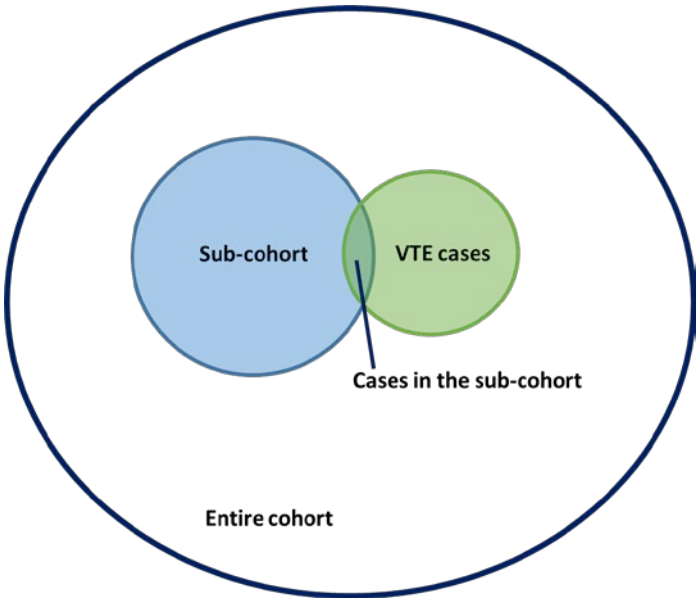
The papers in this thesis use data from the Tromsø Study cohort, with Paper II using the Tromsø 4 Study merged with two additional population-based Scandinavian cohorts. The term "cohort" is derived from the Latin word "cohor". A cohort was the standard military unit of the Roman army, and 10 cohorts made up a Roman Legion. Therefore, each cohort, consisting of between 300 to 800 soldiers each, could be traceable during each battle. Since then, the word cohort has been adopted into epidemiology to define a set of people followed over a period of time. Papers I to IV utilize data from a prospective population-based cohort. In a ***cohort study design***, study participants are followed from the date of inclusion in the study until they are censored, either by the outcome event of interest, the end of the study period, or by other defined censoring events like, in our studies, death or migration. A well-defined population is selected and their exposure status is recorded at study entry, then the outcome of interested is investigated and compared in non-exposed and exposed individuals. In Paper I, subjects were followed from the date of inclusion in 1994 to 1995 (Tromsø 4) or 2007 to 2008 (Tromsø 6) until the end of 2012. In Paper II, subjects were included at study enrollment in 1993 to 1997 and followed up until 2007 to 2012. However, in Papers III and IV, subjects were included at their study entry, but their follow-up began at their incident VTE event and participants were followed up until recurrence or a cancer diagnosis, respectively. Participants with a cancer diagnosis before baseline were excluded from the analysis. The observation-time for cancer-exposed subjects started at the cancer diagnosis date obtained from linkage to the national cancer registries, and cancer was treated as a time-dependent exposure. A cohort study is useful for estimating the absolute and relative risks of a disease. There are several advantages that cohort studies have over other observational studies. As the exposure, or exposures, of interest are determined before the outcome occurs, cohort studies have a temporal framework to assess causality, and the outcome does not influence the

exposure status. Several outcomes can be investigated simultaneously, for instance, in Paper IV, our outcome variables were both cancer and mortality following a VTE event. Furthermore, large numbers of participants are normally included in cohort studies, which allows for generalizability of study results to other populations. However, the large number of needed study participants and the long follow-up duration required for a cohort study, means that this study design can be expensive and time-consuming.

In Paper I, we used a ***case-cohort study design*** with subjects recruited from the fourth and sixth surveys of the Tromsø study. A case-cohort study is a variant of a case-control study, in which the source population is selected from a cohort.[224] Nested within a larger cohort, the study comprised of *cases* (i.e. patients with a VTE) and a randomly selected *sub-cohort* of individuals from the original cohort. The sub-cohort is meant to reflect the occurrence of the exposure in the source population. The case-cohort study design can be visualized in Figure 4. Similar to cohort studies, the case-cohort study has a clear temporal sequence of exposure and outcome, and the probability of obtaining valid and unbiased information from participants is high.[224] In our study 660 VTE cases were included from the Tromsø 4 and 6 surveys, and an age-weighted sub-cohort was randomly selected from the same population. Due to the case-cohort design in which every person in the cohort, including the cases, has the same probability of being selected to the sub-cohort, 68 controls were also cases. The main

Figure 4. Case-cohort study design

The entire cohort is represented by the large outer navy blue circle. Within the entire cohort, cases are selected (represented in green) and a sub-cohort population (represented in blue) is randomly selected. Due to the nature of random selection of the sub-cohort, cases may be included in the sub-cohort population.



advantage of the case-cohort study design over a cohort study is that full covariate data is only required for cases and individuals in the sub-cohort. Thus, the case-cohort design was chosen to limit the costs and time required for genotyping in our study. However, this study design also carries some limitations. In a case-cohort study it is important to consider and account for over-representation of cases in the sample. In our study, the incidence rates of VTE would obviously be over-estimated. However, the incidence rates of cancer would also be over-estimated, as VTE and cancer are strongly associated and the proportion of VTE events in the sample is high. Therefore, for the calculation of absolute risks in Paper I, we used the number of person-years from the original cohort (sample population n=29128) as a basis of calculating the incidence rates of VTE and cancer.

5.1.2 Bias

Errors in estimation can be classified as *random* or *systematic errors.* Random errors can be reduced as the sample size increases, however, systematic errors will remain even as the sample size is infinitely increased. Bias is the term for *systematic error*s in epidemiological research that result in incorrect estimates of the true effect of an exposure on the outcome.[224] The effect of bias will yield observed results that are either over- or underestimated from the true value, depending on the type of systematic error.[225] Biases may be introduced into a study during participant selection, data collection and/or data analysis. Bias may influence both the internal and external validity of a study. Internal validity refers to whether the inferences drawn are true to members of the source population, and external validity, or generalizability, refers to the validity of the inferences to individuals outside of the population studied.[224] Selection and information bias are the two main types of bias, although some overlap can exist between them.

Selection bias occurs when there are systematic errors in the recruitment and/or retention of participants in a study, which can influence the association between the exposure and the outcome.[226] Selection bias are less likely to occur in cohort studies, than in, for instance, case-control studies,

because, in cohort studies both exposed and unexposed study participants are selected before the outcome actually occurs. *Self-selection* is a well-established issue that occurs in epidemiologic studies and is an important issue to acknowledge, as it can threaten the external validity of a study. Self-selection bias arises when individuals "select themselves" into a group, causing a biased sample with nonprobability sampling. For instance, in the six Tromsø Study surveys, either all, or parts, of the population living in the municipality of Tromsø were invited to participate, and the attendance rates were high. Participation rates ranged from 66% in Tromsø 6 survey to 83% in Tromsø 1.[227] Invited subjects who did not attend the Tromsø Study tended to be younger, were more often men and were more likely to be single.[227] The younger (<40 years) and oldest (>80 years) populations had the lowest attendance rates. In the HUNT2 study, the attendance rate was 69%, and similar participation trends were observed with more women, middle-aged and elderly (50 to 70 years) attending and fewest in the youngest (<40 years) and oldest (>80 years) groups.[228] Both VTE and cancer tend to occur more often in the elderly population, and under-representation of this part of the population can diminish the generalizability of the results for that group. In the DCH study, the population of the urban areas of Copenhagen and Aarhus aged 50 to 64 years was invited to participate, and 57 053 of the invited 160 725 people attended (attendance rate 35%).[229] Participants of the DCH Study were more often married and had a higher level of education and socioeconomic status than non-attendees. Cancer is often thought to be more prevalent in persons with a lower socioeconomic status, and this group is underrepresented in this cohort. However, the age standardized incidence rates (SIR) for cancer in Denmark and in the DCH study were essentially the same (SIR 0.97, 95% CI 0.92-1.0s vs. SIR 0.98, 95% CI 0.94-1.03, respectively).[229] Thus, it is not likely that the low participation rates and higher proportion of non-attendees with a lower socioeconomic status and education has a significant impact on the validity of the analysis in this cohort. This is likely because the differences between social classes in Scandinavia is smaller than other parts of the world.

Information bias, or misclassification bias, is introduced when study participants are not categorized into the correct exposure or outcome group. There are two main types of misclassification

bias: differential and non-differential misclassification bias.[224] Differential misclassification occurs when the probability of exposure misclassification is influenced by the actual value of other variables. Differential misclassification can lead to either over- or under-estimation of the true association. Non-differential misclassification occurs when the error does not depend on the value of other variables, and the errors tend to be equally distributed among the cases and the non-cases. Non-differential misclassification tends to occur in prospective studies, as the exposure variables are not related to the outcome of interest, since the baseline information is collected before the outcome occurs. Non-differential misclassification tends to bias the association towards the null hypothesis.[226] In the four papers in this thesis, the main exposure or outcome variables are not based on self-administered questionnaires, but are rather extracted from the patient's medical records by trained professionals, taken from well-validated National registries (i.e. cancer variable) or from laboratory testing. Thus, the degree of misclassification in our studies should be limited. Paper I used information obtained from genotyping of approximately 2500 subjects that participated in the Tromsø 4 and 6 surveys. As in all laboratory testing, there is always a risk of measurement error during the testing process. However, testing of the DNA samples was repeated if the call rates were low. In addition, SNPs that were out of Hardy-Weinberg Equilibrium or those with allele frequencies that were inconsistent with previous reports were excluded. Thus, in the unlikely event that some measurement errors did occur during this process, they would occur by chance and be classified as random errors and not systematic errors. We would expect that these random errors would only have a marginal impact on a cohort that is large in size like ours. Furthermore, in Paper IV, plasma D-dimer levels at incident VTE was the main exposure variable, and these laboratory tests may also be subject to technical measurement errors. These errors would, again, rather be random errors. The effect of random errors decreases as the study population size increases.[224] We used three relatively wide tertiles for the plasma D-dimer levels and a large cohort of 422 patients, therefore, if present, these random errors would likely have little influence on the results.

Medical surveillance bias occurs when a medically relevant exposure or treatment of an exposure leads to closer surveillance for the study outcome of interest.[226] This can, therefore, lead to a higher chance of the outcome being detected in the exposed subjects. Medical surveillance bias tend to result in an over-estimation of the actual effect. Since the bi-directional relationship between VTE and cancer is well-known, the presence of either can influence the probability of the other being detected. In Paper II, the risk of VTE was evaluated by six-month intervals starting from one year before a cancer diagnosis until two years after. Cancer patients are frequently seen and examined by doctors and also receive frequent imaging during the diagnosis, staging, and monitoring of the malignancy. Thus, it is more likely that a VTE diagnosis will be made in a patient under close medical surveillance. However, only symptomatic VTE events were included in our studies, so this would likely not influence our results greatly. Although, imaging could incidentally detect subclinical VTE events that have been diagnosed due to overlapping symptoms with another disease (i.e. shortness of breath in a patient with lung cancer). In Paper IV, the risk of cancer following an incident VTE by plasma D-dimer levels is evaluated. If a VTE event occurs without any obvious provoking factors, a physician may investigate for an underlying malignancy with a more thorough history and physical examination, and sometimes, if appropriate with imaging. Thus, underlying cancers would be diagnosed earlier in these patients. This would likely have little influence on our results, as D-dimer levels at VTE do not influence cancer diagnostics. Although, likely negligible, both of the above issues could be present in Paper III, where the risk of a VTE recurrence is evaluated in patients with no cancer, occult cancer, and overt cancer. Where an incident VTE event could increase the chance of a cancer diagnosis, a recurrent VTE could increase the probability of a cancer diagnosis and finally, a cancer diagnosis could increase the likelihood of a recurrent VTE diagnosis.

5.1.3 Competing risk of death

A distinctive feature of survival analysis is the concept of censoring. In prospective studies, if a subject is lost to follow-up, for example, due to migration, they are censored because it is unknown if

the outcome of interest occurs in this person or not. Death is often handled as a censoring event in prospective studies and death from any cause can prevent the outcome from occurring. An assumption of censored survival time is that it should be non-informative, meaning that at any given point in time, subjects who remain in the study have the same future risk of the outcome as those who are no longer under follow-up (i.e. censored or dropped out from the study). However, cancer patients have higher mortality rates than non-cancer patients and, therefore, censoring affects exposed and non-exposed subjects differently.

Competing risk regression is most often used when the occurrence of one event may alter the chance of another event occurring.[230] Both VTE and cancer are associated with increased mortality, and thus, death could prevent the eventual outcome (whether its cancer or VTE) from occurring. To address this concern, Fine and Gray introduced a statistical model which can account for competing events, like death.[230] In traditional analysis methods, when death occurs, the probability of a subsequent VTE instantly drops to zero. These competing risk of death methods do not censor patients on the date of death. Therefore, the 'true' probability of experiencing the outcome of interest can be presented regardless of the influence of competing mortality, which theoretically leads to unbiased and meaningfully interpretable results.[231]

A study performed by Ay and colleagues using the CATS cohort compared the performance of traditional analysis approaches, like Kaplan-Meier and Cox regression, to competing risk of death analysis on the risk of VTE in a cohort of cancer patients.[232] They found that the risk of VTE was over-estimated when using standard analysis methods (Kaplan-Meier and Cox regression) compared to competing risk of death regression in cancers with high early mortality such as pancreatic, lung, and gastric cancers. Whereas, in cancers associated with lower mortality rates, such as lymphomas and breast cancer, the difference between the methods was minimal. This study concludes that competing risk of death analysis should be considered for biomarker studies with high mortality, randomized trials with interventions with differences in death rates, non-randomized trials with differences in risk

factors for death between groups, and in prognostic studies (i.e. risk score development studies) that can have a potential impact on medical decision making.[232] However, in studies investigating etiological questions, traditional analysis methods can be used.

Competing risk of death analysis was implemented in Papers I to IV, as the risk of VTE (Papers I to III) and cancer (Paper IV) was compared in cancer patients and VTE patients with increased D-dimer levels, respectively, and mortality rates were expected to differ between the groups. As expected, the risk estimates were over-estimated using Cox regression compared to competing risk regression in Papers II and III. This was especially evident in patients with cancers at sites with high early mortality and in patients with occult cancer provoked incident VTE who tend to be diagnosed with more advanced-stage cancers.

However, in Papers I and IV the risk estimates did not differ greatly between Cox regression and competing risk regression. When comparing the presence of risk alleles at FVL and F5 rs4524 in active cancer compared to cancer patients with the wild-type allele, the hazard ratios (Cox regression) and sub-distribution hazard ratios (competing risk regression) were nearly identical for FVL (HR 1.9 versus SHR 1.9) and F5 rs4524 (HR 3.5 versus SHR 3.9). This is not surprising, as we are comparing cancer patients with the SNPs to cancer patients without, and thus, we would not expect the risk of death between the two groups to differ. The presence of risk alleles at FVL and F5 rs4524 is not known to be associated with mortality. However, when evaluating at the risk of cancer-related VTE in subjects with these SNPs compared to cancer-free subjects, the difference then became evident in cancer patients. For instance, in cancer-free subjects with F5 rs4524 the risk estimates did not differ (HR 2.3 versus SHR 2.3), while there was substantial over-estimation in the traditional model in cancer patients with risk alleles at this SNP (HR 21.1 versus SHR 9.5). This difference is driven by the differences in mortality among cancer and non-cancer patients. In Paper IV the HR and SHR did not differ greatly, even though patients in the highest D-dimer level tertile had more advanced-stage cancers at the time

of cancer diagnosis. This is likely explained by the short duration of follow-up in this study (one and two years), and that both high D-dimer levels and cancer are associated with death.

5.1.4 Confounding

The concept of confounding refers to a situation where the association between the exposure and the outcome can be attributed to the influence of a third variable.[226] A confounding factor is an extrinsic variable that correlates with both the exposure and the outcome of interest, and it is not an intermediate variable in the causal pathway. If confounding is present, it may weaken, strengthen or possibly change the direction of the association between the exposure and outcome.[226] In cohort studies, characteristics between exposed versus non-exposed, or diseased versus non-diseased, often differ due to the non-randomized nature of the study design. It is important to assess all observed associations between exposure and outcome for possible confounders. There are several methods to control for confounding in cohort studies.

The most common strategies for dealing with confounding are adjustment for and stratification by the confounding variables.[233,234] Multivariable analysis is a statistical technique where potential confounding variables are included as covariates in the regression model.[226] In Papers I to IV, the Cox regression models are adjusted for age and sex. In Paper III, when investigating the risk of VTE recurrence in patients with occult and overt cancer related incident VTE events, it was questioned whether patients in the overt cancer group were on anticoagulants for a longer duration. Therefore, in addition to sex and age, we adjusted our Cox model for the planned duration of anticoagulation therapy in non-, occult and overt cancer patients, and the risk estimates were unchanged. Thus, duration of anticoagulation was not a confounding variable in these groups of patients. Some variables, such as cancer-related factors like chemotherapy and hospitalization, may also be intermediates in the causal pathway. Adjusting for intermediates in the causal pathway may lead to over-adjustment and could potentially obscure the results.

Another method for dealing with confounding is *stratification*.[226] Stratification involves dividing the study population into strata, or sub-groups, based on the confounder. Stratification also ensures that each group of the strata receives proper representation within the cohort. However, stratification is not always practical when you have small numbers in each sub-group, as it can limit the statistical power, which can lead to insignificant results.

In the case-cohort study that was used in Paper I, a degree of matching between the VTE cases and the sub-cohort was done in order to create a sub-cohort of subjects with comparable characteristics with regards to possible confounding variables. The sub-cohort was randomly selected from the entire Tromsø Study 4 to 6 cohorts, but the selected sub-cohort participants were matched by five-year age categories to the VTE cases. This was done as the average age for a VTE event is higher than the mean age of the Tromsø 4 and 6 participants that the sub-cohort was selected from.

In Paper II, the effect of confounding by age was accounted for by using age as a time-scale. In Cox regression models, time-in-study is traditionally used as a time-scale. In a time-in-study time-scale, subjects start follow-up at the date they are included into a study and are followed-up until the date they are censored, experience the outcome or until the follow-up ends. When age is used as a time-scale, follow-up begins at the age a person is at study inclusion and their age at censoring, outcome, or end of follow-up is the exit-time. The risk of VTE changes more as a function of age than as a function of follow-up time. Accordingly, by using age as a time-scale, the risk of VTE in subjects is compared in subjects at the same age, instead of the same duration of follow-up.[235] This method is considered appropriate in longitudinal studies with large enough study populations that many people at each age are represented.

Even after applying various methods to handle confounding in our analysis, a possibility for *residual confounding* remains. Residual confounding can occur when there are unknown factors that are associated with the exposure or outcome, unspecific definitions of confounding variables, or due to lack of information from insufficient or incorrect data. Experimental studies, like randomized

controlled studies, are the gold standard for establishing causal relationships, as the exposure of interest is randomly assigned to the study participants.

5.1.5 Interaction

In epidemiology, there are two distant types of interaction: statistical interaction and biological interaction. *Statistical interaction* describes a situation where two or more risk factors modify the effect of each other with regard to the occurrence of a given outcome.[226] This phenomenon is also known as *effect modification*. When an interaction is present, it can be approached by stratifying the data on the effect modifying variable. In regression analysis, the presence of statistical interaction is normally assessed by entering a product term into the regression model. Unlike confounding, where the true association may be weakened or strengthened, interaction can result in variation in the risk estimates across the strata.[226] *Biological interaction*, also known as biological interdependence and causal interaction, is the interdependent operation of two or more causes to produce or prevent an effect,[224] meaning that two causes are both required to precipitate a disease and the effect of one is biologically dependent on the presence of another. A biological interaction is not dependent on the underlying statistical model, as it always refers to departure from additivity.[236,237]

In Paper I, we investigated for the presence of synergism between two SNPs in the F5 gene and active cancer on the risk of VTE. *Synergism* refers to the interaction of two or more elements that, when combined, produce a total effect greater than the sum of the individual components. We assessed the presence of synergism by calculating the additive interaction, which was expressed by the relative excess risk due to interaction, or RERI. RERI was calculated as $HR_{11} - HR_{10} - HR_{01} + 1$, where HR_{11} is the hazard ratio for both risk factors present, HR_{10} , the hazard ratio for the first risk factor present (i.e. FVL or F5 rs4525) and HR_{01} the hazard ratio for the second risk factor (active cancer). RERI values <0 signify a negative interaction, values equaling 0 indicate exact additivity, and values >0 indicate a synergistic interaction. We also calculated the attributable proportion due to interaction

(AP) using the equation: AP=RERI/HR_{11}. The AP is interpreted as the proportion of cases in the combined group that is due to interaction between the two exposures. An AP value <0 indicates negative interaction or less than additivity, and an AP value >0 indicates a positive additive interaction. Finally, we investigated an interaction between the F5 variants and cancer on a multiplicative scale by fitting the statistical interaction terms into our Cox regression model adjusted for age and sex. We found significant interaction between the F5 variants and cancer on the risk of VTE on an additive scale (biological interaction), but no interaction on a multiplicative scale (statistical interaction) was observed.

5.1.6 External validity

External validity is thought to be one of the most difficult types of validity to achieve, however, it is at the foundation of every good epidemiological study.[226] A suggested definition for external validity is, "External validity asks the question of generalizability: To what populations, settings, treatment variables and measurement variables can this effect be generalized?"[238] This concept refers to the idea that the study results are applicable to populations, other than the one that was directly studied.[224]

High external validity means that our results can be generalized to the population of Tromsø (Papers I to IV) and Nord Trøndelag and urban Aarhus and Copenhagen (Paper II) as a whole, and then further to Norwegian/Scandinavian populations, or even further to other Western populations. The three studies included in this thesis have relatively high participation rates. The Tromsø Study is a population-based survey with the intent of reflecting the general population of the area, and the attendance rates range from 66% in Tromsø 6 to up to 83% in Tromsø 1.[227] The HUNT2 study has similar participation rates, with 69% of the invited population attending.[228] In these two studies, some groups are better represented than others. For instance, there were lower participation rates for the youngest (<40 years) and oldest (>80 years) populations and men had a lower participation rate compared to

women.[227] It is important to be aware of the lower attendance rates among participants in these age groups when generalizing results. In the instance of our study, the youngest group would not be a large issue, as cancer and VTE are both rare in this age group, however, this could be an issue for generalizability of our results in the oldest group. In the DCH study, the attendance rate was 35%, and a higher proportion of subjects with higher education and a higher socioeconomic status participated in the study.[229] However, the age standardized incidence rates for cancer in Denmark and in the DCH study were comparable.[229] In addition, the age-specific IRs of cancers in both men and women in the Tromsø and the HUNT2 cohorts, were similar to national figures from Norway.[239] Furthermore, the majority of the study participants in the three cohorts used in this thesis are of Caucasian ethnicity. Therefore, the results of our studies may not be representative to other ethnicities.

People that attend health surveys are often considered to be more health conscious compared to the general population. Additionally, subjects who are ill or institutionalized are likely unable to physically attend the survey site for examination or complete a questionnaire as part of a health survey. Individuals who developed cancer in our studies were only those who participated in these health surveys, so it can be appropriate to assume that these subjects are more health-aware than cancer-patients as a whole. People who are more health aware may, for instance, recognize symptoms of a cancer earlier and receive a diagnosis earlier, thereby reducing their risk for a VTE. Also, there are several lifestyle risk factors that can modify the effect of cancer on VTE risk, such as smoking habits, which may differ between survey participants and non-participants. It is important to remember, however, that this bias between health survey attendees and non-attendees is particularly theoretical, and it is not likely that it has a large effect on our results. Further, in Papers I to IV, relative risks (i.e. HRs) rather than absolute risks (i.e. IRs) are predominately reported, thus, our results would not be greatly affected.

The incidence of VTE and the distribution of risk factors in our cohorts is comparable to other similar Western populations. Both inpatient and outpatient VTE events were registered. The incidence

rates of VTE per 1000 person-years was 1.7 (95% CI 1.6-1.9), 1.5 (95% CI 1.4-1.6) and 1.2 (95% CI 1.1-1.3) for the Tromsø, HUNT2 and DCH studies, respectively.[240] This is similar to other studies that present an incidence of VTE ranging from 1 to 2 per 1000 person-years.[3,22,25] Furthermore, the prevalence of cancer in our three cohorts appears to be similar to reports from the Norwegian Cancer Registry and the Danish Cancer Registry.[239,240] These findings provide reassurance that both the cancer and VTE populations in our cohorts are representative of the general population of the Scandinavian countries.

In Paper I, we used genotyping information for two SNPs in the F5 gene (FVL and F5 rs4524). There are large global variations in the human genome as a result of evolutionary events such as migration, natural selection and genetic drift.[241] Therefore, the distribution of SNPs in our population may vary from other populations. Furthermore, Sami are the indigenous people of the northernmost parts of Norway, and thus the genome, and therefore disease risk, in the area may differ from other regions in the country.[242] We calculated the allele frequencies for FVL and F5 rs4524 in our study population and they were coherent with other reference western populations. We compared the prevalence the two SNPs to the HapMap CEU sample of Americans of Western and Northern European descent.[243] The allele frequency of FVL was 0.042 in our cohort and 0.05 in the CEU sample, and the allele frequency of F5 rs4524 was 0.732 in our data and 0.736 in the CEU data. Thus, the distribution of FVL and F5 rs4524 are similar to reference populations and our findings on these SNPs can be generalized to other western European populations.

5.2 Discussion of main results

5.2.1 Joint effects of F5 variants and cancer on the risk of venous thromboembolism (Paper I)

In Paper I, we reported that two F5 gene variants (FVL and rs4524) were associated with a higher risk of VTE and substantially so in patients with active cancer. We observed a synergistic effect, on an additive scale, between these two SNPs and cancer on the risk of VTE. Previous studies have also reported a joint effect between FVL and cancer on the risk of VTE. Blom and colleagues reported a 2-fold (OR 2.2, 95% CI 0.3-17.8) higher risk of VTE in cancer patients with FVL when compared to cancer patients without the mutation, and a 12-fold (OR 12.1, 95% CI 1.6-88.1) higher VTE risk when compared to cancer-free subjects without the mutation.[142] In a larger study using data from the CATS cohort, Pabinger and coworkers also found a 2-fold (HR 2.0, 95% CI 1.0-3.97) higher risk of cancer-related VTE among subjects with FVL.[147] Our findings are in line with both of the above studies. We found a 2-fold (HR 1.9, 95% CI 1.1-1.3) higher risk associated with FVL and cancer when compared to cancer patients without the mutation and a 17-fold (HR 16.7, 95% CI 9.9-28.0) higher risk when compared to cancer-free subjects without risk alleles at FVL. Previous studies found that risk alleles at F5 rs4524 are associated with DVT in middle-aged populations, with VTE in post-menopausal women and with VTE during the antenatal period.[104,244,245] However, to the best of our knowledge, no other studies have investigate the effect of F5 rs4524 on the risk of cancer-related VTE.

Both FVL and F5 rs4524 are thought to be prothrombotic by attenuated down-regulation of activated factor V by activated protein C (APC).[94] Previous studies have also reported that cancer is associated with an acquired APC resistance.[246-248] Therefore, it would be likely to assume that two sources of APC resistance, acquired and inherited sources, would greatly increase the risk of a VTE. This would be comparable to the effect of oral contraceptives in persons with the FVL mutation, as both result in a poor response to activated protein C.[249,250]

The effect of both FVL and F5 rs4524 on the risk of VTE in non-cancer and cancer was driven by the high DVT risk. This finding has been previously described for FVL, and the term *FVL paradox*

describes the different risk of DVT and PE in FVL carriers.[251] This paradox is not observed in other thrombophilic defects such as PT 20210A, antithrombin deficiencies, protein C, and protein S deficiencies.[252] Our study is the first to describe the same pattern for F5 rs4524. Several hypotheses have been proposed to explain this paradox. FVL carriers may have a higher risk of DVT than PE as the clots in these patients may be more resistant to embolization due to a stronger structure[253], because of an impaired anti-fibrinolytic response to APC[254], or simply because fatal PEs may result in fewer diagnoses of FVL carriers.[249]

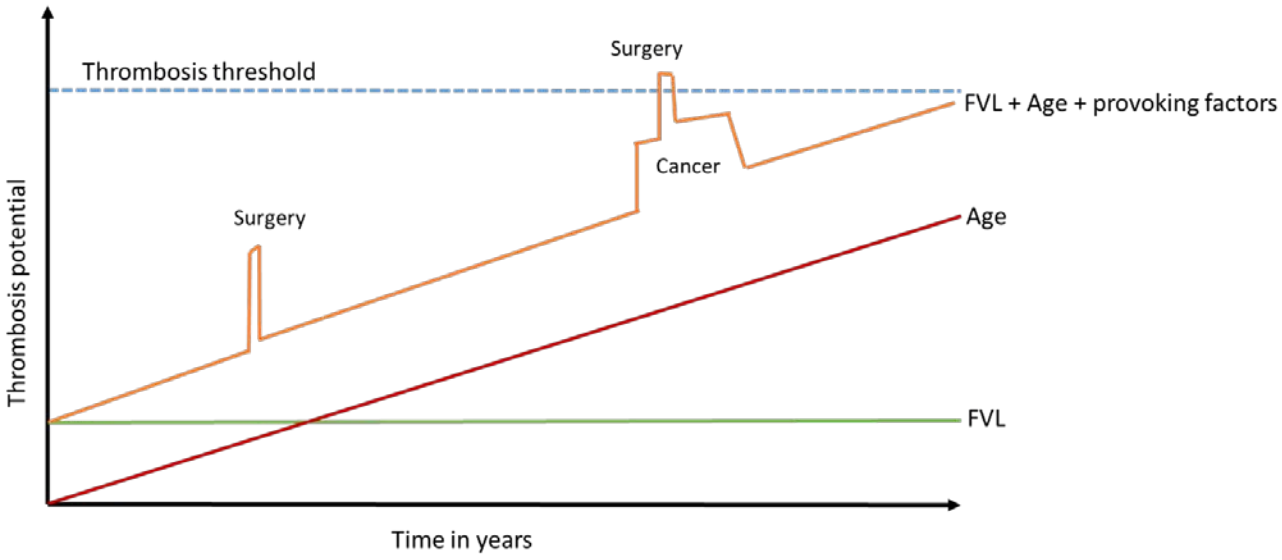


Figure 5. The thrombosis potential model. The green line represents the effect of FVL and the red line represents the effect of age on VTE. The orange line represents the joint effect of age, FVL, and provoking factors on the thrombosis potential. A person with the FVL mutation has a higher baseline thrombosis potential. The development of cancer increases the thrombosis potential substantially, but the addition of surgery as a method of cancer treatment, exceeds the thrombosis threshold and results in a VTE event.

We also found that the presence of risk alleles at FVL and F5 rs4524 had an especially strong impact on cancer-related VTE in the time directly succeeding a cancer diagnosis. The cumulative incidence of VTE among cancer patients increased substantially in the first six months following a cancer diagnosis, and especially so in subjects with risk alleles at FVL and F5 rs4524. These findings are in accordance with the thrombosis potential model (Figure 5).[87] The thrombosis potential model illustrates how several risk factors need to be present concurrently for a VTE event to occur. Alone, inherited risk factors may only mildly increase the VTE risk, however in the presence of cancer, the

thrombosis potential is further increased and may be enough to result in a VTE event. Several cancer-treatments like surgery, chemotherapy, and central venous catheters are known risk factors for VTE, and will further increase the thrombosis potential. These treatment-related factors and their complications (i.e. acute infections) may explain why there is a considerable rise in the incidence of VTE in the initial months following a cancer diagnosis, especially in subjects with risk alleles at FVL and F5 rs4524.

FVL and F5 rs4524 are associated with a moderately high risk of cancer-related VTE, and discriminate patients at risk during the first six months following a cancer diagnosis. Thus, perhaps along with other inherited risk factors, FVL and F5 rs4525 may be attractive candidates to pursue in future VTE risk prediction studies in cancer patients. Further research should address whether information on the presence of risk alleles at traditionally prothrombotic SNPs and novel prothrombotic SNPs can improve the prediction of cancer-related VTE.

5.2.2 Cancer-related VTE in the general population (Paper II)

In Paper II, we investigated the risk of VTE according to time since cancer diagnosis and cancer sites, with and without taking competing mortality into account. In the traditional Cox regression model, the risk of a VTE was already increased 4-fold (HR 4.1, 95% CI 3.0-5.5) in the six months before a cancer diagnosis. There is then a significant increase in the VTE-risk in the first six months following a cancer diagnosis, with a nearly 18-fold (HR 17.5, 95% CI 15.1-20.3) higher risk of VTE, when compared to the reference population. After this time, the risk estimates drop substantially over time. However, when taking competing risk for death into account, the risk estimates were lower at every time-interval, but especially so in the time after a cancer diagnosis. The VTE risk was essentially the same in the six months before (SHR 4.8, 95% CU 3.6-6.4) and six months after (SHR 4.6, 95% CI 3.9-5.4) a cancer diagnosis. These findings challenge results from previous reports that the apparent increased risk in the initial period following a cancer diagnosis is due to an accumulation of cancer-treatment (i.e.

chemotherapy and surgery) and cancer-complication (i.e. immobilization and infections) related factors. The VTE-risk was the same in the six months before and six months after a cancer diagnosis. Iatrogenic and other concomitant factors associated with cancer are not present at this time, as a cancer diagnosis was not yet made. This therefore suggests that cancer itself is a major contributor to the VTE-risk in cancer patients.

Previous studies have also found that standard analysis methods (i.e. Kaplan-Meier estimator, log-rank test and Cox regression) generally overestimate the risk of VTE in cancer. Ay and colleagues reported that the magnitude of bias is proportional to the amount of competing mortality, with considerable bias among cancers with high mortality.[232] Campigotto and colleagues performed simulations comparing Kaplan-Meier estimator (KM) to competing risk of death for the risk of VTE in cancer. Similarly, they found that KM analysis overestimated the VTE risk substantially when comparing a median survival time of five months to one of two months.

Colorectal, lung and prostate cancers accounted for the largest proportions of cancer-related VTE events (12.5%, 11.8% and 9.0%, respectively). Cause-specific hazard ratios for VTE were highest among patients with pancreatic, lung, brain, stomach, and renal cancers, and lowest among those with prostate and breast cancer across all time intervals. Previous studies have reported similar findings, with pancreatic, brain, and lung cancers consistently being associated with the highest risk.[51,55,152] However, accounting for competing risk of death showed that the risk of VTE was substantially overestimated at the cancer sites associated with the highest VTE risk, as they are associated with high rates of early mortality. Using the standard approach (cumulative hazard analysis), the two-year cumulative incidence of VTE ranged between 1 and 10% between all cancer sites. The cumulative incidences of VTE narrowed considerably and ranged from 1 to 4% between all cancer sites when competing risk of death was taken into account. The Khorana risk score is a risk assessment model which uses five clinical (primary cancer site, BMI) and laboratory parameters (hemoglobin levels and prechemotherapy platelet and leukocyte counts) to identify cancer patients at low (0 points),

intermediate (1-2 points) and high risk (≥3 points) of VTE.[65] Two points are assigned for "very high risk" cancer sites (stomach, pancreas, brain) and one point for "high risk" cancer sites (lung, renal, lymphoma, gynecologic, bladder, and testicular). Therefore, cancers at sites traditionally considered "high risk sites" are, at a minimum, included in the intermediate risk group. Our results indicate, however, that the range of the cumulative incidence of VTE between cancer sites was attenuated after applying competing risk of death analysis, as early mortality is high in this group of patients. Therefore, the risk difference between cancer sites isn't as large as previously thought. Future risk prediction models for cancer associated VTE should, therefore, account for competing risk of death and include a broader range of cancer sites.

Risk estimates for VTE among cancer patients decreased as the duration of follow-up was extended, both in Cox regression and competing risk of death analysis, although the magnitude of the differences was greater in the conventional analysis. The magnitude of difference between zero to six months of follow-up and zero to five years of follow-up was greater for cancers with high mortality, like pancreatic cancer (HR 52 vs. 31), than in those with low early mortality, like breast cancer (HR 5.8 vs. 2.9). When the duration of follow-up is extended, cancers with a better prognosis may be cured or enter remission and thereby contribute a large proportion of cured person-time to the cancer-exposed group. This would ultimately result in exposure misclassification and could result in diluted risk estimates during long-term follow-up.

5.2.3 Recurrent venous thromboembolism and mortality after overt and occult cancer related venous thromboembolism (Paper III)

In Paper III, we reported that occult cancer-related VTE is associated with a higher risk of VTE recurrence than overt cancer and non-cancer associated incident VTE. The majority of the VTE recurrences in patients with occult cancer were not related to treatment-related factors, as they

occurred before cancer was diagnosed. Subjects with an occult cancer-related VTE were more often diagnosed with late-stage cancers at cancer sites typically associated with VTE.

Even though, this was the first study to compare patients with a first VTE during a prolonged occult cancer period (i.e. one year) to overt and non-cancer patients, our results are in line with previous studies. In the RIETE registry, patients with a VTE during the three months before a cancer diagnosis had a 5.4-fold higher incidence of three-month recurrence.[13] In a population-based study where active cancer was defined as 92 days before or after the first VTE, the one-year cumulative incidence rate of VTE recurrence was 27%, which was comparable to the one-year cumulative incidence of VTE among patients with active cancer in our study (23%).[60]

The majority of VTE recurrences in patients with occult cancer-related VTE occurred before the date of cancer diagnosis (54%) or within five days of a registered date of cancer diagnosis (69%). Therefore, as cancer-treatment is not initiated yet at this time, as cancer is not yet diagnosed, then these VTE recurrences could not be precipitated by cancer treatment-related factors like chemotherapy and surgery. Data from two studies reported that lung, gastrointestinal, genitourinary, pancreatic, and brain cancers were associated with the highest risk of VTE recurrence.[196,255] Similarly, in our study, lung, gastrointestinal, and hematological cancers, were most often diagnosed in patients with occult cancer and recurrent VTE. Finally, patients with occult cancer-related incident VTEs had more advanced cancers at the time of diagnosis, and several studies have shown that the risk of VTE recurrence is higher in patients with metastatic disease.[196,255,256] Mortality rates were higher among patients with occult cancer-related incident VTE than those with a VTE secondary to overt cancer, with an absolute mortality rate of 58% at one year among this group of patients. This further indicates that the cancers in patients with occult cancer related incident VTE events are more advanced by the time that they are diagnosed. Previous work has shown that the biological aggressiveness of a cancer, which can be manifested by rate of growth or spread of a cancer, is strongly related to VTE risk.[257] Several studies have suggested that VTE may be a marker for biologically aggressive- and fast-growing

tumors.[52,138,139,258] Cancers that progress from local-stage disease to widespread metastatic spread are more likely to be associated with VTE. Thus, cancers that are fast growing are associated with a higher VTE risk, whereas more indolent, slow-growing cancers, like breast and prostate cancer, have a significantly lower risk of VTE.[52,155] These findings are all in line with our findings in Papers I and II that the risk of cancer-related VTE appears to be driven by cancer-related factors, rather than patient- or treatment-related risk factors. The effect of cancer itself, or rather the rate and severity of cancer growth and spread, also appears to have the greatest effect on cancer-related recurrent VTE.

As mentioned above, mortality rates in subjects with overt and occult cancer-related VTE during the year following the cancer diagnosis were high (42% and 60%, respectively). Accordingly, when competing risk of death was taken into account, we found that the one-year recurrence risk was markedly lower for both overt cancer (HR 4.3 versus SHR 2.9) and occult cancer (HR 12.4 versus. SHR 9.6). The difference between Cox regression and competing risk of death regression was even greater when looking at the five-year risk of VTE recurrence in the overt and occult cancer groups. This may be explained by the low five-year survival in cancer, especially when cancer is complicated by VTE.[259]

There is an ongoing debate regarding limited versus extensive screening for cancer in patients with incident VTE. In 2012, an Italian randomized study compared limited and extensive (CT chest, abdomen, and pelvis) cancer screening in patients with idiopathic VTE.[260] They found that extensive screening (with CT alone or together with hemoccult) was not significantly superior in the detection of cancer (10.2% versus 8.2%) when compared to common practice (limited screening). Further, extensive screening did not significantly affect overall mortality and cancer-related mortality (-2.1%, 95% CI -8.0-3.8%, difference in cancer-related mortality). A randomized control trial of 854 patients with an unprovoked VTE compared the effectiveness of limited cancer screening (blood testing, chest x-ray, and screening for breast, cervical, and prostate cancer) versus limited screening in combination with CT scan (abdomen and pelvis) imaging.[217] They found that limited screening plus CT did not lead to fewer missed cancers than only a limited screening strategy. They also found that there were no

differences in the rates of recurrent VTE and mortality between groups. A prospective concurrently controlled cohort study that included 630 patients from ten university hospital clinics in the Netherlands compared limited (baseline screening consisting of history, physical examination, basic laboratory tests, and chest X-ray) and extensive (limited plus chest CT scan and mammography) cancer screening strategies.[261] There was no significant difference the number of cancer diagnoses and cancer-related deaths among patients who had undergone limited screening compared to extensive screening. A recent French multicenter study compared fluorodeoxyglucose (FDG) PET/CT cancer screening to limited screening. [218] They found that limited screening plus FDG PET/CT was not significantly associated with higher rates of cancer diagnosis after unprovoked VTE (absolute risk difference 3.6%, 95% CI -0.4 – 7.9). However, the risk of a subsequent cancer diagnosis was lower in patients who had a negative initial screening with FDG PT/CT than in patients with a negative initial limiting screening (0.5% versus 4.7 %, respectively). The risk of death during follow-up, however, was the same in both extensive and limited screening groups. In our study, even though most cancers were diagnosed within a short time following the incident VTE event (86% of cancers were diagnosed within six months), and the majority of VTE recurrence occurred before a cancer diagnosis (69% diagnosed with cancer before or diagnosed within five days), the mortality among these subjects was high. In fact, 45% of the patients with an occult cancer-related incident VTE died within six months after cancer diagnosis. Therefore, extensive screening for cancer at incident VTE would likely not reduce the morbidity and mortality associated with VTE recurrence, since the recurrent events appear to be associated with advanced cancer stages with high early mortality that occur within a short time following the initial event. In fact, extensive screening could lead to patient suffering from unnecessary medical procedures and would put a greater burden on the healthcare system by using unwarranted resources. Furthermore, extensive screening for cancer at VTE recurrence would also not be of benefit to the patient for the above reasons. Therefore, current society guidelines recommend the use of limited screening strategies in patients with unprovoked VTE. More extensive screening for cancer

should only be performed in cases where a physician has a high suspicion of an underlying malignancy, and should be evaluated on an individual basis.

5.2.4 D-dimer levels at venous thromboembolism and risk of subsequent cancer (Paper IV)

In Paper IV, we found that plasma D-dimer levels >5000 ng/ml at incident VTE were associated with a higher risk of subsequent cancer at one and two years. The one year risk of cancer was 1.6-fold (95% CI 0.5–5.0) higher in subjects in D-dimer tertile 2 (2000 to 5000 ng/ml), and 3.3-fold (95% CI 1.2–9.1) higher in subjects in D-dimer tertile 3 (>5000 ng/ml), when compared to the lowest D-dimer tertile (<2000 ng/ml). The risk persisted when extending the follow-up period to two years.

Previous studies have reported a 2 to 4-fold higher one-year risk of cancer following a VTE when compared to the general population.[6,13,204] The association between plasma D-dimer levels at incident VTE and underlying cancer has not been extensively studied, although two retrospective studies have been performed on this topic. A Dutch study of 218 patients found that D-dimer levels above 4000 μg/L at diagnosis, or during the first days of treatment for DVT, were associated with an increased probability of occult cancer.[262] Recently, Han and colleagues investigated the predictive value of D-dimer for occult cancer in 169 patients with unprovoked VTE, of which 24 developed a subsequent cancer during a median of 5.3 years of follow-up.[263] They found that D-dimer levels >4000 mg/ml were associated with an increased risk of an occult cancer (HR 4.12, 95% CI 1.54–11.04). Although this study was performed in a Korean population with non-Western cancer site distribution, the risk estimates for cancer were similar to those in our study. However, the participants of this study had a notably low age at VTE diagnosis, with 44% of the VTE patients being under 60 years. The mean age among patients who developed cancer was 55.7 year and the incidence of cancer was highest among the below 60 years group.[263] Furthermore, of the 34 patients who developed subsequent cancer, 21 (88%) were diagnosed during the same admission as the VTE diagnosis and the median time to cancer diagnosis of the 3 remaining cancers after the initial hospital discharge was 104 days. The

maximum follow-up time in this study was 8 years, and therefore it seems unusual that no further cancers would be diagnosed during that time.

In our study, in patients who developed cancer within one year, the most common cancer sites were those of the lung and prostate and hematological cancers. Subjects who developed cancer within one year with the highest D-dimer levels, typically had a more advanced cancer at the time of diagnosis, with 80% having some degree of cancer spread. Accordingly, the mortality rates at one and two years were higher among those with higher plasma D-dimer levels at VTE. The one-year risk of death was nearly 6-fold (HR 5.7, 95% CI 2.0-16.5) in D-dimer tertile 3 compared to tertile 1. The median time to death was shorter in the higher D-dimer tertiles in patients who developed cancer at 1020 days, 470 days and 206 days in tertiles 1 to 3, respectively. Correspondingly, recent studies have found that, independent of VTE, D-dimer is associated with mortality, and higher D-dimer levels are a predictor of cancer progression and poor survival.[176,179,264,265] In addition, a study using data from the CATS cohort found that patients with high-grade tumors had higher D-dimer levels (>75th percentile, 1.32 μg/mL) and both tumor grade and D-dimer levels were independently associated with VTE.[257]

Mortality was high in our study participants, and especially so in those with higher D-dimer levels at incident VTE. The relative risk of VTE among cancer patients has been shown to be overestimated as mortality is greater in these patients compared to the general population.[232] As the presence of a competing event (i.e. death) may alter the chance of another event occurring (i.e. cancer) Therefore, we performed competing risk of death analysis using the Fine-Gray model.[230] Unlike in Papers I, II and III, where the difference between the traditional Cox regression model and the competing risk model was large, in this study, the risk estimates were essentially unchanged when competing risk of death was taken into account. This may simply be explained by the short duration of follow-up in this study. As the follow-up time is extended from one year to two years, the difference between the HR and SHR increases slightly. Additionally, as both cancer and high D-dimer levels are associated with death, the between-group differences in mortality wouldn't be as large.

6. Conclusions

- We found that two F5 gene single nucleotide polymorphisms, FVL and F5 rs4524, were associated with a higher risk of VTE, and the risk increased per each additional risk allele at these sites. The F5 SNPs and active cancer displayed synergism, on an additive scale, on the risk of VTE. The effect was greater on the risk of DVT than PE in non-cancer and cancer. The incidence of VTE among cancer patients increased substantially in the six months following a cancer diagnosis, and especially so in patients with the presence of risk alleles at FVL and F5 rs4524.

- In traditional Cox regression models, the risk of VTE is highest the first six months after cancer diagnosis and declines rapidly thereafter. However, when mortality is taken into account, the risk in the period six months before and after cancer diagnosis is similar. This suggests that the cancer itself is a major contributor to VTE risk and that competing risk by death should be taken into account when exploring VTE risk in cancer. The risk of VTE by cancer sites was heavily influenced by mortality rates and the time since cancer diagnosis. Thus, future risk prediction models evaluating risk of VTE among cancer patients should take competing risk of death into account and should address a wider range of cancer sites.

- We found that patients with an incident VTE event during an occult cancer period had a substantially higher rate of VTE recurrence than those with overt cancer and those without cancer. Patients with an occult cancer at incident VTE who experienced a VTE recurrence more often had prothrombotic and advanced cancers at diagnosis. Furthermore, the majority of VTE recurrences in the occult cancer group were not treatment-related as they occurred either before or within five days of a cancer diagnosis. Our findings suggest that the recurrence risk in cancer appears to be due to cancer-related factors, such as tumor type and stage.

- We found that plasma D-dimer levels above 5000 ng/ml at incident VTE were associated with a higher risk of subsequent cancer at one and two years. Mortality was greater among subjects who had higher D-dimer levels at VTE diagnosis. Subjects with higher D-dimer levels at VTE diagnosis who developed cancer within one year, typically had more advanced cancer at the time of diagnosis. D-dimer levels are routinely measured during the diagnostic workup of a suspected VTE. Therefore, D-dimer may be a useful biomarker to consider when evaluating the presence of an underlying cancer.

7. Implications of results and further perspectives

Cancer is a major risk factor for VTE and the incidence of VTE among cancer patients is increasing.[55] Risk stratification of VTE among cancer patients can be broadly done in patient-, treatment- and cancer-related factors. Patient-related risk factors, such as inherited thrombophilias, influence the risk of cancer-related VTE. We found that two F5 gene variants and active cancer had a synergistic effect on the risk of VTE (Paper I). The cumulative incidence of VTE increased substantially in the first six months following a cancer diagnosis, and especially so in patients with risk alleles at FVL and F5 rs4524 (Paper I), which is likely due to cancer treatment-related factors, such as surgery, chemotherapy, and central venous catheters. Throughout this thesis, we have demonstrated that cancer-related risk factors appear to play a strong role on the risk of incident and recurrent VTE. Cancer-related factors such as the primary cancer site (i.e. lung, gastrointestinal, hematological), advanced cancer stage, and time since cancer diagnosis appear to be the main contributors of the VTE risk in cancer. Our findings that the VTE risk is equal in the six months before and six months after a cancer diagnosis (Paper II), that late-stage cancer at diagnosis is a common feature in occult and overt-cancer related VTE (Paper III), and that the majority of occult cancer related incident recurrences occur before a cancer diagnosis is made (Paper III) reinforce this assumption. Further, we found that competing risk by death overestimates the VTE risk when traditional analysis methods like Cox regression and the Kaplan-Meier estimator are used.

Even though cancer patients are at a high risk of VTE and VTE associated mortality, current international guidelines do not recommend prophylactic anticoagulation to all ambulatory cancer patients without additional risk factors due to an uncertain benefit to harm (i.e. anticoagulation-related bleeding risk) ratio.[127,266] Thus, it is vital to recognize patients that are at high risk of cancer associated VTE, in order to identify those who would most benefit from thromboprophylaxis. Current risk prediction scores are thought to have limited clinical usefulness as they have a low potential to identify high-risk patients and a poor ability to predict VTE in the high-risk subjects.[65] These models

only include limited clinical risk factors and are not generalizable to all populations. For instance, although more common in North American populations, patient characteristics like a BMI >35 kg/m^2 will not be a common finding in Western European populations. In fact, in the CATS cohort a BMI >35 kg/m^2 was a rare finding and was not found to be associated with VTE.[135] In Paper I, we found a synergistic effect between two SNPs in the F5 gene (FVL and F5 rs4524) and active cancer on the risk of VTE. These SNPs particularly discriminated patients at risk during the first six months after a cancer diagnosis, and thus could be evaluated at cancer diagnosis. Therefore, these SNPs, and other novel genomic and proteomic risk factors, may be attractive candidates to pursue in future research on prediction models of VTE risk in cancer patients. As demonstrated by the results of this thesis and other studies, future cancer associated risk prediction models should also account for competing-risk by death, as early mortality is high in this group of patients. Future models should also explore further clinical risk factors and include a broader range of cancer sites.

VTE is associated with both short- and long-term cancer. Currently, extensive screening for cancer in unprovoked VTE is not widely recommended. We have found that the risk of one- and two-year cancer is especially high in patients with higher plasma D-dimer levels at incident VTE. As D-dimer levels are routinely taken during the diagnostic work-up for VTE, it may be a useful surrogate marker for the presence of an underlying malignancy. Moreover, further studies are warranted to explore additional existing (i.e. C-reactive protein, platelet, and leukocyte count) and novel (i.e. SNPs, proteomics) biomarkers as predictive markers for cancer among patients with VTE.

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