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Shared risk factors for arterial cardiovascular diseases and venous thromboembolism

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List of papers

I. Ischemic stroke and risk of venous thromboembolism in the general population: the Tromsø study.
Rinde LB, Småbrekke B, Mathiesen EB, Løchen ML, Njølstad I, Hald EM, Wilsgaard T, Brækkan SK, Hansen JB.
Journal of the American Heart Association. 2016 November; 5 (11): e004311

II. Repeated measurements of carotid atherosclerosis and future risk of venous thromboembolism: the Tromsø Study.
Småbrekke B, Rinde LB, Hald EM, Njølstad I, Mathiesen EB, Johnsen SH, Hansen JB, Brækkan SK, Lijfering WM.
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III. Atherosclerotic risk factors and risk of myocardial infarction and venous thromboembolism; time-fixed versus time-varying analyses. The Tromsø Study.
Småbrekke B, Rinde LB, Hindberg K, Hald EM, Vik A, Wilsgaard T, Løchen ML, Njølstad I, Mathiesen EB, Hansen JB, Brækkan SK.
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IV. Impact of prothrombotic genotypes on the association between family history of myocardial infarction and venous thromboembolism
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Summary

Extensive evidence support an association between arterial cardiovascular disease (CVD, i.e. myocardial infarction [MI] and ischemic stroke), and subsequent venous thromboembolism (VTE, i.e. deep vein thrombosis [DVT]) and pulmonary emboli [PE]). However, the mechanism behind the associations remains unclear. The aim of this thesis was to investigate the impact of ischemic stroke on VTE and to investigate potential shared risk factors for arterial CVD and VTE.

All papers in this thesis utilize data from the Tromsø Study. The study populations for Paper I, II and III were recruited from the fourth, fifth and sixth survey of the Tromsø study. In Paper IV, we recruited a subgroup participants with genetic information from the fourth survey of the Tromsø Study and from the second survey of the Nord-Trøndelag Health (HUNT) Study. Participants were followed from the first survey they attended to the date of an incident event (i.e. VTE, MI or ischemic stroke), the date of death or migration, or until end of follow-up in 2008/2012.

Ischemic stroke was associated with a transient increased risk of VTE, and the risk was particularly high for provoked events. The association persisted after adjusting for potential confounders, indicating that the stroke itself increased the VTE risk. We found no association between formation, presence or progression of atherosclerosis and VTE in time-varying analyses, indicating that atherosclerosis does not represent the missing link for the association between arterial CVD and VTE. Except for body mass index, none of the traditional cardiovascular risk factors increased the risk of VTE, and risk estimates for MI and VTE based on a single baseline measurement and repeated measurements corresponded well. Lastly, we showed that the association between a family history of MI (FHMI) and VTE is not explained by prothrombotic genotypes, and that the combination of FHMI and prothrombotic genotypes had an additive effect on VTE risk.

Our findings imply a strong and transient increased risk of VTE after ischemic stroke and that the association between arterial CVD and VTE cannot be explained by atherosclerosis. Of the well-known cardiovascular risk factors, only age, obesity and FHMI are associated with VTE. The association between arterial CVD and subsequent VTE is only partly explained by shared risk factors. The remaining association is likely mediated by risk factors following the arterial cardiovascular event, such as immobilization and infection, and direct effects of the arterial cardiovascular event, such as activation of the coagulation system.

Sammendrag

Det er gode holdepunkter for en sammenheng mellom arteriell kardiovaskulær sykdom (hjerteinfarkt og iskemisk hjerneslag) og påfølgende risiko for venøs tromboembolisme (VTE, fellesbetegnelsen på dyp venetrombose [DVT] og lungeemboli [LE]), men mekanismen for denne sammenhengen er ukjent. Formålet med denne avhandlingen har vært å undersøke hvordan hjerneslag påvirker risikoen for VTE og å undersøke potensielle felles risikofaktorer for kardiovaskulær sykdom og VTE.

Artiklene i avhandlingen bruker data fra Tromsøundersøkelsen. Studiedeltakerne i artikkel I, II og III ble rekruttert fra den fjerde, femte og sjette undersøkelsen (Tromsø 4, 5 og 6). De inkluderte i artikkel IV besto av en undergruppe som fikk utført genetiske analyser. Disse deltok i Tromsø 4 eller i den andre Helseundersøkelsen i Nord-Trøndelag (HUNT 2). I samtlige artikler ble deltakerne fulgt fra første undersøkelse de deltok i til en kardiovaskulær hendelse eller VTE oppsto, til de døde eller flyttet, eller til studieslutt i 2008/2012.

Iskemisk hjerneslag ga en forbigående økt risiko for VTE, og risikoen var særlig høy for provosert VTE. Sammenhengen vedvarte etter justering for potensielle konfoundere, noe som indikerer at det var hjerneslaget, eller tilstander relatert til hjerneslaget, som økte risikoen for VTE. Det var ingen sammenheng mellom nydannelse, tilstedeværelse eller progresjon av aterosklerose og VTE i analyser med oppdaterte målinger, noe som tyder på at aterosklerose ikke kan forklare sammenhengen mellom kardiovaskulær sykdom og VTE. Foruten kroppsmasseindeks ga ingen av de tradisjonelle kardiovaskulære risikofaktorene økt risiko for VTE, og risikoestimater for hjerteinfarkt og VTE basert på én måling og repeterte målinger korresponderte godt. Vi viste også at sammenhengen mellom familiær predisposisjon for hjerteinfarkt (FHMI) og VTE ikke kunne forklares av gener som øker trombosetendensen, og at kombinasjonen av FHMI og protrombotiske gener hadde additiv effekt på risiko for VTE.

Våre funn tyder på at det er en midlertidig økt risiko for VTE etter iskemisk hjerneslag, og at assosiasjonen mellom kardiovaskulær sykdom og VTE ikke kan forklares av aterosklerose. Blant velkjente kardiovaskulære risikofaktorene var det bare alder, overvekt og FHMI som hadde sammenheng med VTE. Felles risikofaktorer kan dermed bare delvis forklare sammenhengen mellom kardiovaskulær sykdom og VTE. Resten av sammenhengen kan trolig forklares av at komplikasjoner etter den kardiovaskulære hendelsen, som for eksempel immobilisering og infeksjoner, øker risikoen for VTE, eller at den kardiovaskulære hendelsen fører til aktivering av koagulasjonssystemet og dermed økt trombosetendens.

Abbreviations

AP	attributable proportion due to interaction
APC	activated protein C
BMI	body mass index
CCA	common carotid artery
CI	confidence intervals
CRP	C-reactive protein
CT	computed tomography
CTPH	chronic thromboembolic pulmonary hypertension
CVD	cardiovascular disease
DALY	disability-adjusted life-years
DVT	deep vein thrombosis
ECG	electrocardiography
ECM	extracellular matrix
F	factor
FHMI	family history of MI
FVL	factor V Leiden
HDL	high-density lipoprotein
HR	hazard ratio
HUNT	Nord-Trøndelag Health Study
IMT	intima media thickness
ICA	internal carotid artery
ICD	International Classification of Diseases
LDL	low-density lipoprotein
LMWH	low-molecular-weight heparin
MI	myocardial infarction
MRI	magnetic resonance imaging
OR	odds ratio
PE	pulmonary embolism
PTS	post-thrombotic syndrome
PVD	peripheral vascular disease

PY	person-years
RERI	relative excess due to interaction
RCT	randomized controlled trial
SI	synergy index
SNP	single nucleotide polymorphism
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TPA	total plaque area
UNN	University Hospital of North Norway
VTE	venous thromboembolism
vWF	von Willebrand Factor
WC	waist circumference
WHO	World Health Organization

1. Introduction

Venous thromboembolism (VTE) is the collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT is the formation of a thrombus (i.e. "blood clot") in the deep veins, typically in the large and deep veins of the lower extremities. Other more unusual sites of thrombus formation are the deep veins of the upper extremities, the vena cava, the portal vein, the mesenteric veins and the venous sinuses in the brain. Classical signs and symptoms of DVT include pain, swelling, edema, and redness of the affected extremity. PE is usually a complication of DVT and occurs when a thrombus in a deep vein detaches from its original site and travels with the bloodstream to the lungs, where it lodges and interrupts normal blood flow. However, recent studies suggest that PE can also occur without an associated DVT. [1, 2] Classical signs and symptoms of PE include chest pain, tachypnea, dyspnea, and coughing. In severe cases, PE can lead to circulatory collapse and death. VTE is usually treated with anticoagulant agents. These drugs prevent further thrombus formation, while the already existing thrombus is degenerated by innate fibrinolytic systems in the body. Severe cases can be life-threatening (e.g. large PE), and treatment with thrombolytic agents, which breaks down the thrombus, may be necessary. Treatment type and duration depends on the type of VTE event and presence of provoking factors.

Arterial and venous thrombosis have traditionally been considered as two separate diseases, with different pathophysiology and treatments. In a case-control study from 2003, Prandoni and colleagues reported a higher prevalence of carotid plaques in patients with unprovoked VTE, compared with patients with provoked VTE and controls.[3] Although later prospective studies did not show any association between atherosclerosis and subsequent VTE,[4-6] the findings supported the hypothesis of an association between arterial and venous thromboembolic diseases. Several studies have reported an increased risk of VTE after myocardial infarction (MI).[7, 8] The risk of VTE seemed to be highest the first year following the MI, and the risk of PE was higher than the risk of DVT.[7] In addition, studies investigating the association between stroke and VTE found a high prevalence of VTE the first months after the stroke,[9-11] and identified risk factors included severe strokes and lower limb paresis.[12, 13] Furthermore, studies have reported an increased risk of arterial thrombotic disease (both MI and stroke) after VTE, [14-16] and the risk remained elevated for 20 years after the VTE event.[15] Thus, there is growing evidence of a bidirectional association between arterial and venous thromboembolic diseases. The mechanism behind the associations remains unclear, but shared risk factors, mediators and a direct causal interrelation have been proposed as possible mechanisms.[15]

Of the traditional cardiovascular risk factors, only age and obesity have consistently been associated with VTE.[17-20] Diabetes, hypertension, dyslipidemia, and smoking have been associated with

VTE in some, but not all studies.[20-25] It is uncertain if the conflicting results are a consequence of different study populations or different study designs. In addition, family history of MI (FHMI) have been shown to increase the risk of VTE in several studies,[26-29] indicating that shared environmental risk factors or genetic disposition in certain families can cause both arterial cardiovascular disease (CVD) and VTE.

Cardiovascular diseases are the most common cause of death globally,[30, 31] and stroke is an important cause of disability.[32] The health burden of these diseases is immense,[33, 34] and it is of great importance to identify possible mechanisms behind the association between arterial CVD and VTE. Topics of the present thesis will be the relationship between arterial CVD and VTE, and the association between cardiovascular risk factors and VTE.

1.1 Epidemiology of venous thromboembolism

VTE is the third most common cardiovascular disease, after MI and stroke.[18] The incidence in the general population is 1 to 2 per 1,000 per year,[35, 36] and the incidence increases with increasing age to nearly 1% per year in those > 80.[35, 37] The incidence of VTE is increasing, mainly because of a substantial increase in incidence of PE.[35, 38] In the Tromsø Study, the age-adjusted incidence rates (IR) of VTE increased from 158 per 100,000 person-years (PY) in 1996/1997 to 210 per 100,000 PY in 2010/2011, and IR of PE increased from 45 to 113 per 100,000 PY in the same period.[35] However, the increasing incidence of PE, the minimal change in mortality and the decreased case-fatality points towards an increase in diagnosis of clinically insignificant PE or false-positive results,[39] rather than a true increase in disease.[40] Women of reproductive age have a higher incidence of VTE than men at the same age, whereas men have a higher incidence in the elderly.[37, 41] This may relate to differential exposure to clinical risk factors by age and sex, such as pregnancy, puerperium, and use of oral contraceptives among younger women.[37]

Approximately two-thirds of VTE events are diagnosed as DVT alone, and one-third as PE with or without concurrent DVT.[35, 42, 43] Studies including autopsy reports tend to report a higher proportion of PE.[44] PE was previously believed always to be a complication of DVT, occurring when a part of a thrombus of the deep veins dislodged and embolized to the lungs. However, in up to 50% of patients with PE, no DVT is found with ultrasound or magnetic resonance imaging (MRI).[1, 2, 45] Possible explanations are that the thrombus can dislodge completely, that a PE can have a cardiac origin or that the PE originates from local thrombus formation in the lungs.[2, 46, 47] VTEs are classified as provoked or unprovoked, depending on the presence of environmental provoking factors at the time of the VTE event. The estimated proportion of provoked events varies with definitions of unprovoked and

provoked events, but most population-based studies have estimated that 50-60% of VTE cases are associated with a provoking factor.[35, 36, 43] An additional classification of provoking factors into minor transient, major transient and persistent provoking factors have been suggested (Table 1). Unprovoked events occur in the absence of any provoking factor.[48] The risk of recurrent VTE is lowest for those who experienced a VTE triggered by a major transient risk factors and highest for patients with unprovoked VTEs or VTEs triggered by a persistent risk factor.[44] Minor transient provoking factors are associated with a 3-10-fold increased risk of VTE and 15% will get a recurrent event after five years, while major transient risk factors are associated with a greater than 10-fold increased risk of incident VTE, and 3% recurrence after five years.[49]

Table 1. Categorization of provoked VTE events.

Transient risk factors		**Persistent risk factors**
Minor	Major	
• Surgery with general anesthesia < 30 minutes • Admission to hospital for less than three days with acute illness • Confined to bed out of hospital for at least three days with acute illness • Estrogen therapy • Pregnancy and puerperium • Leg injury with reduced mobility for at least three days	• Surgery with general anesthesia > 30 minutes • Confined to bed in hospital for at least three days with acute illness • Cesarean section	• Active cancer • Non-malignant conditions (e.g. inflammatory bowel disease)

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

In addition to short-term complications such as symptoms of DVT or PE and acute death, VTE has several long-term complications. A recurrent VTE may occur at any time after an incident VTE, and around 30% of VTE patients will experience a recurrent event within the first 10 years after an incident event.[50-52] The risk of recurrence is highest the first 6-12 months following an incident VTE,[41, 52] and independent risk factors of recurrence include male sex, increasing body mass index (BMI), neurological disease with paresis and active malignancy.[43, 52-55] Furthermore, a meta-analysis concluded that recurrent VTEs tend to occur as the same type of clinical event as the initial event, i.e. patients with an incident PE tend to suffer from a recurrent PE.[56] Chronic pain, venous stasis, skin changes, skin ulcers and heaviness are symptoms of the post-thrombotic syndrome (PTS), occurring in 20-50% of DVT patients.[50, 51, 57] It is the most common complication of DVT and risk factors for developing PTS include increasing age and BMI, female sex, previous ipsilateral DVT and a proximal thrombus (as compared with more distal thrombi).[57] Among patients with PTS, 4-10% develop severe

PTS.[50, 51, 57]A serious long-term complication of PE is chronic thromboembolic pulmonary hypertension (CTPH), caused by high blood pressure in the arteries of the lungs due to chronic obstruction. Pulmonary hypertension forces the right side of the heart to work harder than normal and can lead to right-sided heart failure. Hence, symptoms of CTPH include dyspnea, chest pain and symptoms of right-sided heart failure (e.g. dependent edemas, increasing abdominal circumference due to ascites and nocturia). CTPH affects 1-4% of patients within two years after a first episode of symptomatic PE, and risk factors for CTPH are previous PE, younger age, unprovoked PE and larger perfusion defect at presentation.[58, 59]

VTE has major consequences for the affected individual and for the society. In a large Norwegian population-based cohort study, participants with VTE had higher rates of work-related disability compared with participants without VTE (crude IR were 37.5 vs. 13.5 per 1,000 PY, respectively). In age- and sex-adjusted analyses, the hazard ratio (HR) of work-related disability after VTE was 1.62 (95% confidence interval [CI] 1.29-2.04), and the risk was especially high after DVT (HR 1.80, 95% CI 1.37-2.36).[60] A systematic review on the global disease burden of VTE concluded that VTE was the leading cause of hospital-related disability-adjusted life-years lost (DALYs), being responsible for more DALYs lost than nosocomial pneumonia and adverse drug events.[61] Furthermore, VTE is associated with high mortality and fatality. A recent study using data from the Tromsø study found an overall 1-year all-cause mortality rate of 29.9 (95% CI 25.7-34.8) per 100 PY in VTE patients, and a rate of 23.6 (95% CI 17.8-31.3) per 100 PY in cancer-free VTE patients.[41] Reported 30-day all-cause mortality ranges from 6% to 10%, and 1-year all-cause mortality from 21% to 33%.[36, 62] 1-year all-cause mortality was approximately 60% in patients with cancer-associated VTE and 15% in cancer-free VTE patients, indicating that cancer itself is an important cause of death among VTE-patients.[36, 41, 62] The 30-day case-fatality was higher in patients with PE than DVT (15% vs. 9%), and higher in patients with VTE provoked by cancer (25%) compared with individuals with VTE provoked by other factors than cancer (7%).[43]

1.2 Pathophysiology of venous thromboembolism

In 1856, Rudolph Virchow postulated that abnormalities in blood flow (stasis), hypercoagulability of the blood and injury to the vessel wall could lead to thrombus formation.[63] These factors are collectively termed *Virchow's triad*, and they remain important and relevant for our understanding of thromboembolic diseases.

Physiological hemostasis prevents blood loss after vessel damage. Primary hemostasis denotes the process of platelet activation and adhesion, and secondary hemostasis refers to the initiation of the coagulation cascade and fibrin formation. The coagulation cascade is a complex cascade of proteins

increasing (procoagulant proteins) and decreasing (anticoagulant proteins) the fibrin formation, which is the end product of the cascade and the main component of a venous thrombus. The coagulation cascade consists of the intrinsic, extrinsic and the common pathway (Figure 1). The pathways are multiple series of reactions where the activated form of a protein activates the next protein in the cascade. Tissue factor (TF), expressed in monocytes, monocyte-derived microvesicles and possibly by activated endothelial cells triggers the extrinsic pathway (TF and FVIIa), while cellular RNA and polyphosphate expressed by activated platelets and bacteria trigger the intrinsic pathway FXIIa, FXIa, FXIa, and FVIIIa). The common pathway consists of FXa, FVa, and thrombin (FIIa), which converts fibrinogen to fibrin.[64] The coagulation cascade is regulated by different anticoagulant pathways. Tissue factor pathway inhibitor (TFPI) blocks FXa and the TF/FVIIa complex, activated protein C (APC) inactivates FVa and FVIIIa and antithrombin inhibits all procoagulant proteins.[64] The coagulation cascade is thoroughly regulated, and disorders of the coagulation proteins can lead to excessive bleeding or thrombus formation. For example, an animal study showed that mice deficient in proteins of the extrinsic or common pathway die during embryonic development or shortly after birth. Further,

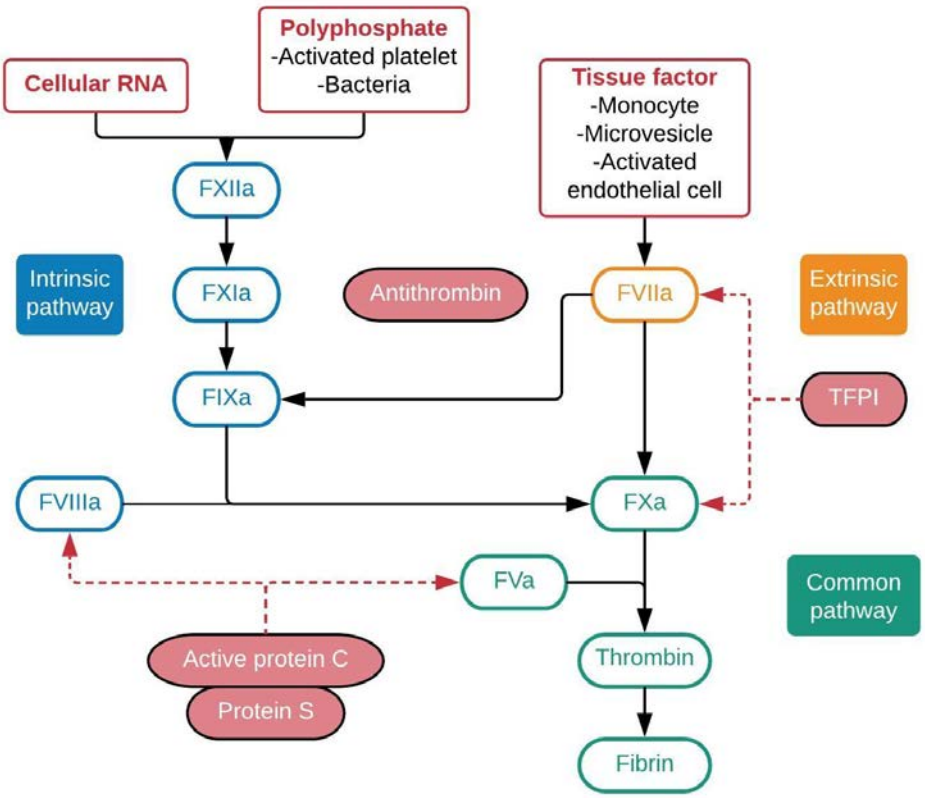


Figure 1. A simplified figure of activation of the coagulation cascade. Pathological activation of the extrinsic pathway (FVIIa and TF) is via expression of TF in monocytes, monocyte-derived microvesicles and possibly by activated endothelial cells. Cellular RNA and polyphospate released by activated platelets and bacteria activate the intrinsic pathway (FXIIa, FXIa, FIXa and FVIIIa). (Adapted from Mackman, Journal of Clinical Investigation 2012)

mice lacking one of the three major anticoagulants do not survive, indicating that all of the pathways are required to regulate the clotting cascade.[65]

Under normal conditions, blood flows from arteries, through capillaries and returns to the heart via the veins. While the pressure is high in arteries, the veins are a low-pressure system in which the blood moves against gravity, and blood flow is maintained by skeletal muscle contractions squeezing blood through the veins while the venous valves prevent back-flow. In situations or conditions preventing normal function of the skeletal muscles and normal blood flow, a generalized venous stasis may occur. Immobilization, surgery, hospitalization, and pregnancy are all well-known risk factors for VTE that may cause reduced blood flow and stasis. A localized stasis in the venous valve pockets is likely to play an important role in the pathogenesis of VTE as autopsy and radiology studies have shown that venous thrombi originate in the venous valves.[66] This is emphasized by the increased risk of DVT with increasing numbers of venous valves.[67] Blood flowing past the venous valves creates a vortex flow in the valvular pockets, causing stasis and hypoxia in the bottom of the valves (Figure 2). Possibly, hypoxia activates the valvular endothelium, monocytes, and platelets, which further triggers the coagulation cascade.[66, 68] In addition, platelets and leukocytes may be activated by malignancies or infection.[69-71]

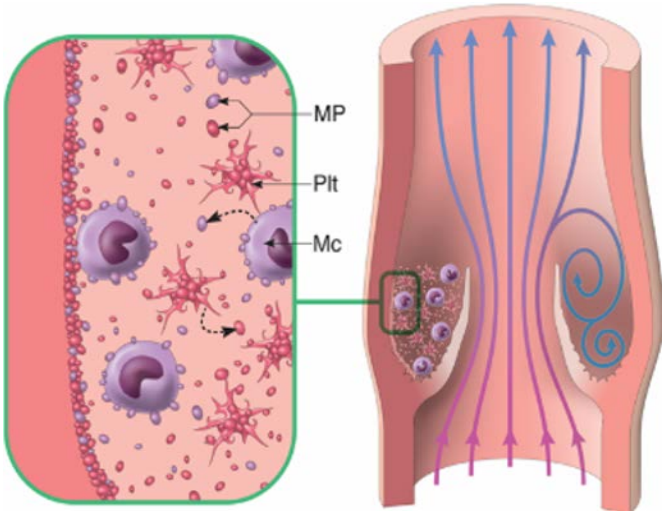


Figure 2. The pathophysiology of thrombus formation in the venous valves. Due to vortexes, blood is trapped in recesses of the valves pockets. The hypoxia that accrues promotes prothrombotic processes in the endothelial cells, platelets (Plt) and leukocytes (monocytes [Mc] in particular). Activated monocytes and platelets bud off microparticles (MP) containing TF, which triggers the extrinsic pathway of the coagulation cascade. (Figure by Roy Lysså)

Plaque formation and plaque rupture play a key role in the pathogenesis of arterial CVD, but the role of vessel wall injury in the development of VTE is less clear. The vessel wall may be injured (for example due to trauma,[72] surgery or central venous catheters) and cause thrombosis through exposure of TF and cellular RNA.[73] However, a histological study found no evidence of endothelial damage for most thrombi.[74] Although there is no direct injury, alterations in the valvular endothelium (due to hypoxia, as described above) and imbalance between pro- and anticoagulant factors may explain why

thrombi can occur.[64] Brooks and colleagues showed that vascular endothelial proteins important for activation of protein C (endothelial protein C receptor and thrombomodulin) were increased in valvular pocket endothelium compared to endothelium of the vein lumen. Variations in the up and down-regulation of anticoagulant proteins in valvular pockets may be associated with thrombus formation.[75] In addition, activated endothelial cells can downregulate the expression of endothelial protein C receptor and thrombomodulin, and upregulate expression of TF.[76]

Hypercoagulability**,** or thrombophilia, is the term used for the increased tendency of thrombus formation. Thrombophilia can be inherited or acquired, and mechanisms include an increased concentration of procoagulant proteins, the presence of variant clotting proteins that are more procoagulant, decreased concentration or deficiency of anticoagulant proteins and/or decreased fibrinolysis.[64] For example, a mutation in the *F5* gene leads to a variant of FV (Factor V Leiden) that is more resistant to APC, and mutations causing antithrombin or protein C or S deficiency leads to reduced levels or functionality of the anticoagulant proteins (see Figure 1).[77]

1.3 Risk factors of venous thromboembolism

A risk factor can be defined as any attributes, characteristics or exposures of an individual that increases the likelihood of developing a disease or injury.[78] VTE is considered a multicausal disease,[79] and several acquired and inherited risk factors have been described.[80] The complex interactions between risk factors, causing VTE in some individuals, but not others, may be explained by the thrombosis potential model, first described in 1999.[79] The model shows how combinations of different risk factors and provoking factors may cause the thrombosis potential to exceed the thrombosis threshold (Figure 3).

The person in Panel A has an underlying thrombophilic trait (e.g. FVL), and risk is increasing with increasing age. Early in life, there is a major transient provoking factor (e.g. surgery), but the thrombosis potential does not exceed the thrombosis threshold. Later in life, the same person experiences another major transient provoking factor (e.g. acute illness with immobilization), the thrombosis potential rises above the threshold, and the person experiences a VTE. The thrombosis potential remains increased following the incident VTE event, and a subsequent minor provoking factor (e.g. estrogen therapy) is enough to cause a recurrent VTE. Note that the combination of age and FVL exceeds the additive effects of age and FVL, indicating positive interaction between the two risk factors. In Panel B, there is no underlying thrombophilic trait, and neither a minor nor a major provoking factor is enough to push the potential over the threshold early in life. Later in life, however, the person gets a persistent provoking factor (e.g. cancer). Although the persistent provoking factor

alone is not enough for the potential to exceed the threshold for this person, an additional (minor or major) transient factor pushes the potential higher than the threshold, and the person experiences an incident VTE.

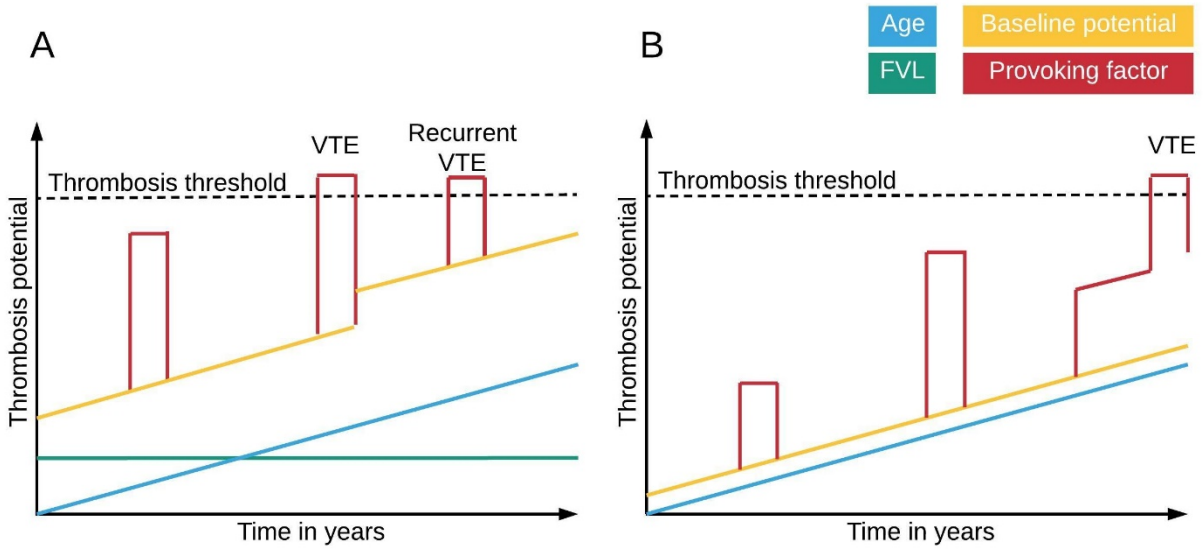


Figure 3. The thrombosis potential model. The blue line represents a risk factor that increases over time, e.g. age, and the green line represents risk factors that are stable over time, e.g. Factor V Leiden (FVL) or other thrombofilic traits. The yellow line represents the combination of age and FVL and red bars represent provoking factors. Early in life, the provoking factor is not enough to reach the thrombosis threshold and the subjects remains free of VTE. Later in life, provoking factors may cause the thrombosis potential to exceed the thrombosis threshold, dependent on the subject's baseline potential (i.e. baseline risk). The subjects may experience an incident or recurrent VTE. (Adapted from Roach et al, Journal of Thrombosis and Haemostasis 2015 and Rosendaal, Lancet 1999)

Minor transient provoking factors are associated with low risk of incident VTE, but high risk of recurrent VTE,[49] indicating that subjects who experience an incident VTE triggered by a minor risk factor have a higher baseline risk (i.e. their baseline thrombosis potential is closer to the thrombosis threshold) and thereby a higher risk of recurrent VTE. The same could be true for patients with unprovoked VTE. Conversely, major transient provoking factors are associated with a high risk of incident VTE because the trigger is strong enough to push the thrombosis potential over the thrombosis threshold, independent of the baseline risk. However, when the risk factor is removed and the thrombosis potential is back to normal, the risk of recurrence is low.[49, 81]

1.3.1 Hereditary risk factors

There is a high heritability of VTE. A family history of VTE is an independent risk factor for VTE,[82] and family and twin studies indicate that genetic risk factors account for 50-60% of VTE risk.[83-86] ***Inherited thrombophilia*** can be caused by two main mechanisms: gain-of-function of procoagulant factors and loss-of-function of anticoagulants.[77] The following section will focus on the different mechanisms of inherited thrombophilia and exemplify how and to what extent well-known prothrombotic genotypes affect the risk of VTE.

Gain-of-function is caused by mutations in genes coding for procoagulant proteins, leading to increased production and concentration of a normal protein (e.g. prothrombin G20210A and non-O blood type), impaired down-regulation of a normal protein (e.g. FVL) or, rarely, synthesis of a hyperactive protein (factor IX Padua).[77] The rs1799963 mutation in the *F2* gene, more commonly known as prothrombin G20210A, leads to a high plasma level of prothrombin (see Figure 1),[87] and possibly reduced inactivation of factor FVa by APC.[88] The prothrombin G20210A variant is associated with a 1.5 to 3-fold increased risk of VTE,[87, 89] and it is present in 2% of the normal population.[90] The non-O blood type is one of the most common genetic risk factors for VTE, and the risk is probably mediated through increased levels of von Willebrand factor (vWF) and FVIII.[91] Non-O blood type is associated with 1.5 to 2-fold increased risk of VTE,[89, 92] and the variant is present in about 60% of the Norwegian population.[93] An example of a mutation causing impaired down-regulation of a normal protein is the rs6025 mutation in the *F5* gene, commonly known as FVL. The mutation leads to a missense mutation (the amino acid arginine is replaced by glutamine) in FV, leading to APC-resistance and consequently reduced inactivation of FVa.[94] The mutation is present in about 5% of the healthy population,[95, 96] and is associated with a 2.2 to 3-fold increased risk of VTE.[89, 96, 97] A mutation in the *F9* gene, causing hyperfunctional FIX (8-fold the normal activity), was detected in an Italian family with juvenile VTE.[98] The mutation has been named factor IX Padua, and has not been found in other cohorts of patients with VTE.[77]

The other mechanism of inherited thrombophilia is loss-of-function of the anticoagulant proteins (see Figure 1). These mutations are associated with a higher risk of VTE than the gain-of-function mutations, but are less frequent.[99] Deficiency in antithrombin, protein S or protein C is caused by reduced concentration and/or low protein activity.[77] The prevalence of antithrombin deficiency is approximately 0.02% in the general population and up to 2% in VTE patients.[90] The prevalence of protein S and protein C deficiency is about 0.2% in the general population and 2-3.4% in VTE patients.[90] Deficiency of the anticoagulant proteins are associated with a 10 to 20-fold increased risk of VTE.[100, 101]

In 1965, Olav Egeberg described the first family with an identified thrombophilia, caused by antithrombin deficiency.[102] During the 1980s and 1990s, more prothrombotic genetic variants, such as protein C and S deficiency, FVL and prothrombin G20210A, were discovered.[87, 94, 103] Since then, genome-wide association studies (GWAS) have allowed identification of more single nucleotide polymorphisms (SNPs) associated with VTE.[99, 104] Although the new SNPs display weaker associations with VTE, the SNPs may be of clinical significance if they interact with other risk factors of VTE, giving supra-additive risk estimates. For example, a recent study reported that the combinations of cancer and variants in the *F5* gene (rs6025 and rs4524) yielded a synergistic increase of VTE risk.[105] Furthermore, the combination of these may improve prediction models of VTE.[106] In 2013, de Haan and colleagues proposed the inclusion of selected SNPs in a VTE prediction model. The genetic score based on the 5 SNPs most strongly associated with VTE performed as well as the score of 31 SNPs, and combining the genetic and non-genetic risk scores improved the diagnostic accuracy of the prediction model.[106] Nonetheless, the authors concluded that subgroups of high-risk persons, in whom genetic profiling will be cost-effective, must be identified for the genetic risk scores to become clinically relevant.[106]

In total, 17 genes have been robustly demonstrated to be associated with VTE,[107] however, they only explain 15-20% of the VTE heritability.[85] This suggests that much remains to be done to understand the genetics and epigenetics of VTE.

1.3.2 Acquired risk factors

There are several well-established acquired risk factors for VTE. These include, but are not limited to, age, obesity, cancer, hospitalization, surgery, trauma, acute medical conditions, immobilization, pregnancy and puerperium, and estrogen treatment.[108-110] Cardiovascular risk factors and risk of VTE will be discussed in section 1.3.3.

Advancing age is a strong risk factor for VTE, and the incidence increases with increasing age. Studies have reported an annual incidence around 800 per 100,000 in those ≥ 80.[35, 37] In a sex-adjusted analysis, people > 70 years had an 11-fold increased risk of VTE (HR 10.5, 95% CI 7.8-14.2) compared with those < 50 years of age.[17] The reasons for the increased risk in the elderly is not fully understood. Although the increased risk cannot be attributed to a higher incidence of cancer,[111] cumulative clustering of other risk factors with increasing age may explain some of the excess risk. Increased levels of D-dimer, C-reactive protein (CRP), vWF, tissue plasminogen activator, FVIII and fibrinogen in the elderly may indicate increasing activation of blood coagulation and inflammation.[112-114] Further, the increased risk in the elderly may be attributed to age-related degeneration of venous valves and

decreased compliance of the vein walls.[66] Lastly, some of the excess risks in the elderly may be due to reduced muscle strength and a less effective skeletal-muscle pump.[115]

Obesity, defined by the World Health Organization (WHO) as BMI ≥ 30 kg/m^2,[116] is associated with a 2 to 3-fold increased risk of VTE compared with subjects with BMI < 25 kg/m^2.[17, 117] Using a population-based cohort, Heit and colleagues estimated that 33% of unprovoked VTEs could be attributed to overweight and obesity.[118] Other measures of obesity, such as waist circumference (WC), hip circumference and waist-hip ratio, were also associated with increased risk of VTE.[119, 120] In fact, WC showed higher risk estimates for VTE and identified more subjects at risk of VTE than BMI.[119, 121] In addition, weight gain itself has been shown to increase the risk of VTE, especially in already obese subjects.[122] As obesity is associated with elevated iliofemoral venous pressure[123] and because venous flow in the lower extremities differs significantly between healthy obese and non-obese individuals, obesity-induced stasis has been suggested as a mechanism behind the association between obesity and VTE.[124] Other possible mechanisms include obesity-driven chronic inflammation and impaired fibrinolysis.[125-127]

In 1865, Armand Trousseau described an association between ***cancer*** and VTE. Since then, many studies have confirmed the association. Subjects with cancer have a 4 to 7-fold increased risk of VTE compared with subjects without cancer,[128-130] and overall, approximately 20% of VTE cases could be attributed to malignancy.[109] Risk of VTE is highest the initial 3-12 months after cancer diagnosis,[128, 130, 131] and several scientists argue that therapeutic interventions (e.g. surgery or chemotherapy) and hospitalizations are possible explanations for this.[128, 132] Risk of VTE seems to vary among different types of cancer and cancer stage, with risks being highest for patients with cancers of the pancreas, brain, and lung,[130, 133] and for patients with more advanced cancer.[128, 132, 134] Of note, HRs of VTE were substantially reduced when competing risk by death was taken into account.[131] This suggested that the high risk of VTE in certain cancer types may be due to high mortality in these cancers and that the apparent high risk immediately after diagnosis is explained by poor prognosis.[131] Furthermore, the risk of VTE was similar in the periods six months before and six months after cancer diagnosis, and as it is reasonable to assume that subjects were unexposed to treatment-related factors in the prediagnostic period, the study implies that cancer itself is an important risk factor for VTE.[131] The pathophysiology of cancer-related VTE can be explained by Virchow's triad. Cancer causes a hypercoagulable state with increased activation of the coagulation cascade,[135] tumor invasion or cancer treatment can lead to vessel wall injury,[136] and tumors can cause venous stasis by direct compression of blood vessels.[137]

Hospitalization is a strong risk factor for VTE. One study found that the age- and sex-adjusted incidence rate of VTE in hospitalized patients were 960 per 10,000 PY, while the incidence rate in the

community was 7.1 per 10,000 PY.[138] Furthermore, calculations showed that 59% of VTE cases could be attributed to institutionalization, and hospitalization for surgery and for medical conditions accounted for similar proportions of the cases (24% and 22%, respectively).[109] Several risk factors can be present during hospitalization, such as surgery, acute medical conditions, and immobilization. As previously mentioned, ***surgery*** is categorized as minor or major transient provoking factors, depending on the type of surgery and duration of general anesthesia. Major surgery, broadly defined as operations requiring ≥ 30 minutes of general anesthesia, carries a high risk of VTE. Procedures conferring highest risk of VTE were invasive neurosurgery (HRs ranging from 4 to 40) and orthopedic surgery (HRs ranging from 3 to 12).[129, 139] Several ***acute medical conditions*** are associated with increased risk of VTE, including myocardial infarction and stroke (discussed in section 1.4), infections, respiratory diseases, congestive heart failure, and autoimmune diseases.[8, 129, 140-143] Institutionalization due to an acute medical condition was associated with an 8-fold increased risk of VTE (HR 8.0, 95% CI 4.5-14.2).[129] Although risk assessment models (e.g. the Padua Prediction Score for medical patients),[144] have been developed to help discriminate between patients at high and low risk of VTE, studies show that only 60-65% of surgical patients and 35-40% of medical patients with high risk of VTE received appropriate prophylaxis.[145-147]

Immobilization leads to stasis, which is one of the main causes of VTE. Immobilization accompanies many surgical and medical conditions and probably mediates some of the association between these conditions and VTE. The definition of immobilization and strengths of risk estimates varies. In one study, immobilization, defined as total confinement to bed and/or armchair, was associated with a 6-fold increased risk of VTE (HR 5.6, 95% CI 2.3-13.7).[148] However, another study found a 1.8-fold (HR 1.76, 95% CI 1.27-2.44) increased risk of VTE in patients with total body immobility.[149] In the Tromsø study, immobilization, defined as bedrest for at least three days, Eastern Cooperative Oncology Group (ECOG) score of 4 or other specified immobilizing factors, was associated with a 38-fold increased risk of VTE. Immobilization and infection had synergistic effects on VTE, yielding an odds ratio (OR) of 141 (95% CI 66-298).[141] In a study from 1972, Warlow and colleagues reported that stroke patients who did not receive anticoagulation had a venous thrombus in 60% of the paralyzed legs and in 7% of non-paralyzed legs.[150] Although some degree of immobilization occur during prolonged travel, the association between VTE and prolonged travel is controversial.[80, 148, 149] A case-control study reported that traveling for more than four hours was associated with a 2-fold increased risk of VTE, and the risk was similar in those traveling by plane, car, bus or train. This indicates that it is the immobilization, rather than the plain travel itself, that increases the risk of VTE.[151]

1.3.3 Cardiovascular risk factors

Shared risk factors have been proposed as a possible mechanism for the association between arterial CVD and VTE.[152] Many studies have investigated the association between traditional cardiovascular risk factors and VTE, but the results are conflicting. Only age, obesity and FHMI have consistently been associated with VTE.[17-19, 27, 28, 117] Age and obesity as risk factors for VTE have been discussed in detail, and the following section will focus on some of the remaining cardiovascular risk factors and risk of VTE.

Results regarding the association between ***sex*** and incident VTE are conflicting. While some studies have shown similar incidence and risk of VTE in men and women,[17, 37, 153] others have found an overall higher incidence and risk of VTE among men.[19, 20, 37, 43] As previously mentioned, women of reproductive age have a higher incidence of VTE than men at the same age, whereas men have a higher incidence in the elderly.[36, 37] This may relate to differential exposure to clinical risk factors by age and sex, such as risk factors related to pregnancy and contraception, among younger women.[37] In a population-based case-control study, Roach and colleagues showed that the risk of incident VTE was twice as high in men as in women when female reproductive risk factors were taken into account, supporting that male sex is a risk factor for incident VTE.[154] Furthermore, because the age-specific incidence is different in men and women, the risk related to the sex would depend on the age distribution of the study population. Lastly, the sex difference in risk of VTE may partly be explained by an increased risk of VTE with increasing body height.[155, 156]

Evidence support that there is no association between ***hypertension*** and VTE. One case-control study reported a reduced risk of VTE in subjects with blood pressure in the highest quintile,[28] and a cohort study reported a HR of 1.51 (95% CI 1.13-2.01) in men with diastolic blood pressure in the highest quartile.[20] Nevertheless, most studies found no association between hypertension and VTE.[17-19]

Dyslipidemia is the collective term for abnormal levels of lipids (i.e. high levels of low-density lipoprotein [LDL], low levels of high-density lipoprotein [HDL] and/or high levels of triglycerides) in the blood. Although some case-control studies have reported an association between dyslipidemia and VTE,[22, 25] the majority of studies show no association with VTE.[17-20, 157] The positive results in the case-control studies may be due to limitations of the study design, such as reverse causation, selection bias or unmeasured confounders.

Diabetes is a strong risk factor for arterial CVD, but not for VTE. A few studies have reported an association between diabetes and VTE, however, authors were not able to adjust for BMI.[19, 21] The majority of studies found no association between diabetes and VTE when analyses were adjusted for

BMI.[17, 18, 20, 23, 158, 159] The metabolic syndrome is a cluster of cardiovascular risk factors, including abdominal obesity, insulin resistance, hypertension and dyslipidemia,[160] associated with increased risk of CVD and mortality.[161] The syndrome has been associated with unprovoked VTE[162] and recurrent VTE.[163] However, two studies demonstrated that the risk of VTE was mediated by abdominal obesity and that none of the other components of the metabolic syndrome, alone or in cluster, was associated with increased risk of VTE.[164, 165]

Some studies found an association between current/former ***smoking*** and VTE,[24, 166, 167] and some found a dose-dependent association,[20, 167-169] whereas several studies have failed to find an association between smoking and VTE.[17-19, 28] A meta-analysis from 2013 reported a 1.3-fold (95% CI 1.24-1.37) increased risk of VTE in current smokers compared with never smokers, and a dose-dependent association with 6% increased risk of VTE per additional pack-year, in models adjusted for BMI. The risk was increased for both unprovoked and provoked VTE.[170] In contrast, a large Danish cohort study found an association between current smoking and provoked VTE, but not between smoking and unprovoked VTE or VTE provoked by provoking factors other than cancer.[167] Furthermore, a study including participants from the Tromsø study reported an association between heavy smoking and provoked VTE. However, the association disappeared when a cause-specific model was applied (i.e. eliminating possible mediation by MI and cancer), suggesting that smoking-attributable diseases or other predisposing factors may mediate the apparent association between smoking and VTE.[171] Proposed mechanisms for the association between smoking and VTE include a smoking-induced procoagulant state, increased inflammation, and reduced fibrinolysis.[170, 172]

Results regarding a possible association between ***physical activity*** and VTE are diverging. Some studies have shown a protective effect of physical activity on risk of VTE,[173] and provoked VTE in particular.[18] Some studies found an increased risk of VTE in those physically active,[18, 174] while other studies have failed to find an association.[19, 20, 175] In several of the studies, authors were unable to adjust for BMI.[18-20] The lack of standardized assessment methods and definitions of physical activity complicates the interpretation of the existing results. Plausible mechanisms for a beneficial effect of physical activity might be improved function of the calf muscle pump function and increased fibrinolysis.[176, 177]

Socioeconomic status, often measured by education, occupation and income, is closely related to health, and coronary heart disease in particular.[178, 179] However, few studies have investigated the association between ***education level*** and VTE, and results are conflicting.[19, 20]

Growing evidence suggests an association between ***FHMI*** and VTE. In 2008, Brækkan and colleagues were the first to address the association and found a 1.3-fold increased risk of VTE in a

multivariable-adjusted analysis (HR 1.27, 95% CI 1.01-160).[26] One case-cohort and one case-control study confirmed the association with equal magnitude of risk estimates,[28, 29] but the study stratifying by ethnicity found no association between FHMI and VTE in blacks (not further specified).[29] The association between FHMI and VTE could potentially be mediated by an increased risk of MI. To address this problem, the authors in one study applied a cause-specific model and found a 1.3-fold increased risk of VTE in analyses adjusted for cardiovascular risk factors.[27] The risk was particularly high for unprovoked VTE and increased with increasing numbers of affected relatives, which pointed towards shared environmental or genetic risk factors.[26, 27] In contrast, subjects with a parental history of MI had a 3% increased risk of VTE (standardized incidence ratio of 1.03, 95% CI 1.01-1.04) in a large registry-based study.[180] However, this study defined FHMI as MI in a first-degree relative regardless of the relative's age at the event, whereas the other studies defined FHMI as MI in a first-degree relative below the age of 60. This, in addition to limited information on potential confounders, might explain the diverging results.

Results regarding the associations between many of the cardiovascular risk factors and VTE are inconsistent. Overall, the majority of studies that found an association between cardiovascular risk factors and VTE were retrospective,[21, 22, 25, 28, 173] whereas most prospective studies reported no association.[17-20, 23, 159, 175] In most cohort studies, risk factors are assessed at baseline and related to outcomes occurring several years later. However, the status of a risk factors can change over time. For example, people can gain weight, stop smoking or get increased blood pressure during follow-up. Random measurement errors, temporary fluctuations, and changes in exposure over time generally lead to regression dilution bias,[181] a phenomenon that results in an underestimation of the true association between exposure and outcome. As most of the cardiovascular risk factors are modifiable, changes during follow-up may have influenced the risk estimates of VTE cohort studies. Thus, we cannot exclude that there are weak associations between the cardiovascular risk factors and VTE, which we are unable to detect because of regression dilution bias. Regression dilution bias can be addressed by performing time-varying analyses (requires repeated measurements of all participants) or correct the risk estimates by a regression dilution ratio (requires repeated measurement of a subsample of the participants).[182, 183] Using the latter approach, a previous study reported that a single baseline measurement of cholesterol and diastolic blood pressure resulted in a 47% and 76% underestimation of the association with coronary heart disease risk in the third decade of follow-up, respectively.[184]

1.4 Association between arterial cardiovascular disease and venous thromboembolism

1.4.1 Arterial cardiovascular disease and risk of venous thromboembolism

Arterial CVD and VTE have traditionally been considered as separate diseases. However, several studies performed during the last decades have pointed towards a potential bidirectional association between arterial CVD and VTE.[11, 14-16, 185]

A growing amount of evidence support an association between arterial CVD and subsequent VTE. Some studies investigating the association between MI and VTE show that patients with MI have a 1.3 to 1.5-fold higher risk of subsequent VTE.[5, 7, 186] However, others have failed to find a relationship,[187, 188] and one cohort study reported a reduced risk of VTE in patients with arterial events.[6] When the positive associations were investigated in detail, the risk of VTE was higher when the MI occurred less than three months before the VTE diagnosis, as compared with more than three months.[7, 185] Furthermore, the risk was higher for PE than DVT,[7, 185] and reported risks for unprovoked and provoked events were similar.[7, 186] The results from these studies must be interpreted with caution, as many of them are retrospective and therefore unable to determine causality,[7, 185-188] or because they have limited validation of CVD, VTE, and potential confounders.[7, 185, 186, 188]

Furthermore, there seems to be a strong association between stroke and subsequent VTE. A study in which stroke patients were screened for thrombosis (using ^{125}I fibrinogen) showed that around 50% developed DVT within 2 weeks in absence of thromboprophylaxis,[189] and a small cohort study of 111 Asians detected DVT in 30% of patients after 10 days and in 45% of patients after 30 days.[10] In the CLOTS trial, which investigated the effect of compression stockings in stroke patients, DVT was detected in 11.4% of patients after eight days, and 14.5% after 28 days.[9] In a large case-control study, the OR of VTE was 1.31 (95% CI 1.17-1.48) in patients with a previous hospital diagnosis of stroke, and the risk was substantially higher if the stroke occurred within three months before the VTE (OR 4.41, 95% CI 2.92-6.65).[7] Risk factors for developing VTE included severe stroke,[11, 13] lower limb paresis,[12, 190] age[10, 190] and CRP.[191] VTE after stroke is associated with high mortality. PE account for 13-25% of early deaths after stroke,[189] and one study showed that sudden death occurred in 50% of PE patients with previous stroke.[192] There are several evident limitations potentially explaining the imprecise results, including different study designs, small study populations with different ethnicity, limited validation of exposures, outcomes and potential confounders and missing information on the use of anticoagulant prophylaxis (yes/no, type and duration). Limiting data exists regarding the association between ischemic stroke and VTE in the general population.

Few studies have investigated the association between peripheral vascular disease (PVD) and VTE. An autopsy study, which found no association between coronary thrombosis and VTE, found an increased risk of VTE in relation to the presence of PVD (OR 1.7, 1.6-1.9).[188] A retrospective cohort of 302 patients and controls investigated the risk of VTE after arterial events but did not give a specific risk estimate after PVD due to a low number of outcomes (n=1).[193]

1.4.2 Atherosclerosis and venous thromboembolism

Atherosclerosis is characterized by the presence of atherosclerotic plaques. The vessel walls have three concentric layers - *intima*, *media,* and *adventitia*. The *intima* is the innermost layer (i.e. closest to the vessel lumen) and consists of endothelial cells and underlying extracellular matrix (ECM). It is separated from the *media*, which mainly consists of smooth muscle cells and ECM, by an elastic membrane. Atherosclerotic plaques are intimal lesions and are considered as a chronic inflammatory response of the arterial wall to endothelial injury.[194, 195] The pathogenesis include endothelial dysfunction, accumulation of lipoproteins, platelet adhesion, monocyte adhesion and migration into the vessel wall, smooth muscle cell recruitment and proliferation, and excessive production of ECM.[195, 196] Clinical consequences of atherosclerosis include mechanical obstruction in the vascular lumen, plaque rupture with acute vascular thrombosis and aneurysm formation due to weakening of the underlying vessel wall.[194]

Atherosclerosis is often measured by ultrasound assessments of total plaque area (TPA) and intima-media thickness (IMT) in the carotid artery. The prevalence of carotid atherosclerosis in the general adult population is approximately 25%,[197] and the prevalence increases with increasing age.[198] Although both TPA and IMT are independent risk factors for stroke and MI, [198-201] a meta-analysis of population-based studies showed that the presence of carotid plaques had a higher diagnostic accuracy for the prediction of future arterial CVD, compared with IMT.[202] Furthermore, studies have shown that there is no significant difference between the prevalence of atherosclerosis in the right and left carotid artery,[203, 204] and that carotid atherosclerosis correlates well with the general extent of atherosclerotic disease in an individual.[205, 206] Although the association between atherosclerosis and arterial CVD is well established, the association between atherosclerosis and VTE remains controversial.

In a case-control study from 2003, Prandoni and colleagues found a higher frequency of carotid plaques in patients with unprovoked VTE (47%) compared with patients with provoked events (27%) and controls (32%). The multivariable-adjusted OR for carotid plaques in patients with unprovoked VTE, compared with patients with provoked events and controls, were 2.3 (95% CI 1.4-3.7) and 1.8

(95% CI 1.1-2.9), respectively.[3] In the following years, the association between atherosclerosis and VTE was confirmed by other case-control studies, with 91-300 participants. Unprovoked VTE was significantly associated with coronary artery calcium on CT angiography,[207] increasing IMT[208, 209] and presence of plaques[208, 209] after adjusting for cardiovascular risk factors. Suggested mechanisms for the possible association between atherosclerosis and VTE are shared risk factors and common pathophysiological mechanisms, such as endothelial dysfunction, inflammation, platelet activation, and coagulation activation.[207-209]

Prospective studies have not shown an association between subclinical atherosclerosis and VTE.[4-6] In a study with nearly 16,000 participants aged 45-64 recruited from the general population, there was no association between atherosclerosis, as measured by IMT and TPA, and VTE in the adjusted models.[5] In another cohort study, with participants above 65 years of age, any subclinical atherosclerosis was associated with a reduced risk of VTE (adjusted HR 0.60, 95% CI 0.39-0.90). This was mostly explained by an inverse association of high-risk carotid plaques and VTE.[6] To ensure appropriate measurement and classification of atherosclerosis and to eliminate possible mediation of MI, Hald and colleagues calculated and compared risks of MI and VTE associated with atherosclerosis, and applied a cause-specific model. In a study of 6,300 participants aged 25-84 recruited from the general population, they found a strong association between carotid atherosclerosis and future MI, but not VTE.[4] The follow-up time in the cohort studies ranged from 11.7 to 15.4 years.[4-6] The association between the formation and progression of atherosclerosis and risk of VTE has not been investigated.

The evident discrepancy in results between the case-control and cohort studies can possibly be explained by differences in study design. In the case-control studies, atherosclerosis was measured after the VTE event occurred. Thus, it is not possible to determine the temporal sequence between atherosclerosis and VTE (an inherent limitation of case-control studies). Furthermore, the case-control studies were prone to selection bias, especially because the control groups were small (48 cases and 44 controls in the smallest study).[208] In the cohort studies, measurements of atherosclerosis were performed before the outcome, and a temporal sequence could be established. However, atherosclerosis may develop over time and a true association between atherosclerosis and VTE may have been underestimated due to regression dilution bias.

2. Aims of the thesis

The aims of the thesis were:

- To investigate the overall and time-dependent risk of VTE by ischemic stroke in a population-based cohort with validated information on exposure, outcome and potential confounders (Paper I)

- To investigate the association between the presence, formation, and progression of carotid atherosclerosis and VTE using a prospective cohort with repeated measurements, in participants recruited from the general population (Paper II)

- To investigate whether the use of repeated measurements of atherosclerotic risk factors influenced the risk estimates for VTE and MI compared with baseline measurements only, in a prospective cohort recruited from the general population (Paper III)

- To investigate if the association between a family history of myocardial (FHMI) infarction and VTE were explained by the presence of prothrombotic genotypes and to assess the combined effects of FHMI and prothrombotic genotypes on the risk of VTE in a case-cohort study recruited from the general population (Paper IV)

3. Methods

3.1 Study population – The Tromsø Study and the HUNT Study

The Tromsø study is a single-center population-based cohort study with repeated health surveys of the inhabitants of the municipality of Tromsø. It was initiated in 1974 to determine the causes of the high cardiovascular mortality in Norway and to develop interventions to prevent MIs and strokes.[210] Seven surveys have been conducted and the study now includes a wide range of examinations and diseases. The surveys used for the papers in this thesis were conducted in 1994-1995 (Tromsø 4), 2001-2002 (Tromsø 5) and 2007-2008 (Tromsø 6). To these surveys, the entire (Tromsø 4) or parts of the population (Tromsø 5 and 6) aged 25 years or older were invited to participate, and 27,158, 8,130 and 12,984 participants attended in Tromsø 4, 5 and 6, respectively. Attendances were high, ranging from 79% in Tromsø 5 to 66% in Tromsø 6. In the fourth survey, all inhabitants aged 55-74 years and a random 5-10% sample in the other age groups were invited to a second, more extensive examination. Subjects who attended the second visit in Tromsø 4, in addition to random samples within different age-groups of the fifth and sixth surveys, were eligible for the second visit of Tromsø 5 and Tromsø 6. In all papers, participants with a history of VTE before baseline were excluded.

The Nord-Trøndelag Health (HUNT) Study was primarily designed to determine the prevalence of hypertension, diabetes and undiagnosed tuberculosis, and to evaluate the quality of health care provided to these patients. The first survey was conducted in 1984-1986, and 74,599 participated (attendance of 88%). In 1995-1997, the second survey of the HUNT Study (HUNT 2) was conducted. The main objectives of this survey focused on important public health issues, such as cardiovascular disease, diabetes, obstructive lung disease, osteoporosis, and mental health. In HUNT 2, all individuals at the age of 20 and older living in Nord-Trøndelag County were invited, and 66,140 participated (71%).[211] The third survey of the HUNT Study was completed in 2008, and the fourth survey started in 2017.

Paper I and III in the thesis were based on information from Tromsø 4-6, and the participants were followed from enrollment in 1994-1995 until December 31, 2010. In Paper I, participants who developed ischemic stroke during the study period contributed with unexposed person-time from inclusion to the date of ischemic stroke, and then with exposed person-time from the date of ischemic stroke and onwards. Paper II includes participants attending one or more extensive examination in Tromsø 4-6, and participants were followed from the date of enrollment until December 31, 2012. Paper III included subjects enrolled in the fourth survey who attended or was supposed to attend the fifth and sixth survey. Subjects who were re-invited after Tromsø 4, but failed to attend one or more

visits were excluded from the follow-up, while subjects who moved or died were included and censored at the date of migration or death. Paper IV was based on information from Tromsø 4 and HUNT 2, and participants were followed from inclusion (1994-1995 in Tromsø 4 and 1995-1997 in HUNT 2) until December 31, 2008, in HUNT 2 and December 31, 2012, in Tromsø 4.

3.2 Exposure assessment

3.2.1 Ischemic stroke

Ischemic stroke was defined according to the WHO definition (i.e. an acute disturbance of focal or global cerebral function with symptoms lasting ≥ 24 hours or leading to death of presumed vascular origin),[212] when CT or MRI scans or autopsy had ruled out brain hemorrhage. The national 11-digit identification number allowed linkage to national and local diagnosis registries. Possible cases of incident ischemic stroke were identified by searching hospital discharge diagnosis registry at the University Hospital of North Norway (UNN) and the National Causes of Death Registry at Statistics Norway, which covers participants living in Norway at the time of death regardless of the place of death. The 9th revision of the International Classification of Diseases (ICD-9) codes 340 to 438 was used from 1994 to 1998, and the 10th revision of ICD codes (ICD-10) I60 to I69 were used thereafter. Manual text searches were used until 2001 when medical records became digital, and electronic text searches were used thereafter. To ensure case completeness, manual and/or electronic text searches were performed in all participants with ICD-9 codes 410-414, 427 and 789-799 and ICD-10 codes I20-I25, I47.1, I48, R96, R98, and R99. Medical records, autopsy records and death certificates were retrieved for case validation by an independent end-point committee.

3.2.2 Cardiovascular risk factors

Information on cardiovascular risk factors was collected by physical examination, non-fasting blood sampling, and self-administered questionnaires, and the collection of data was repeated at each survey. Height and weight were measured with participants wearing light clothing and no shoes. BMI was calculated by the weight in kilograms (kg) divided by height in meters (m) squared (kg/m^2). Overweight (BMI 25-29.9 k/m^2) and obesity (BMI ≥ 30 kg/m^2) was defined according to the WHO.[116] Blood pressure was measured three times with an automatic device (Dinamap Vital Signs Monitor in Tromsø 4 and Dinamap 845XT [Critikon] in HUNT 2) in a sitting position after two minutes of rest. The average of the two last readings was used in the analyses. Subjects were defined as hypertensive if they had systolic blood pressure ≥ 140, or diastolic blood pressure ≥ 90 or if they reported current use

of antihypertensive medication. Total cholesterol, triglycerides, and HDL were measured in blood samples collected from an antecubital vein. Detailed information on handling and analyses of the blood samples have been published elsewhere.[17, 211] Hypercholesterolemia was defined as total cholesterol ≥ 6.5 mmol/L or use of lipid-lowering drugs. Low HDL cholesterol was defined as ≤ 1.03 mmol/L in men and ≤ 1.30 mmol/L in women, according to the National Cholesterol Education Program - Adult Treatment Panel III guidelines.[213] The questionnaires were used to obtain information on current smoking (yes/no), diabetes mellitus, physical activity, education and medication use, including the use of antihypertensive medication and lipid-lowering drugs.

3.2.3 Carotid atherosclerosis

The ultrasound examination was a part of the second and extensive examination at each survey. High-resolution B-mode and color Doppler ultrasonography were used to scan the right carotid artery longitudinally from the level of the clavicle, through the carotid bulb (bifurcation segment) and the proximal internal carotid segment (ICA) as far downstream as possible. An Acuson Xp10 128 ART ultrasound scanner equipped with a 7.5-MHz linear-array transducer was used in Tromsø 4 and 5, and a GE Vivid 7 with a linear 12-MHz transducer was used in Tromsø 6. Still images were reported for each plaque and digitized using the Matrox Meteor II frame grabber card and Matrox Intellicam (Matrox Imaging, Montreal, QC, Canada). With the use of Adobe Photoshop 7.0, measurements of plaque area were made by outlining the perimeter of the plaque, and the plaque area was calculated as pixel values. To ensure equal and standardized examination techniques and measurement procedures, all sonographers completed a two-month pre-study training protocol. In addition, subjects were randomly distributed among the different sonographers, who were blinded to data from the questionnaires and blood samples.[214] Inter-observer reproducibility of the ultrasound examinations was found to be good.[198]

A plaque was defined as a localized protrusion of the vessel wall into the lumen of at least 50% compared with the adjacent IMT. In each subject, a maximum of six plaques were registered in the near and far walls of the distal part of the common carotid artery (CCA), bifurcation, and ICA, respectively. TPA was calculated as the sum of all plaques. IMT was defined as the average of the mean IMT values of the near and far wall of the CCA and far wall of the bifurcation. To minimize variability in IMT during the cardiac cycle, the image capturing was standardized by recording images at the top of the R wave in an electrocardiographic (ECG) signal. Plaque initiation was defined as development of new plaques at follow-up in vessels without plaques at the previous examination, and plaque

progression as the difference in TPA between two measurements. Participants with negative progression were included in the no progression group.[198, 215]

3.2.4 Family history of myocardial infarction

To identify FHMI, subjects were asked to report whether their mother, father, sister, brother, child or none in the family had a history of MI before the age of 60 years in the self-administered questionnaires. A positive FHMI was regarded as ≥ 1 first-degree relative with a history of MI before the age of 60 years. Parental FHMI was regarded as ≥ 1 parent with a history of MI before the age of 60 years. The questionnaire picks out 80% of the confirmed MI-positive family histories.[216, 217]

3.2.5 Prothrombotic genotypes

The following SNPs were genotyped and used in Paper IV: rs8176719 in *ABO* (non-O blood type), rs6025 in *F5* (FVL), rs1799963 in *F2* (prothrombin G20210A), rs2066865 in *FGG* and rs2036914 in *F11*. In the Tromsø Study, rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*) and rs2036914 (*F11*) were genotyped with the Sequenom platform, and rs2066865 (*FGG*) with the TaqMan platform, as previously described.[218] The HUNT Study performed genotyping using the Illumina HumanCore Exome array.

Participants were considered carriers of the prothrombotic risk gene when one or two risk alleles were present. We did not differentiate in hetero- and homozygotes due to few homozygote study participants. The only genetic variant with a minor allele associated with a reduced risk of VTE was the rs2036914 in *F11*, and in this case, we considered the common allele as the risk allele.[219] For rs8176719 (*ABO*), zero risk alleles were classified as O blood type, whereas one or two risk alleles were classified as non-O blood type. The 5-SNP score conceived by de Haan and colleagues was created by summarizing the number of risk alleles from the five sequenced SNPs.[106]

3.3 Outcome assessment

3.3.1 Venous thromboembolism

In the Tromsø Study, all incident VTE events were identified by searching the hospital discharge diagnosis registry, the autopsy registry and the radiology procedure registry at UNN. UNN is the only hospital in the Tromsø region, and provides all relevant radiological procedures and hospital care. The discharge diagnosis codes used from 1994-1998 were 325, 415.1, 451-453, 671.3, 671.4 and 671.9

from ICD-9. From 1999 to 2012, the relevant codes from ICD-10 were I26, I80-I82, I67.6, O22.3, O22.5, O87.1, and O87.3. Manual text searches were used until 2001 when medical records became digital, and electronic text searches were used thereafter. In the HUNT Study, incident VTE events were identified by searching the hospital discharge diagnosis registry and the radiology procedure registry at the two local hospitals in the county (Levanger Hospital and Namsos Hospital) and by searching the discharge diagnosis registry at the tertiary-care center of the region, St. Olav's Hospital in Trondheim (Sør-Trøndelag County). The discharge diagnosis codes used were ICD-9 codes 415, 451-453, 325, 362.3, 433, 557.0, 634-638 (with decimals 6 and 7), 639.6, 639.8, 639.9, 671, 673, 674 and 997.2, and ICD-10 codes I26, I80-I82, I63.6, I67.6, K55, H34.8, O08, O22, O87 and O88.

The medical record for each potential case of VTE was reviewed by trained personnel. In the Tromsø Study, a VTE was verified and recorded when clinical signs and symptoms of DVT or PE were combined with objective confirmatory tests (i.e. compression ultrasound, venography, spiral computed tomography [CT], perfusion-ventilation scan, pulmonary angiography, and autopsy), and resulted in a diagnosis made by a physician that required anticoagulant treatment (i.e. low-molecular-weight heparin [LMWH], vitamin K antagonists or similar agents, thrombolytic therapy or vascular surgery). DVTs were recorded in the upper and lower extremities, including the inferior vena cava, and at unusual sites (i.e. the mesenteric veins, portal vein and in the venous sinuses). VTE cases from the autopsy registry were recorded when the death certificate indicated VTE as the cause of death or as a significant condition contributing to death. In the HUNT study, a VTE diagnosis required positive objective confirmatory tests (ultrasonography, venography, CT, perfusion ventilation scan or echocardiogram).

VTEs were classified as DVT or PE, and if DVT and PE occurred simultaneously it was recorded as a PE. Furthermore, the VTEs were classified as unprovoked or provoked, depending on the presence of provoking factors at the time of diagnosis. In the Tromsø Study, provoking factors included surgery, trauma or acute medical conditions (i.e. MI, ischemic stroke or major infections) within the last three months, active cancer, immobilization (i.e. bed rest for more than three days, wheelchair use or long-distance travel exceeding four hours within the last 14 days prior to the event) or any other factors described by a physician in the medical records (e.g. intravascular catheter). In the HUNT Study, provoking factors were active cancer at the event or within six months after the event, trauma, surgery or marked immobilization (paresis, paralysis, prolonged bedrest due to an acute medical illness or travel for more than eight hours) within the last three months, pregnancy or puerperium at the time of the event and oral contraceptives used at the time of the event or up to one month prior to the event. In Paper I, acute medical conditions were not included as a provoking factor.

3.3.2 Myocardial infarction

Incident MI events were validated according to modified WHO MONICA/MORGAM criteria, including clinical signs and symptoms, findings in electrocardiograms, values of cardiac biomarkers and autopsy reports when applicable.[220] We included all events classified as definite, probable and possible MI (Table 2).[221] The unique nation 11-digit identification number allowed linkage to national and local diagnosis registries. Possible cases of incident MI were identified by searching the hospital discharge diagnosis registry at the UNN by searching for ICD-9 codes 410-414, 430-438 and 798-799 in the period 1994-1998, and ICD-10 codes I20-I25, I60-I69, and R96, R98 and R99 thereafter. Manual text searches were used until 2001 when medical records became digital, and electronic text searches were used thereafter. In addition, the National Causes of Death Registry at Statistics Norway was searched, allowing identification of fatal MI events that occurred as out-of-hospital deaths, including deaths that occurred outside the municipality of Tromsø. Medical records, autopsy records and death certificates were retrieved for case validation by an independent end-point committee.

Table 2. Classification algorithm for myocardial infarction (MI) in the Tromsø Study.

Definite MI	Defined by one of the following conditions: • Typical, atypical or inadequately described symptoms *and* a definite new infarction in ECG recordings • Typical symptoms *and* significantly higher myocardial enzyme and/or troponin levels • Atypical or inadequately described symptoms *and* significantly higher myocardial enzyme and/or troponin levels *and* a probable new infarction on ECG recordings • Postmortem evidence of recent MI or thrombosis
Probable MI	Defined by one of the following conditions: • Typical, atypical or inadequately described symptoms *and* a probable new infarction in ECG recordings *and* moderately increased myocardial enzyme and/or troponin levels • Typical symptoms *and* moderately higher myocardial enzyme and/or troponin levels • Atypical or inadequately described symptoms *and* significantly higher myocardial enzyme and/or troponin levels • Atypical or inadequately described symptoms *and* moderately higher myocardial enzyme and/or troponin levels *and* probable new infarction on ECG recordings • Sudden death with no evidence of non-coronary cause of death
Possible MI	An event that can be dated and for which secondary data of typical history in combination with ECG findings and/or echocardiography and/or autopsy are consistent with MI but for which no primary data source is available
Unstable angina	Angina at rest of minimal exertion and ST-depression or negative T-wave in ECG
Unclassifiable	Increase in troponins or enzymes in relation to cardiac revascularization procedures (percutaneous coronary intervention or coronary artery bypass grafting) or otherwise unclassifiable
Silent MI	Defined as one of the following, in combination with the absence of clinical symptoms: • New diagnostic Q-wave in incidental ECG • Evidence of MI on echocardiography and/or multigated acquisition scan • Evidence of MI at autopsy
No MI	The conclusion after the validation procedure is that the event does not fulfill the criteria for an acute coronary event

(Adapted from Skjelbakken et al, J Am Heart Assoc, 2014)

4. Main results

4.1 Paper I

Ischemic stroke and risk of venous thromboembolism in the general population: the Tromsø Study

Even though clinical data support a relation between stroke and VTE, the strength and time dependence of the association remains to be settled at the population level. We therefore aimed to investigate the association between ischemic stroke and VTE in a prospective population-based cohort. Participants (n=30,002) were recruited from 3 surveys of the Tromsø Study (conducted in 1994-1995, 2001-2002 and 2007-2008) and followed to December 2010. All incident events of ischemic stroke and VTE during follow-up were recorded. Cox regression models with age as time-scale and ischemic stroke as a time-dependent variable were used to calculate HR of VTE, adjusted for cardiovascular risk factors. During a median follow-up time of 15.7 years, 1,360 participants developed ischemic stroke and 722 had an incident VTE event. 57 participants experienced an ischemic stroke and a subsequent VTE event. The risk of VTE was highest the first month (HR 19.7, 95% CI 10.1-38.5) and from one to three months after the ischemic stroke (HR 10.6, 95% CI 5.0-22.5), but declined rapidly thereafter. The risk estimates were approximately the same for DVT and PE with HRs of 19.1 (95% CI 7.8-38.5) and 20.2 (95% CI 7.4-55.1), respectively. Ischemic stroke was associated with a higher risk of provoked (HR 22.6, 95% CI 12.5-40.9) than unprovoked VTE (HR 7.4, 95% CI 2.7-20.1) the first three months. In conclusion, the risk of VTE was increased during the first three months after an ischemic stroke. The particularly high risk of provoked VTE suggests that additional predisposing factors related to the stroke itself, such as immobilization, may potentiate the risk of VTE in patients with ischemic stroke.

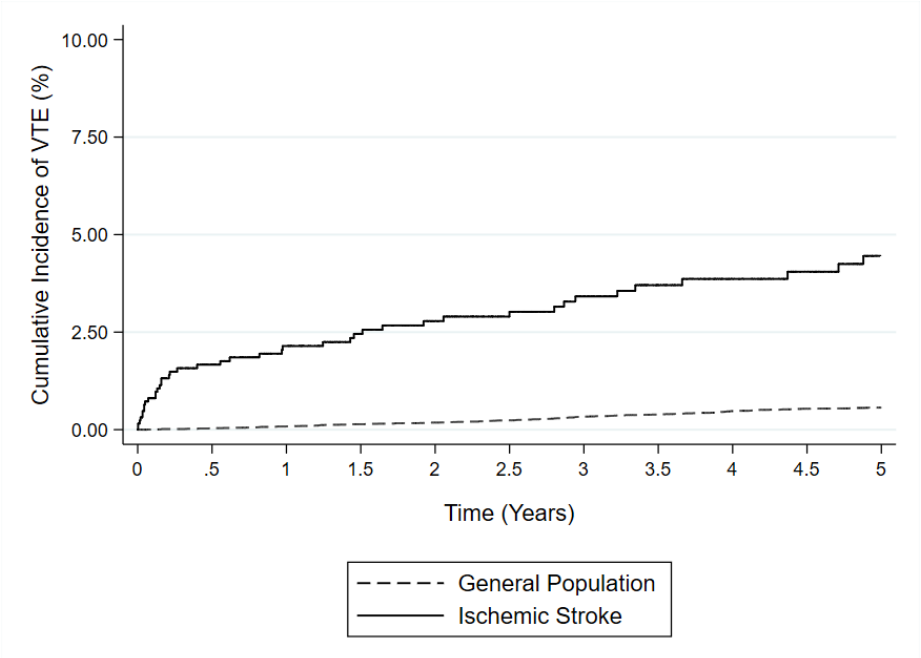
4.1.1 Erratum – Paper I

In November 2016, we published the paper *Ischemic stroke and risk of venous thromboembolism in the general population. The Tromsø Study* in the Journal of American Heart Association.

Recently, we were made aware that figure 2 in the paper was incorrect. In the figure, the values on the y-axis range from 1 to 100, while the values were supposed to range from 1 to 10. As a consequence, the cumulative incidence displayed in the figure is higher than it should be. Except for the corrected y-axis, the new figure is identical to the published figure, and the implication of the results remains the same.

The text describing the figure and the cumulative incidence is also incorrect, and a higher cumulative incidence than actually observed is reported. The correct text should be: *The cumulative incidences of VTE in subjects without and with ischemic stroke are shown in Figure 2. There was a notable increase in the cumulative incidence of VTE during the initial 3 months following an incident stroke as displayed by the substantially steeper slope in the incidence curve for subjects with ischemic stroke compared to those without ischemic stroke. The cumulative incidence of VTE was 1.5% during the first 3 months in subjects with ischemic stroke, compared with 0.02% in the general population during the same time period. The incidence curves for VTE remained essentially parallel in the period more than 6 months after the incident ischemic stroke event (Figure 2).*

Corrected figure:



4.2 Paper II

Repeated measurements of carotid atherosclerosis and future risk of venous thromboembolism: the Tromsø Study

Whether a relationship between atherosclerosis and subsequent VTE exists is controversial. Previous case-control studies have reported an association between carotid plaques and VTE, whereas cohort studies have not shown any association between carotid atherosclerosis and subsequent VTE. Because atherosclerosis may develop over time, regression dilution bias can lead to underestimation of a true association in cohort studies. We aimed to investigate the association between carotid atherosclerosis and VTE by using repeated measurements of IMT and TPA in participants recruited from the general population. Participants were recruited from the fourth (1994-1995), fifth (2001-2002) and sixth (2007-2008) surveys of the Tromsø Study. In total, 10,426 participants were included, for whom measurements of carotid IMT and TPA and potential confounders were updated at each available survey. Time-varying Cox regression models were used to calculate HR of VTE across levels of IMT and TPA, adjusted for age, sex and BMI. During a median follow-up of 10.8 years, there were 368 incident VTE events. Participants with increasing IMT were older and had a less favorable cardiovascular risk profile. There was no association between TPA and risk of VTE, and increasing IMT was not associated with increased risk of VTE (HR 0.96, 95% CI 0.86-1.07). Neither plaque formation nor plaque progression was associated with VTE (HRs of 1.00, 95% CI 0.98-1.02 and 0.96, 95% CI 0.84-1.11, respectively). Additional adjustments for traditional cardiovascular risk factors had a negligible effect on the risk estimates. In conclusion, our study shows that carotid IMT and TPA were not associated with an increased risk of VTE using a time-varying analysis with repeated measurements. Furthermore, there was no association between plaque formation of plaque progression and subsequent VTE. The findings suggest that atherosclerosis is not an intermediate for the association between arterial cardiovascular disease and VTE.

4.3 Paper III

Atherosclerotic risk factors and risk of myocardial infarction and venous thromboembolism; time-fixed versus time-varying analyses. The Tromsø Study

Single measurements of modifiable risk factors may underestimate associations with outcomes in cohorts due to regression dilution bias, especially if follow-up is long. We aimed to compare risk estimates of MI and VTE by atherosclerotic risk factors during long follow-up using time-fixed and time-varying analysis. The study included 5,970 subjects enrolled in the fourth survey of the Tromsø Study (1994-1995). Atherosclerotic risk factors, including blood pressure, lipid levels, BMI, diabetes, and smoking status, were measured at baseline, and subjects still alive at the fifth (2001-2002, n=5,179) and sixth (2007-2008, n=4,391) survey were re-measured. Time-fixed and time-varying Cox regression models were used to estimate HR for MI and VTE adjusted for age and sex. Until December 2012, there were 714 and 214 incident MI and VTE events, respectively. During a median follow-up time of 15.7 years, we found that variations in BMI, blood pressure and lipid levels were small. For these risk factors, risk estimates of MI and VTE were similar in the time-fixed and time-varying analyses. For MI, variables that changed considerably over time yielded the greatest changes in risk estimates. For example, HR for smoking was 1.80 (95% CI 1.55-2.10) in the time-fixed and 2.08 (95% CI 1.78-2.42) in the time-varying analysis. For VTE, there was a significant association with BMI and hypertension in both the time-fixed and the time-varying model. However, the association with hypertension disappeared when adjusting for BMI in addition to age and sex. For BMI, the risk of VTE was slightly lower in the time-varying analysis compared with time-fixed analysis. Our findings suggest that for MI and VTE, risk estimates based on baseline and repeated measurements correspond well. Furthermore, misclassification is a problem only in situations where the association is between exposure and outcome is strong and the exposure varies greatly during follow-up. Of the traditional atherosclerotic risk factors, only BMI was associated with VTE, suggesting that underestimation of risks by regression dilution bias is not explaining the lack of association between atherosclerotic risk factors and VTE.

4.4 Paper IV

Impact of prothrombotic genotypes on the association between family history of myocardial infarction and venous thromboembolism

A family history of myocardial infarction (FHMI) increases the risk of venous thromboembolism (VTE). We aimed to investigate the effect of prothrombotic genotypes on the association between FHMI and VTE in a case-cohort recruited from a general population. In a case-cohort analysis, cases with a first VTE (n=1,493) and a sub-cohort (n=13,072) were sampled from the Tromsø study (1994-95) and the Nord-Trøndelag Health (HUNT) Study (1995-1997). DNA-samples obtained at baseline were genotyped for rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*) and rs2036914 (*F11*). Participants not officially registered as inhabitants in Tromsø or Nord-Trøndelag at baseline (n=3) were excluded. Furthermore, we excluded participants with missing information on SNP variables (n=175), FHMI (n=2,769) and BMI (n=52). Cox regression models were used to estimate hazard ratios (HRs) for VTE and all analyses were adjusted for age, sex, and BMI. There were 1,169 incident VTEs during a median follow-up time of 12.3 years. FHMI was associated with a 1.3-fold increased risk of VTE (HR 1.32, 95% CI 1.16-1.50) and 1.5-fold increased risk of unprovoked VTE (HR 1.47, 95% CI 1.22-1.78). The risk of VTE by FHMI did not alter in analysis adjusted for the five genotypes. The combination of FHMI and the different prothrombotic genotypes did not result in an excess VTE risk. For instance, having both FHMI and non-O blood type (rs8176719) was associated with a 1.8-fold increased risk of VTE (HR 1.78, 95% CI 1.49-2.13), which approximated the sum of having only FHMI (HR 1.35, 95% CI 1.07-1.71) or non-O blood type (HR 1.38, 95% CI 1.19-1.59). Thus, FHMI and the prothrombotic genotypes had an additive effect (i.e. no biological interaction) on the risk of VTE. In conclusion, our findings suggest that the association between FHMI and VTE is not explained by rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*) and rs2036914 (*F11*). FHMI combined with prothrombotic genotypes had an additive effect on VTE risk.

5. General discussion

5.1 Methodological considerations

5.1.1 Study design

The papers in this thesis are based on data from the Tromsø Study and the HUNT Study, two prospective population-based cohort studies.

In Paper I-III, we used a ***cohort study*** design. In a cohort study, a predefined population is followed from the date of inclusion in the study until an outcome of interest occurs, or until migration, death or end of study period. An advantage with cohort studies is that it is possible to relate one or more characteristics to future outcomes, and thus study the natural history of risk factors and diseases in individuals.[222] Temporality is, among others, one of the criteria needed to provide epidemiologic evidence for causality.[223] Other criteria for determining a causal relationship are strength of the association, consistency with other studies, a plausible mechanism between cause and effect and biological gradient (dose-response relationship).[223] Thus, results from one cohort study is not enough to conclude on causality. Although a randomized controlled trial (RCT) would be the best study design to determine causality, it requires large amounts of resources, carries considerable ethical considerations and it may be impossible to carry out. For instance, it would be unethical and impossible to inflict carotid atherosclerosis on people in order to study the association between atherosclerosis and VTE.

Another advantage of the cohort study design, as compared with other observational study designs, is that several exposures and outcomes can be investigated simultaneously. The nature of the Tromsø Study allowed us to investigate the association between risk factors and two different outcomes (MI and VTE). Furthermore, if cohort studies are based on a defined and well-characterized population, the incidence rates can be extrapolated beyond the study group to similar populations elsewhere (discussed in section 5.1.7).[222] This is in contrast to case-control studies and the majority of RCTs with highly selected study participants.

Some limitations of the cohort study design merit consideration. First, cohort studies are inefficient for studying incidence and associations of rare outcomes because it would require large populations and/or many years of follow-up, and thus be very resource-demanding. However, the outcomes used in the papers of the present thesis (MI and VTE) are common in the general population. Second, change in exposure during follow-up can lead to regression dilution bias and underestimation of associations. Third, cohort studies, as well as other types of observational study designs, are prone

to bias and confounding. Advantages and limitations of the cohort study design will be discussed in the following sections.

In Paper IV, we used a ***case-cohort study*** design with subjects recruited from Tromsø 4 and HUNT 2. A case-cohort study is a variant of a cohort study, in which participants (cases and a sub-cohort) are recruited from a parent cohort after baseline. Case-cohort studies are often used when large cohorts are needed to observe enough cases, but it is not feasible to collect data on covariates for the whole cohort. As genotyping is time-consuming and expensive, the case-cohort design is optimal for investigating the aims in Paper IV.

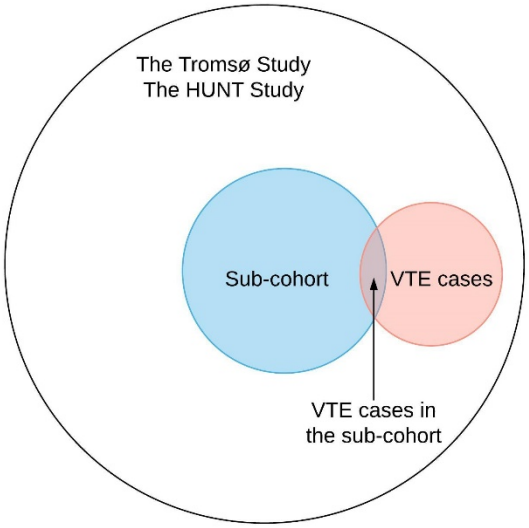


Figure 4. Case-cohort study.

Because assessments of risk factors were done at baseline, before the outcome occurred, there is a temporal sequence of exposure and outcome in the case-cohort studies. As with cohort studies, incidences, absolute risks, and relative risks can be calculated. The sub-cohort is reflecting the source population, and every person in the cohort has an equal chance of being included in the study as a control, regardless of how much time that person contributed with and whether the person developed the disease.[224] Thus, with appropriate sampling and analyses, the risk estimates in a case-cohort study are similar to risk estimates derived from the full cohort.[225] For Paper IV, all incident cases of VTE (n=1,493) and a randomly selected sub-cohort (n=13,072) from Tromsø 4 and HUNT 2 were included in the study (Figure 4). As every person had an equal chance of being included in the sub-cohort, the sub-cohort included 217 cases. After exclusion of participants not officially registered as inhabitants in Tromsø or Nord-Trøndelag, and participants with missing values for at least one of the risk alleles studied, FHMI or BMI, the study consisted of 11,618 participants with 1,169 VTE events. Because of the size of the sub-cohort, we did not make adjustments to the partial likelihood in the Cox regression analyses.[226]

5.1.2 Bias

Bias is the term for systematic errors in epidemiological research that results in incorrect estimates of the true association between an exposure and an outcome. Depending on the types of systematic errors, bias can lead to overestimation or underestimation of risk estimates. There are several types of biases, and they can be classified as either selection bias or information bias.[222]

Selection bias is a result of systematic errors in the recruitment of participants, and occur when individuals have different probabilities of inclusion in the study sample according to relevant study characteristics, i.e. the exposure and outcome of interest.[227] This type of bias is less likely to occur in cohort studies compared to case-control studies, because participants, exposed or unexposed, are recruited before the outcome develops. Nevertheless, cohort studies are prone to a type of selection bias called non-response bias (or participation bias). Non-response bias is introduced if participation rates differ between study participants with certain traits that affect the outcome (i.e. the study participants are systematically different from the target population).[228, 229] In general, participation in epidemiological studies have declined over the past years, and attendees are more likely to be female, have higher socioeconomic status, higher education, and be married.[228] In accordance to this, participation in the Tromsø Study have declined from around 83% in Tromsø 1-3 to 77% in Tromsø 4, 79% in Tromsø 5 and 66% in Tromsø 6, and non-attendees tended to be younger, were more often men and unmarried. In both the Tromsø Study and the HUNT Study, people < 40 years of age and > 80 years of age had lowest attendance.[210, 211] After HUNT 2 was completed, a non-participation study was conducted. A random sample of non-participants was contacted by telephone or letter to investigate the reasons for non-attendance. In the younger age groups, the main reasons to not participate were lack of time or having moved out of the county. In the older age-groups, many reported to have regular follow-up by a general practitioner or at the hospital and therefore did not need to attend a health survey. Approximately 10% could not attend because they were immobilized due to disease.[211] Reduced attendance in population-based studies preclude generalizability to whole populations, and results regarding the youngest and oldest populations must thus be interpreted with caution. Furthermore, there is a strong relationship between socioeconomic status and MI,[179] and low attendance among those with low socioeconomic status may have affected associations between cardiovascular risk factors and MI. However, non-response bias is of greater concern when estimating absolute risks (compared with relative risks), and most studies have found little evidence of substantial bias as a result of non-participation.[222, 228] Nonetheless, it is important to maintain a high degree of participation, and the challenge for future surveys of the Tromsø Study will be to develop methods to increase recruitment and feasibility to optimize participation.

Another type of selection bias in cohort studies is bias due to differential loss to follow-up. This type of bias would occur when the different exposure groups have a different probability of completing the study and is always of concern in cohort studies.[230] In survival analysis, subjects are censored when they are lost to follow-up, for example, due to migration or death, because it is unknown if the outcome occurs in that person or not. An assumption of censored survival time is that participants who remain in the study have the same risk of the outcome as those who are no longer under follow-up

(called non-informative or independent censoring). In all papers in the present thesis, participants were censored when they moved from the municipality of Tromsø or when they died. As there is no reason to suspect that participants that moved from Tromsø had a different risk of MI or VTE than those who stayed, simple censoring at the date of migration is adequate. Conversely, death prevents the outcome of interest to occur, and the censoring becomes informative. This situation, in which death is a competing event, is called competing risk by death and is of special concern when investigating older populations[231] and exposures related to high mortality, such as cancer.[232] The absolute risk and cumulative incidence of an event are dependent on the rate of the event and the mortality rate. Hence, competing risk by death must be taken into account when dealing with absolute risks and cumulative incidences in prognostic research.[232, 233] However, when investigating causality between an exposure and an outcome (etiological research), the exposed and unexposed individuals alive and actually at risk of developing the event of interest are compared. Censored participants contribute with exposed or unexposed person-time before the censoring event, and do not affect the hazard ratio after being censored.[233] As the papers included in the present thesis investigated etiological associations between risk factors (i.e. stroke, atherosclerosis, cardiovascular risk factors, and FHMI) and VTE or MI, competing risk of death was not taken into account.

In Paper III, we included participants who attended all three surveys, or was supposed to attend all three surveys, but died or moved during follow-up. This was to avoid selective inclusion of participants who survived the entire study period as these would more likely be healthier than those who died. However, we had to exclude participants without repeated measurements to investigate our aim, and we cannot rule out that those who were excluded differed from those who were included. Although the main aim of the study was to compare different methods, we cannot be certain that the selection did not affect the estimates.

Systematic errors in a study's data collection may lead to ***information bias***. Misclassification bias is a type of information bias which occurs if included participants are incorrectly placed in different exposure or outcome categories. There are two types of misclassification: differential misclassification and non-differential misclassification.[224] Differential misclassification occurs when the probability of misclassification differs with regards to exposure or outcome status, whereas non-differential misclassification occurs when all participants have the same probability of misclassification. As perfect tools to gather information rarely exists, most studies must assume a certain degree of misclassification.[230] Differential misclassification can lead to both over- and underestimation of the true association, whereas non-differential misclassification consistently results in an underestimation of the true association. Consequently, non-differential misclassification is generally more "accepted" than differential misclassification.[227] In cohort studies, differential outcome misclassification can occur

if exposure status affects the probability of getting diagnosed with a disease. To avoid differential outcome misclassification bias in our studies, the end-point committee was blinded to the participants' baseline risk.

Several of the variables used in our studies are self-reported through questionnaires (e.g. smoking, physical activity, and diabetes) and are potentially prone to misclassification. For example, self-reported information on smoking have shown to yield reliable estimates of true the smoking prevalence,[234] whereas the reliability and validity of self-reported physical activity are worse.[235] Although self-reported diabetes have been shown to be reliable,[236] the prevalence of self-reported diabetes in the Tromsø study is lower than expected. In 2016, WHO estimated that the prevalence of diabetes in Norway was 6.6%,[237] however, the prevalence of self-reported diabetes ranged from 2% in Tromsø 4 (1994-1995) to 5% in Tromsø 6 (2007-2008). The increasing prevalence is likely a result of a true increase in the prevalence of diabetes during the last decades and increasing awareness of diabetes in the population and among doctors. The discrepancy between the self-reported prevalence and true prevalence of diabetes is probably due to underdiagnosing of type 2 diabetes due to few symptoms. As awareness and testing of diabetes has increased during the last decades, the discrepancy between self-reported and true prevalence has decreased. Nevertheless, the degree of misclassification related to self-reported variables will be similar in those who experience the outcome and those who do not (i.e. non-differential) because baseline measurements are collected before the outcome occurs. This will lead to an underestimation of true results.

Validation of the FHMI variable in the Tromsø Study showed high concurrence between reported and confirmed diagnoses,[216] and another study validating self-reported FHMI found high specificity (97%) and lower sensitivity (68%) of a positive FHMI.[238] Furthermore, measurement errors in the physical examinations may occur, for instance, if blood pressure was measured with a defect sphygmomanometer. However, as participants answered the questionnaires and underwent the physical examinations at the start of the study, and were thus unaware of future disease, the misclassification of the self-reported variables are non-differential. To minimize non-differential misclassification, examinations were standardized, e.g. blood pressure was measured three times and the average of the last two was used, and height was measured without shoes.

Medical surveillance bias can occur if an exposure leads to closer surveillance and an increased probability of detection of an outcome.[239] This is of special concern if the outcome of interest is subclinical and exposed individuals are more likely to be examined. For instance, patients with suspected PE are examined with CT, which may also detect (subclinical) pulmonary diseases. The pulmonary diseases may be just as prevalent in the unexposed, and the apparent association is caused

by increased surveillance of patients with PE. Medical surveillance bias may be of concern in Paper I, as it is plausible that stroke patients were more closely monitored for VTE and had a higher probability of getting a VTE diagnosis compared with participants without stroke. Although VTE was thoroughly validated and the diagnosis required signs and symptoms of VTE and objective confirmatory tests, it is likely that stroke patients with signs of VTE were more likely to undergo diagnostic tests for VTE compared with unexposed participants with similar signs. This may have overestimated the incidence rate and HR among the exposed.

5.1.3 Modifiable risk factors and regression dilution bias

Regression dilution bias is a potential limitation of a cohort study with a single measurement. Regression dilution bias is caused by random (non-differential) measurement errors, temporary fluctuations *and* true changes in variables over time, and results in an underestimation of the true association between exposure and outcome.[230, 240] In agreement with this, previous studies have shown that the use of single baseline measurements of cardiovascular risk factors greatly underestimated the true association with coronary heart disease.[241, 242] Methods to reduce random measurement errors during study conduction include using standardized measurement approaches and using the average of several measurements. In study analyses, regression dilution bias can be addressed by performing time-varying analyses if repeated measurements are available for the entire or parts of the cohort.[182, 183]

The main aim of Paper III was to investigate whether the use of repeated measurements of cardiovascular risk factors influenced the risk estimates for VTE and MI compared with baseline measurements only, and if the lack of association between cardiovascular risk factors and VTE in previous cohorts could be explained by regression dilution bias. We concluded that risk estimates for MI and VTE based on baseline measurements and time-fixed analyses corresponded well with risk estimates based on repeated measurements and time-varying analyses. Only BMI was associated with VTE, indicating that possible underestimation of risks due to regression dilution bias did not explain the lack of association between cardiovascular risk factors and VTE. The risk estimates based on a single baseline measurement were generally reliable, and dilution of risk estimates was a problem in situations where the association between exposure and outcome is strong, and when the exposure status varies greatly during follow-up.

In Paper I and II, information on possible confounders was updated at each survey for those who participated in more than one survey. Repeated measurements of carotid atherosclerosis also allowed us to assess if the initiation or progression of atherosclerosis increased the risk of VTE. As we

did not have updated measurements for any of the participants of the HUNT study, we used single baseline measurements and traditional time-fixed analyses for Paper IV. Furthermore, genotypes are not modifiable, and, as Paper III suggested, risk estimates of VTE based on single measurements and repeated measurements corresponded well for BMI.

5.1.4 Confounding and mediation

Confounding is often considered as one of the main categories of bias. The concept of confounding refers to a situation where the association between an exposure and an outcome can be attributed to the influence of a third, known or unknown, variable (Figure 5).[227] A variable is a confounder if (i) it is an independent risk factor for the outcome, either causal or a surrogate for a causal factor, (ii) it is associated with the exposure, and (iii) is not an intermediate variable between the exposure and the outcome.[224, 227, 243] When investigating the presumably causal association between a risk factor *a* and an outcome *c,* an additional variable *b* would be a confounder if it is an independent risk factor for *c,* associated with *a* and not an intermediate between *a* and *c.* For instance, age is an obvious confounder for the association between grey hair and mortality. Confounders are of special concern in etiological research, in which causal relationships are investigated.[244] Mediation closely resembles confounding, but the criteria for mediation is that the mediator is a presumed causal effect of the risk factor of interest (i.e. *a* causes *b*, see Figure 5).[243] Adjusting for a mediator in regression analysis will show the direct effect of a risk factor on an outcome, by removing the indirect effect caused by the mediator. However, the mediator does not act as a *confounder* for the association, it is the reason *why* the risk factor and the outcome are associated, and exposure *a* is still causally related to outcome *c*. Mathematically, there is no difference between a confounder and a mediator, and it is not always clear whether a variable is a confounder or a mediator. If an association between a risk factor and an outcome diminishes after adjusting for a variable, we cannot conclude that the

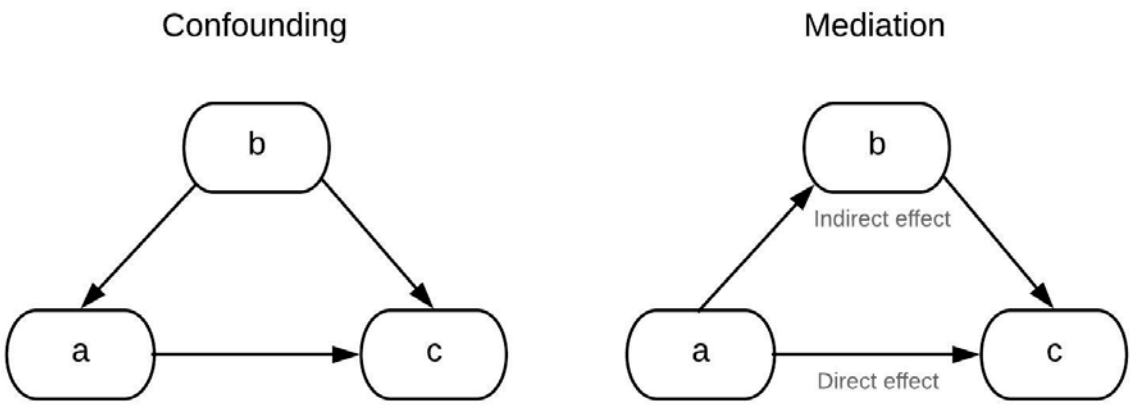


Figure 5. The concept of confounding and mediation.

unadjusted association was an artifact due to confounding. The variable could have been a mediator in the causal pathway between the risk factor and an outcome, and adjusting for the variable would underestimate the true association between the risk factor and the outcome.[243] Mediation analyses can be performed to investigate to what extent an association is mediated through a third variable.[245] Although it might be difficult, it is important to use non-statistical arguments to decide whether the third variable is a confounder or a mediator.

In RCTs, participants are randomly assigned to treatment groups, balancing potential confounding factors between the groups. Observational studies do not randomize the exposure, and failing to adjust for confounding in the analyses can result in associations that are overestimated, underestimated or even reversed, compared with the true association.[227] Strategies to minimize confounding include, among others, stratified analyses in which different strata of an exposure are analyzed separately, and regression modeling with confounders included in a multivariable model.[246, 247] In stratified analyses, participants are divided into strata (i.e. sub-groups) of the confounder, and the effect of the risk factor is measured within each sub-group. Disadvantages of stratification include reduced statistical power due to fewer participants in each sub-group, and possible additional confounding if other characteristics are imbalanced between the sub-groups. In Paper I-IV, we used multivariable analyses to determine the independent contribution of each risk factor, thereby estimating the effect of a risk factor on the outcome, adjusted for confounders.[248] It is important to note, that even though preventive measures to minimize confounding were applied, we can never rule out that unknown confounding factors may be present and lead to *residual confounding*.[249]

In all papers, analyses were adjusted for age, an important confounder for the association between the risk factors and outcomes studied. In the analyses, age was used as time-scale, with the participants' age at study enrollment being defined as entry time, and age at the VTE or censoring event (i.e. migration, death or study end) being defined as exit time. This is considered the superior way of eliminating confounding by age, as compared to age adjustments, if the hazard of the outcome is expected to change more as a function of age than as a function of time-on-study.[250]

In Paper I, we adjusted for additional risk factors in different models. Sex and BMI are known risk factors of both stroke and VTE, and thus important confounders. This is emphasized by the substantially attenuated HRs after adjustment. As previously discussed, whether other cardiovascular risk factors are independent risk factors of VTE or not is controversial. Adjusting for these risk factors

had a marginal impact on the association between stroke and VTE, suggesting that they are not confounders or mediators for the association. Unfortunately, we did not have information on immobilization or infections, which we believe are mediators for the association between stroke and VTE (Figure 6). If we were able to adjust for these variables, we could estimate the direct effect of stroke on VTE. However, it is important to remember that stroke and VTE can be *causally* related, even though potential mediators exists. Immobilization and infections would not act as confounders for the association, but they would be the *reason why* stroke and VTE are associated.

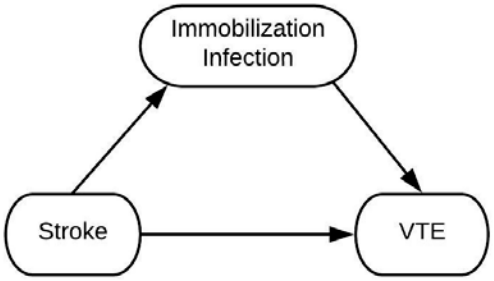


Figure 6. Immobilization and infection as possible mediators for the association between stroke and VTE.

In Paper II, we adjusted for age (as time-scale), sex and BMI. These are independent risk factors of both atherosclerosis and VTE. Other cardiovascular risk factors were added in the multivariable model, however, they did not alter the estimates. In Paper III, the main aim of the study was to compare different analyses, not to evaluate the magnitude of the risk estimates. Thus, the analyses were adjusted for age (as time-scale) and sex. We found an apparent association between blood pressure and VTE, which diminished after further adjustments for BMI, indicating that the association was confounded by BMI.

In Paper IV, we used a case-cohort design in which the sub-cohort was randomly selected from the full cohort. We adjusted all analyses for age (as time-scale), sex and BMI, all of which are possible confounders for the association between FHMI and VTE. The main aim of the study was to assess if the association between FHMI and VTE was explained by prothrombotic genotypes, i.e. if prothrombotic genotypes was a confounder, causing clustering of MI in families and VTE (Figure 7). However, when we included the prothrombotic genotypes in a multivariable analysis (together with other confounders), the association between FHMI and VTE was not affected, suggesting that the prothrombotic genotypes are not confounders and that something else drives the association.

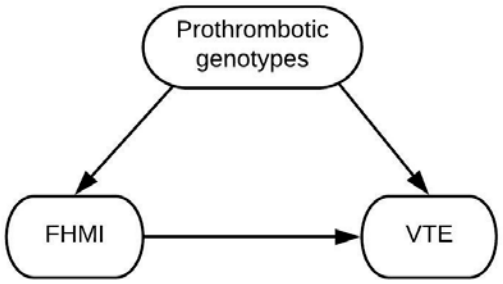


Figure 7. Prothrombotic genotypes as possible confounders for the association between FHMI and VTE.

5.1.5 Interaction

To investigate whether the effect of one risk factor on an outcome differs across the strata of another risk factor, the presence of interaction between the risk factors can be examined.[251] Interaction is also known as effect modification, and the third variable is often called the effect modifier.[227] Statistical interaction can be evaluated on an additive (absolute risk) and a multiplicative (relative risk) scale, depending on the statistical model being used. Furthermore, HRs derived from multiplicative models, e.g. Cox regression models, can be used to examine the presence of interaction on an additive scale (i.e. biological interaction).[252] In Cox regression analyses, the presence of interaction on a multiplicative scale can be assessed by entering a product term into the regression model.[251] If statistical interaction is present, data should be stratified on the effect-modifying variable. Even if there was no statistical interaction on a multiplicative scale, there might be interaction on an additive scale.[251]

An important assumption of the Cox regression model, which is used in all papers of this thesis, is the proportional hazard assumption. This assumption indicates that exposure to a certain risk factor is associated with a fixed relative increase in the risk of the outcome of interest compared with a reference hazard (i.e. among the unexposed). In other words, the Cox models assume that at any given time, the hazard in the exposed individuals is a multiple of the underlying hazard.[227] When using age as time-scale, a test for proportional hazard assumption will determine if the risk of an outcome among the exposed individuals is constant over time (i.e. increasing age), compared with the unexposed. Thus, a test for the proportional hazard assumption will also test for interaction with age.

In Paper IV, we investigated the presence of additive interaction between FHMI and the different SNPs on the risk of VTE. Synergism refers to the (positive) interaction of two or more variables that combined gives a greater effect than the sum of the individual variables. The presence of synergism between two exposures was assessed by calculating the relative excess risk due to interaction (RERI), the attributable proportion due to interaction (AP) and the synergy index (SI) according to Andersson *et al.*[253] RERI was calculated as $HR_{11} - HR_{10} - HR_{01} + 1$, where HR_{10} is the hazard ratio for the first risk factor (i.e. FHMI) in the absence of the second risk factor (i.e. prothrombotic risk alleles), HR_{01} is the hazard ratio for the second risk factor in the absence of the first risk factor, and HR_{11} is the hazard ratio when both risk factors are present. AP was calculated as $RERI/HR_{11}$ and can be interpreted as the proportion of cases in the combined group that is due to the interaction between the two exposures. Lastly, the SI was calculated as $[HR_{11} - 1]/ [(HR_{10} - 1) + (HR_{01} - 1)]$. SI can be interpreted as the excess risk from exposure to both risk factors when interaction is present, relative to the risk from exposure when interaction is absent.[252] RERI and AP equal to 0 and SI equal to 1.0 mean no interaction.[253, 254]

5.1.6 Missing data

Despite careful planning and execution of studies, missing data are common in epidemiological research, and may introduce bias.[255] Data can be missing if participants do not respond to questions in the questionnaires, if the equipment used for physical examinations fails, by loss or errors in laboratory handling of blood samples, if participants are lost to follow-up, or for other known or unknown reasons. The prime concern is always whether the available data is biased or not.[255] If an observation is missing independent of the unobserved value and other available data, the observation is missing completely at random. This would be the case if a blood sample is lost by accident. If the data missing is independent on the missing value itself, but not to other variables, the data is missing at random. This would be the case if the elderly were more likely to have measured blood pressure than the younger population. Finally, data could be missing not at random if it is dependent on the unobserved values, for instance, if a question in a questionnaire is (systematically) not answered because it is too difficult. Data missing not at random can cause bias.[255] There is no optimal way to handle missing data, but different approaches exist. Firstly, participants with missing data on covariates can be omitted from the analysis. Secondly, participants with missing information can be excluded from the study. This gives complete case analyses but can lead to decreased statistical power and selection bias if participants excluded differ from those not excluded (which will be the case unless the data are missing completely at random). Lastly, it is possible to estimate (impute) what the missing values were, based on other known covariates in the study.[255, 256] Imputation requires that the data is missing at random.

In the present thesis, missing data were handled by omitting participants with missing values for variables in the statistical analysis of that specific variable, or by excluding participants with missing information from the study. When using the first approach, the number of study participants in each analysis varied slightly depending on variables included (< 2%). In Paper II, participants who attended the ultrasound examination, but had missing information on IMT or TPA (n=490) were excluded. It is uncertain why these measures were missing and how they affected risk estimates. Given the data were missing at random or completely at random, e.g. due to defect equipment a certain day, the results would not be affected. However, if the data were not missing at random, i.e. dependent on unobserved values, results could be biased. This would be the case if, for instance, those with missing information were too sick to move to the examination bench, or to obese to obtain valid measurements.

In Paper IV, we excluded participants with missing values for at least one of the risk alleles studies (n=175) and those with missing information on FHMI (n=2,769). As with all laboratory testing, there are risks of errors in measurements and handling of the blood samples used for genotyping. The

missing values for the SNPs are most likely missing completely at random, i.e. independent of the genotypes and other available variables, and exclusion of these participants would not bias the results. Assuming the participants with missing information on the FHMI variable did not answer the question, simply because they did not know or because they did not have FHMI, the values would be missing at random or not at random, respectively. The latter alternative could give biased results, and to investigate this possibility, we performed a sensitivity analysis in which participants with missing information on FHMI were included in the study and categorized as not having FHMI. The association between FHMI and VTE, albeit slightly attenuated, remained when participants with missing data on FHMI were classified as having no FHMI, indicating that those with missing information did not substantially differ from those without missing information.

5.1.7 External validity

External validity is the extent to which a study can be generalized to a population.[257] External validity is of great importance in research where the purpose is to improve public health.[258] Internal validity refers to the extent to which bias and confounding are minimized so that any difference between groups can be truthfully attributed to the exposure.[257] Both internal and external validity are essential for epidemiological research. There is not external validity without internal validity, but the presence of external validity does not guarantee internal validity (i.e. the participants are representative of the population, but there is confounding in the study).[230] Although RCTs are considered to be the best study design for minimizing the effect of bias and confounding, and thus maximizing internal validity, the external validity is usually limited due to strict inclusion and exclusion criteria.[246] Cohort studies are non-experimental, and the absence of random allocation reduces the internal validity. However, high-quality cohort studies with well-defined inclusion and exclusion criteria, as well as high attendance, enhances the chance of high external validity.

In the surveys of the Tromsø Study used in the present thesis, the entire or parts of the population were invited to participate, and the attendances were high, ranging from 79% in Tromsø 5 to 66% in Tromsø 6.[210] To HUNT 2, all individuals at the age of 20 and older living in Nord-Trøndelag County were invited to participate, and the attendance was 71%. The distribution of risk factors and incidence of VTE in the Tromsø and HUNT Study are similar to other Western populations, indicating a high degree of external validity. As previously noted, the participation rates were lower among those < 40 years of age, those > 80 years of age, and among men compared with women, threatening the generalizability in these subgroups. In Paper II, we used data from participants attending the second and extensive examination. All inhabitants aged 55-74 years and a smaller random sample in other age

groups were invited. This weakens the generalizability of the results to the underrepresented age-groups. Further, cohort studies are prone to non-response bias. Participants may be more health conscious than those who did not participate, and institutionalized elderly and ill patients are unlikely to attend health examinations. Consequently, participants in cohort studies are usually healthier than the general population, and it is likely to assume that this applies to our studies as well. However, as discussed previously, non-participation does not seem to introduce substantial bias. The population in Tromsø and Nord-Trøndelag are homogenous Caucasian populations,[210, 211] with a small Sami minority in Tromsø, and our results are likely to be generalizable to other Caucasian populations. However, the incidence of VTE and MI,[259-261] as well as the distribution of SNPs,[262, 263] differs between ethnicities, and generalizing our results to populations with other ethnic compositions must be done with caution.

5.2 Discussion of main results

5.2.1 Ischemic stroke and risk of venous thromboembolism

Prior to the present thesis, evidence for an association between stroke and VTE was mainly derived from small cohort studies on selected populations,[9-13, 191, 264, 265] and trials assessing the protective effect of different treatment strategies.[266-269] The reported incidence of DVT after stroke ranges from 4-11% during the first 14 days after the stroke,[9, 190, 191, 265] and 15-45% 20-30 days after the stroke.[9-11] However, these studies screened stroke patients for DVT, and many reported a high proportion of asymptomatic DVTs,[9-11, 190, 265] and distal DVTs.[9, 10, 191, 265] The clinical significance of asymptomatic and distal DVTs is uncertain. For instance, proximal DVT is more commonly associated with PE than distal DVT,[270, 271] and an RCT comparing placebo to LMWH treatment in patients with distal DVT found no difference in thrombus extension or symptomatic PE (the trial was terminated early due to slow recruitment and expiry of study drug).[272] Current guidelines from the American College of Chest Physicians recommend that patients with isolated distal DVT without severe symptoms or risk factors for extension are followed up with serial imaging after two weeks, and recommend anticoagulation only if the thrombus extends.[44] Although symptomatic PE occurs in only 1-5% of patients during the first 14 days after an acute stroke,[11, 189, 273] PE may account for up to 25% of deaths after acute stroke.[189, 192]

The only previous population-based study investigating the association between stroke and VTE was a large registry-based case-control study from Denmark, including almost 6,000 patients and 60,000 controls. The study revealed that patients with a history of stroke had a 4.4-fold (95% CI 2.9-6.7) increased risk of VTE during the first three months after the stroke. The risk decreased but remained slightly elevated (HR 1.18, 95% CI 0.95-1.46), after the initial three months.[7] However, the

events in this study were found by searching hospital registries for VTE-related ICD codes without further validation, and the study lacked information on possible confounders, including BMI. Thus, we cannot exclude that the entire, or parts of the association was non-causal and due to confounding factors.

In Paper I, we reported that subjects who developed ischemic stroke had an increased risk of VTE, compared with those without ischemic stroke in the general population. VTE events were thoroughly validated and potential confounders were collected at baseline and updated during follow-up for participants attending more than one survey. The risk of VTE was substantially increased the first month after the ischemic stroke, with a multivariable-adjusted HR of 19.7 (95% CI 10.1-38.5). The risk of VTE declined to 10.6 (95% CI 5.0-22.5) one to three months after the ischemic stroke and remained slightly elevated after three months (HR 1.5, 95% CI 1.1-2.2). Analyses stratified on types of VTE displayed a higher risk of provoked than unprovoked events, and provoking factors included, among others, immobilization within the last 14 days prior to the event. Risk estimates for DVT and PE were approximately the same (HR 19.1 and 20.2, respectively). The association between ischemic stroke and VTE remained significant after adjustment for age, sex, BMI and other cardiovascular risk factors.

Our results are in accordance with a registry-based cohort study assessing the association between stroke and VTE in the general population published in 2016.[274] The study followed 200,000 stroke patients and a comparison cohort of almost 1 million members of the general population for five years and computed cumulative risks, rates, and HRs of VTE. Reported 5-year cumulative incidence of VTE was 2.1% (95% CI 2.1-2.2) in the stroke cohort, 2.3% (95% CI 2.2-2.4) in patients with ischemic stroke and 1.9% (95% CI 1.9-2.0) in the comparison cohort. 5-year VTE rates were 7.2 per 1,000 PY in the stroke cohort and 5.0 per 1,000 PY in the comparison cohort, yielding a HR of 1.5 (95% CI 1.5-1.6). The HR of VTE during the initial three months after the stroke was 4.8 (95% CI 4.4-5.2). The HRs were higher for PE (5.8, 95% CI 5.2-6.6) than DVT (4.2, 95% CI 3.7-4.7), and higher for provoked events (5.0, 95% CI 4.6-5.5) than unprovoked events (2.1, 95% CI 1.4-3.0).[274] Provoking factors were defined as previous cancer or fracture, trauma, surgery, infection, pregnancy, delivery or immobilization within 90 days before the event. Of note, the stroke patients and VTE events were detected by searching registries, and the study had limited information on possible confounders, such as BMI. The specific type of strokes were registered (ischemic, hemorrhagic or subarachnoid hemorrhagic), but remained unspecified for 45%. The ischemic strokes were associated with a higher cumulative incidence of VTE than the hemorrhagic strokes, but the HRs were based on the entire stroke cohort. These limitations could, at least to some extent, explain why our study yielded higher risk estimates for VTE.

No recent studies have investigated VTE risk in stroke patients in the absence of anticoagulant treatment. As current knowledge and guidelines support routine thromboprophylaxis in hospitalized patients with reduced mobility and ischemic stroke,[275] it would be unethical to perform RCTs to assess the risk of VTE without prophylactic treatment. Although only 37-50% of patients with ischemic stroke received appropriate thromboprophylaxis (based on predefined criteria),[145, 146] it is reasonable to believe that VTE risk would be substantially higher if none received anticoagulation. Furthermore, standard management of stroke patients include lipid-lowering treatment with statins and antiplatelet therapy with aspirin and/or other antiplatelet agents.[276] Statins have been shown to reduce the risk of VTE in some,[277-279] but not all studies.[280, 281] A large meta-analysis published in 2012 did not support a large protective effect, but the authors concluded that a moderate reduction in risk could not be ruled out.[282] Aspirin has previously been associated with decreased risk of recurrent VTE,[283, 284] but not with decreased risk of incident VTE.[285] However, results from a recent RCT found that a more intensive antiplatelet therapy reduced the risk of incident VTE.[286] Treatment with anticoagulation, and possibly statins and aspirin, would underestimate the observed association between stroke and subsequent VTE.

5.2.2 Atherosclerosis and risk of venous thromboembolism

Atherosclerosis is an independent risk factor for stroke and MI, [198-201] and after Prandoni and colleagues reported a higher frequency of carotid plaques in patients with unprovoked VTE compared with controls, it was hypothesized that atherosclerosis might be the missing link between arterial CVD and VTE. The association between atherosclerosis and VTE was later investigated in several studies, with different results depending on study design. Case-control studies reported an association between atherosclerosis and VTE, and unprovoked VTE in particular.[3, 207-209] In contrast, three large population-based cohorts found no association, and a cause-specific model excluded MI as a potential confounder or mediator.[4-6] Several factors may explain the divergent results between cohort and case-control studies. Firstly, recruitment of controls that are not fully representative of the source population from which the cases were derived can lead to overestimation of the true effect. None of the case-control studies selected controls from the general population.[3, 207-209] Secondly, carotid atherosclerosis was defined in different ways in the different studies. For instance, a plaque was defined as a protrusion into the lumen of at least 1.5 mm in some studies,[3, 5, 209] and as a localized thickening of the vessel wall of > 50% compared to adjacent IMT in other studies.[4, 208] Lastly, case-control studies are inherently prone to reverse causation, and it is not possible to determine if atherosclerosis caused VTE or vice versa. Although this is an unlikely explanation in the three studies in which assessment of atherosclerosis was performed in close proximity to the VTE diagnosis,[3, 207, 208]

it might be the case in the study where assessment of atherosclerosis was done within three years from the VTE diagnosis.[209] Hald and colleagues calculated and compared risks of MI and VTE associated with atherosclerosis to ensure that the measurement and classification of atherosclerosis were appropriate. They found an association between atherosclerosis and MI with a similar magnitude as shown earlier, but no association between atherosclerosis and VTE.[4] The remaining concern was that atherosclerosis might have developed over time and that a true association with moderate effect size could have been underestimated due to regression dilution bias. The main argument against the cohort studies has, indeed, been the long time between the baseline measurements and outcome.[287]

In Paper II, we assessed if the negative results in cohort studies were due to regression dilution bias by using repeated measurements of participants recruited from the general population. VTE events were thoroughly validated and potential confounders were updated during follow-up for participants attending more than one survey. In time-varying analyses, we found no association between the initiation, presence or progression of atherosclerosis and VTE, and adjustment for potential confounders did not alter the results. Our study confirms the results from previous cohort studies and provides further evidence of a non-causal relationship between atherosclerosis and VTE.

A recently published study using data from the Tromsø Study assessed whether an incident VTE was associated with subsequent formation and progression of carotid atherosclerosis.[288] Participants attending two or more ultrasound examinations in the Tromsø Study were eligible for the study, and 150 subjects with incident VTE were identified. Subjects with carotid plaque(s) at the first visit had 4.1 mm^2 (95%CI -1.7 to 10.0) larger change in TPA between the first and second visit compared with subjects without VTE. The association persisted after adjusting for potential confounders, including CRP, and after restricting the analyses to VTE diagnosed in the first half of the time interval between ultrasound examinations. No association between VTE and subsequent novel plaque formation was found.[288] The results must be interpreted with caution due to limited statistical power, and larger studies are warranted. Nonetheless, increased risk of plaque progression after VTE could potentially explain the previously diverging results between study designs (i.e. case-control studies detected an increase in atherosclerosis after VTE) and to some extent mediate the association between VTE and subsequent risk of arterial CVD.

5.2.3 Shared risk factors for arterial cardiovascular diseases and venous thromboembolism

Of the traditional cardiovascular risk factors, only age and obesity has consistently been associated with VTE. Whether other risk factors, such as diabetes, hypertension, dyslipidemia, and

smoking, increases the risk of VTE is controversial. As previously mentioned, the majority of studies that found an association between cardiovascular risk factors and VTE were retrospective, whereas most prospective studies reported no association. While case-control studies are limited by possible reversed causation and high risk of recall and selection bias, cohort studies are limited by potential regression dilution bias due to a long time between exposure and outcome.

In Paper III, we reported that risk estimates for VTE and MI based on a single baseline measurement corresponded well with risk estimates based on repeated measurements. Except for BMI, none of the atherosclerotic risk factors increased the risk of VTE, neither in the time-fixed model based on baseline measurements nor in the time-varying analyses based on repeated measurements. The results suggest that regression dilution bias does not explain the lack of association between cardiovascular risk factors and VTE in the cohort studies.

Our results are in agreement with the majority of previously published cohort studies. Further, the results are in accordance with those from a large meta-analysis published in 2017, investigating the association between cardiovascular risk factors and VTE. The study by Mahmoodi and colleagues was based on data from 9 large cohorts and included approximately 250,000 participants with 5,000 VTE events.[289] In models adjusted for age, sex, and BMI, there was no association between VTE and hypertension, hyperlipidemia or diabetes. Current smoking was associated with a 1.2-fold increased risk of VTE (HR 1.19, 1.08-1.32), and subgroup analyses revealed that smoking was associated with provoked VTE (HR 1.36, 95% CI 1.22-1.52), but not unprovoked VTE (HR 1.08, 95% CI 0.90-1.29). The increased risk of provoked VTE is potentially mediated by cancer, which is a well-known risk factor for VTE, or hospitalization and immobilization due to other smoking-related diseases, such as MI and chronic respiratory illnesses.[289] This is supported by the lack of association between smoking and VTE in cause-specific analyses, eliminating the mediating effect of cancer and MI.[17, 18] In order to assess whether the long follow-up in the included studies could have diluted the associations, sensitivity analyses with follow-up restricted to five years were performed. Results in the sensitivity analyses were comparable to the original analyses with long follow-up. Surprisingly, the meta-analysis found an inverse association between systolic blood pressure and VTE. The authors discuss that competing risk of comorbid conditions, such as atrial fibrillation, might explain the results as atrial fibrillation is strongly associated with hypertension and routinely treated with anticoagulant drugs.[289] Nonetheless, the study concluded that previously reported associations between cardiovascular risk factors and VTE are likely to be non-causal due to confounding.[289]

Previous studies have shown an association between FHMI and VTE.[26-29] The family history itself is not a risk factor but indicates clustering of genetic and environmental risk factors of VTE in

certain families. Age and BMI explains some of the association between FHMI and VTE,[27] but other cardiovascular risk factors have little impact on the association.[26-28] Due to the particularly increased risk of unprovoked VTE, and that the risk of VTE increased with increasing numbers of affected relatives, it was hypothesized that the association between FHMI and VTE was caused by shared genetic risk factors. In accordance with previous studies, we found a 1.3-fold increased risk of VTE in individuals with a FHMI in Paper IV. However, the association between FHMI and VTE could not be explained by rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*), and rs2036914 (*F11*), as adjustments for these prothrombotic genotypes had a negligible effect on the risk estimates. Furthermore, combinations of FHMI and the prothrombotic genotypes had additive effects on the risk of VTE. For instance, having both FHMI and rs8176719 (*ABO*) was associated with a 1.8-fold increased risk of VTE, which was equal to the sum of having only FHMI or rs8176719 (*ABO*). Similar results were found for FHMI in combination with the other individual SNPs and the combined 5-SNP score. Our results suggest that FHMI and the prothrombotic genotypes are unrelated risk factors of VTE and that these prothrombotic genotypes do not affect the association between FHMI and VTE.

The mechanism(s) for the association between FHMI and VTE remains unknown. Two risk factors acting through the same pathophysiological pathway can have both synergistic and additive effects on an outcome. For instance, obesity and rs6025 (*F5*), which are associated with hypercoagulability, had synergistic effects on VTE risk.[97] Similarly, the risk of VTE in obese women using oral contraceptives has been shown to exceed the sum of the effects of the individual risk factors.[290] However, a cohort study of 66,000 genotyped participants found additive effects on VTE risk when different prothrombotic genotypes, all causing hypercoagulability, were combined.[89] Consequently, our results do not allow us to determine the mechanisms behind the association between FHMI and VTE and do not exclude the possibility that other unrecognized genetic variants can partly explain the association between FHMI and VTE.

Even though the genotypes studied in Paper IV do not explain the association between FHMI and VTE, results from Paper IV and previous studies indicate that genetic risk factors are one of the main contributors to the association. In addition, environmental risk factors clustering within families may potentially act as confounders or mediators for the association. Although the association between FHMI and VTE is independent of traditional cardiovascular risk factors,[26-28] other environmental risk factors related to both MI and VTE, such as stress and socioeconomic status,[20, 178, 291] might partly explain the association.

On the basis of the papers in the present thesis and results from previously published studies, it is possible to conclude that, of the well-known cardiovascular risk factors, only age, obesity and FHMI are shared risk factors between arterial CVD and VTE. In addition, the association between smoking

and VTE observed in some studies seems to be mediated by cancer and other smoking-related diseases, such as MI and chronic respiratory diseases.

5.2.4 Possible mechanisms for the association between arterial cardiovascular diseases and venous thromboembolism

The underlying mechanism explaining the observed association between arterial CVD and VTE is unknown, but different mechanisms have been suggested. In essence, the association can be non-causal due to shared risk factors (i.e. confounders), or causal (Figure 8, Panel A). If a causal relationship exists, the effect of arterial CVD on VTE can be indirect (i.e. mediated through other factors) or direct (Figure 8, Panel B). Furthermore, the association between arterial CVD and VTE can be due to confounders, mediators *and* a direct effect, and thus be partially non-causal and partially causal (Figure 8, Panel C).

The association between arterial CVD and VTE would be non-causal if shared risk factors explained the association. As previously discussed, a confounding variable for the association between

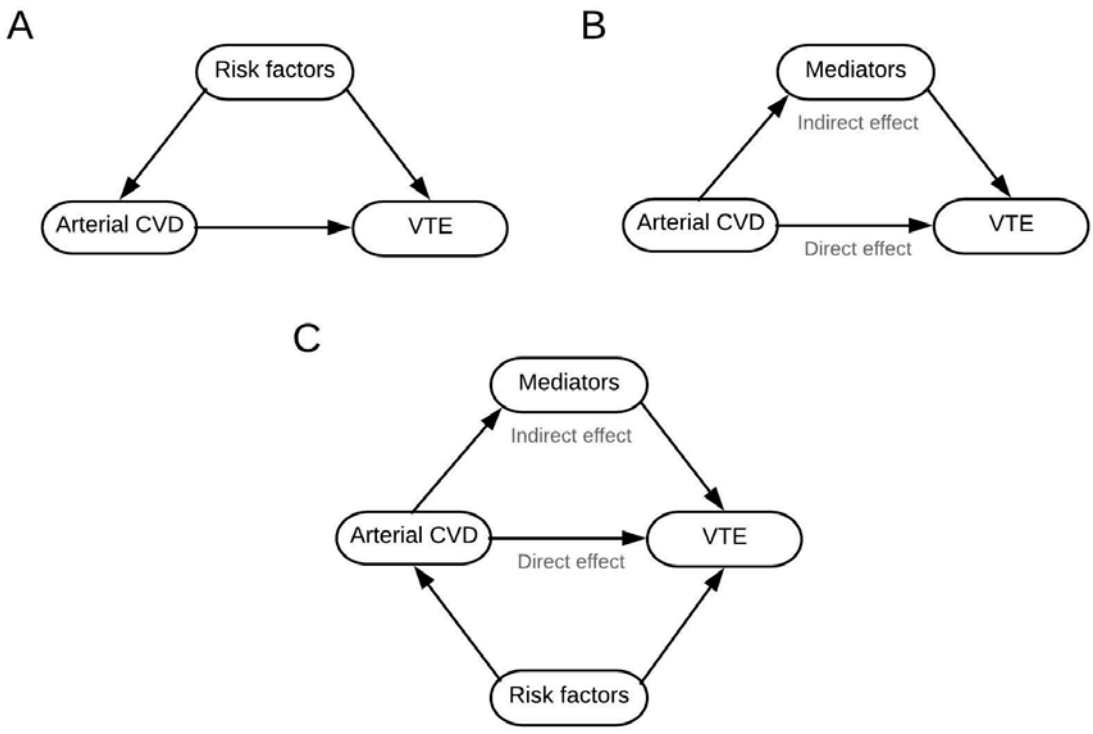


Figure 8. Possible mechanisms for the association between arterial CVD and VTE. Panel A represents a non-causal relationship caused by shared risk factors (i.e. confounders), and Panel B represents a causal relationship. The association that remains after removing the indirect effect caused by mediator(s) represents the direct effect of arterial CVD on VTE. Panel C demonstrate the combined non-causal and causal relationship with shared risk factors, mediators and a direct effect of arterial CVD on VTE.

arterial CVD and VTE is a variable that is an independent risk factor for VTE, associated with arterial CVD and not an intermediate variable between the arterial CVD and VTE. In the papers included in the present thesis, we demonstrated that there is no association between atherosclerosis and VTE and that BMI is the only traditional cardiovascular risk factor associated with VTE. Thus, atherosclerosis and the other cardiovascular risk factors (e.g. hypertension, dyslipidemia, diabetes) do not meet the criteria to be confounders, and cannot explain the association. This is emphasized by the small effects of adjusting for cardiovascular risk factors and FHMI on the risk estimates for VTE.[8, 143, 186] If the association between arterial CVD and VTE was explained entirely by cardiovascular risk factors, adjusting for these risk factors would completely attenuate the association. Consequently, some of the association is likely to be caused by other, unknown, shared risk factors (i.e. residual confounding), synergistic effects of known and unknown risk factors, or by indirect or direct factors.

Furthermore, if shared risk factors were important for the association between arterial CVD and VTE, there would be a permanent, and not transient, increased risk of VTE. In fact, the VTE risk would be expected to increase over time after diagnosis of arterial CVD, as risk factors tend to accumulate over time and age. However, studies investigating the risk of VTE by time since an arterial CVD event demonstrated that the VTE risk was substantially increased during the first three to six months after the arterial event, but declined rapidly thereafter.[7, 8, 185] This suggests that mechanisms related to the arterial event itself increase the risk of VTE.

Several lines of evidence point towards a causal relationship between arterial CVD and subsequent VTE. From a clinical and pathophysiological perspective, it is likely to assume that the majority of the association between arterial CVD and VTE is caused by mediators. For instance, we know that hospitalization and immobilization are important risk factors for VTE,[109, 129, 138, 148] and a potential consequence of arterial CVD. In accordance with this, some studies have shown that arterial events are especially associated with subsequent provoked VTE events.[8, 143, 186] Further, Barsoum and colleagues found a significant association between MI and VTE in crude analyses (OR 1.84, 95% CI 1.25-2.71) in a case-control study, but the OR was attenuated to 1.64 (95% CI 1.05-2.57) after they adjusted for hospitalization for major surgery or medical illness, and nursing home confinement in addition to age and BMI.[187] Neurological deficits with accompanying immobilization are common complications of stroke,[292] and a risk factor for VTE.[11, 129] In a population-based case-crossover study, Morelli and colleagues investigated stroke and other triggers for incident VTE.[293] Stroke was registered in 4.2% of the hazard periods (90 days before the VTE event), compared with 0.2% of the control periods (18 to 6 months), resulting in a 20-fold increased risk of VTE (95% CI 8.3-48.1). The risk was attenuated to 6-fold (95% CI 1.6-22.1) when immobilization and infection were taken into account, and a mediation analysis revealed that 68% of the total effect of stroke on VTE risk was mediated by immobilization

and infection. In a study by Sørensen and colleagues, the risk estimates of VTE four months to five years after MI and stroke were 1.01 (95% CI 0.78-1.31) and 1.18 (95% CI 0.95-1.46), respectively. After five years, the risk was similar for MI and stroke patients (RR of 1.3).[7] The higher long-term risk in stroke patients might reflect the increased risk of prolonged immobilization due to paralysis or paresis. Supporting this hypothesis, studies have shown that different measures of stroke severity are strongly associated with VTE risk,[11, 190] and that most DVTs after stroke affects the paretic leg.[9]

However, studies have demonstrated an increased risk of unprovoked VTE after arterial CVD as well, and some studies found similar risk estimates for unprovoked and provoked VTE.[7, 185] Thus, it is unlikely that hospitalization and immobilization explain the entire association between arterial CVD and VTE. Other medical complications are frequent among MI and stroke patients,[294] and potential mediators for the association between arterial CVD and VTE include infections due to prolonged hospital stays,[141, 295] heart failure,[129, 148, 296] atrial fibrillation,[46] and surgery (e.g. coronary artery bypass grafting).[139] Infections have been shown to increase the VTE risk independent of immobilization,[141, 295] suggesting that other factors, such as local inflammation and activation of coagulation, contribute to the increased risk of VTE. While both heart failure and atrial fibrillation has the potential to induce stasis and subsequent VTE, it has also been suggested that atrial fibrillation can lead to right-sided atrial thrombi that can dislodge and cause PE.[46] This hypothesis is supported by the particularly increased risk of PE after MI,[7, 8, 185] and it might (partly) explain why up to 50% of patients with PE do not have concurrent DVT.[1, 2, 45]

The direct effect of arterial CVD on VTE would be the association that persists after adequate adjustments for confounders and mediators. However, indirect and direct effects can be hard to distinguish from each other, especially for multifactorial diseases without a specific and known mechanism, such as VTE. For the association between arterial CVD and VTE, the direct effect would be the basic pathophysiological mechanisms in which arterial CVD leads to coagulation activation and thrombus formation. For instance, studies have demonstrated alterations in concentrations of pro- and anticoagulant proteins in the acute phase of ischemic stroke,[297, 298] and a bidirectional association between inflammation and coagulation can possibly induce thrombus formation.[299] However, it might be difficult to differentiate whether these alterations are a result of the arterial event itself or other medical complications following the arterial event.

Finally, the association between arterial CVD and VTE might be a result of medical surveillance bias. As previously discussed, it is a type of bias that can occur if an exposure leads to closer surveillance and an increased probability of detection of an outcome. We cannot exclude that patients with MI and stroke are under stronger surveillance, and are more likely to undergo diagnostic procedures for DVT

and PE, than the general population. Including only symptomatic VTE events will, to some extent, reduce the risk of this bias.

The association between arterial CVD and VTE is a result of a complicated interplay between non-causal and causal mechanisms. To unravel the total causal effect of arterial CVD on VTE, analyses must be adjusted for confounders, and to investigate the direct effect of arterial CVD on VTE, analyses must be adjusted for confounders and mediators (see Figure 8). However, this might be difficult as there may be reasonable doubt as to whether a variable is in the causal pathway between arterial CVD and VTE or not, and because a variable might be a confounder *and* a mediator. For instance, one can argue that obesity increases the risk of both arterial CVD and VTE, and thus be a confounder for the association. Conversely, one can also argue that obesity is a result of an inactive lifestyle, a possible consequence of an arterial event. In this case, obesity is in the causal pathway between MI and VTE, and can thus be classified as a mediator. Nevertheless, the most important initiative to improve patient care is to acknowledge arterial CVD as a risk factor for VTE, to avoid complications of arterial CVD related to increased VTE risk, and to give appropriate anticoagulation in situations where thromboprophylaxis is warranted. As current data on the prediction of VTE in stroke patients are scarce, better knowledge regarding risk factors and triggers for VTE in stroke patients are important to develop future risk assessment models.

6. Conclusions

- Subjects who developed ischemic stroke had an increased risk of VTE compared with those without stroke in the general population. The risk of VTE was especially high during the first three months after ischemic stroke and declined rapidly thereafter. Ischemic stroke yielded higher risk of provoked VTE than unprovoked VTE, and adjustments for cardiovascular risk factors did not attenuate risk estimates. This suggests that mechanisms or conditions related to the stroke itself contribute substantially to the association between ischemic stroke and VTE

- Carotid atherosclerosis, measured by IMT and TPA in time-varying analyses, was not associated with future risk of VTE. Furthermore, there was no association between plaque initiation or plaque progression and VTE. Our findings suggest that atherosclerosis is not an intermediate for the association between arterial CVD and VTE

- We found that risk estimates for VTE and MI based on a single baseline measurement and time-fixed analyses corresponded well with risk estimates based on repeated measurements and time-varying analyses. Except for BMI, none of the cardiovascular risk factors were associated with VTE, suggesting that the lack of association between cardiovascular risk factors and VTE in previous prospective cohort studies cannot be explained by regression dilution bias. For MI, the difference between risk estimates from the time-fixed and time-varying analyses was greatest for variables that changed much during follow-up and for variables with strong associations with MI

- The known association between FHMI and VTE was not explained by rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*) or rs2036914 (*F11*). Combinations of FHMI and the prothrombotic genotypes displayed an additive effect on VTE risk, indicating no biological interaction between the risk factors

7. Final remarks and future perspectives

The findings in this thesis support a strong association between stroke and subsequent VTE. The risk is especially high during the first three to six months after the stroke, indicating that factors related to the stroke itself increase the risk of VTE. Thus, it is important to avoid complications of the stroke related to increased VTE risk, such as immobilization and infections, and to use thromboprophylaxis. Current international and national guidelines recommend prophylactic-dose LMWH or intermittent pneumatic compression stockings in patients with acute ischemic or hemorrhagic stroke and restricted mobility.[275] Unfortunately, evidence suggests that these guidelines are not routinely followed. Although rates are somewhat higher in most European countries,[145] only 37-50% of stroke patients worldwide receive appropriate thromboprophylaxis.[145, 146] To improve patient care, it is important to increase the rates of appropriate use of thromboprophylaxis in stroke patients and to develop prediction models to accurately discriminate between patients at high and low risk of VTE.

Our results support the findings from previous cohort studies reporting that there is no association between atherosclerosis and VTE. Thus, atherosclerosis cannot explain the association between arterial CVD and VTE. Furthermore, we have reported that, among the well-known cardiovascular risk factors, only age, obesity and FHMI are associated with VTE. In addition, there seems to be an association between smoking and provoked VTE which is mediated by cancer and other smoking-related diseases.[289] The association between arterial CVD and VTE persists in analyses adjusted for cardiovascular risk factors, suggesting that there is a causal relationship between arterial CVD and VTE. Further research to unravel the mechanisms behind the association between arterial CVD and VTE are warranted to understand the complex interplay between shared risk factors, mediators and direct effects.

MI, stroke, and VTE are the three most common cardiovascular diseases, with a high risk of mortality and disability. Shared risk factors are important targets for interventions, as there is great potential to decrease the burden of several diseases. Obesity is, as discussed in this thesis, a shared risk factor for arterial CVD and VTE. In 2016, more than 1.9 billion adults were overweight and over 650 million adults were obese,[116] and the prevalence of both overweight and obesity is increasing.[116, 300] A high proportion of arterial CVD and VTE events can be attributed to obesity,[118, 301, 302] and interventions to reduce obesity are important to reduce the large impact of MI, stroke, and VTE at an individual and population level.

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Paper I

ORIGINAL RESEARCH

Ischemic Stroke and Risk of Venous Thromboembolism in the General Population: The Tromsø Study

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***Background*—**Even though clinical data support a relation between ischemic stroke and venous thromboembolism (VTE), the strength and time dependence of the association remain to be settled at the population level. We therefore aimed to investigate the association between ischemic stroke and VTE in a prospective population-based cohort.

***Methods and Results*—**Participants (n=30 002) were recruited from 3 surveys of the Tromsø study (conducted in 1994–1995, 2001, and 2007–2008) and followed through 2010. All incident events of ischemic stroke and VTE during follow-up were recorded. Cox-regression models with age as time scale and ischemic stroke as a time-dependent variable were used to calculate hazard ratios (HR) of VTE adjusted for cardiovascular risk factors. During a median follow-up time of 15.7 years, 1360 participants developed ischemic stroke and 722 had a VTE. The risk of VTE was highest the first month (HR 19.7; 95% CI, 10.1–38.5) and from 1 to 3 months after the stroke (HR 10.6; 95% CI 5.0–22.5), but declined rapidly thereafter. The risk estimates were approximately the same for deep vein thrombosis (HR 19.1; 95% CI, 7.8–38.5), and pulmonary embolism (HR 20.2; 95% CI, 7.4–55.1). Stroke was associated with higher risk for provoked (HR 22.6; 95% CI, 12.5–40.9) than unprovoked VTE (HR 7.4; 95% CI, 2.7–20.1) the first 3 months.

***Conclusions*—**The risk of VTE increased during the first 3 months after an ischemic stroke. The particularly high risk of provoked VTE suggests that additional predisposing factors, such as immobilization, potentiate the VTE risk in patients with ischemic stroke.
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Key Words: epidemiology • ischemic stroke • risk factor • venous thromboembolism

Ischemic stroke is a major challenge to public health and healthcare systems due to frequent hospitalizations, frequent medical complications, disability, dependency, nursing home confinement, and a high mortality rate.[1–3] Even though clinically overt pulmonary embolism (PE) occurs in only 1% of stroke patients during the first 14 days after an acute stroke,[4–6] PE may account for up to 25% to 50% of deaths after acute stroke.[6–8]

Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and PE, is a common disease with serious short- and long-term complications, such as development of the post-thrombotic syndrome after a DVT, or death due to circulatory collapse secondary to PE.[9,10] VTE is a multifactorial disease, and advancing age and obesity are recognized as shared atherosclerotic risk factors for VTE and ischemic stroke.[11] In addition, immobilization, particularly in an in-hospital setting, is associated with high risk of VTE.[12–15] Therefore, neurological deficits entailing immobilization and other medical complications secondary to acute ischemic stroke may predispose for VTE.[2,16,17]

Several randomized trials including selected patients with acute ischemic stroke have assessed the risk of symptomatic VTE in patients without and with antithrombotic treatment.[4,5] Data from a meta-analysis displayed that the incidence of asymptomatic and symptomatic DVT was 17% among 1186 patients with stroke, whereas the incidence of symptomatic PE was 1.0% among 10 997 patients who did not receive antithrombotic therapy during follow-up (controls).[5] However, limited data exist regarding the association between ischemic

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stroke and risk of VTE in the general population. A registry-based case–control study recruited from the general population in Denmark revealed that patients with a history of stroke had a short-term 4.4-fold increased risk of subsequent VTE during the first 3 months after stroke.[18] Results from registry-based studies should, however, be interpreted with caution due to the lack of validation of exposure and outcomes and inability to adjust for obvious confounders such as body mass index (BMI).

The aim of the study was to investigate the overall and time-dependent risk of VTE by ischemic stroke in a population-based cohort with validated information on exposure (ischemic stroke), end point (VTE), and potential confounders.

Methods

Study Population

The Tromsø Study is a single-center, prospective, population-based study, with repeated health surveys of the inhabitants of Tromsø, Norway. Study participants were recruited from the fourth, fifth, and sixth survey of the Tromsø Study, conducted in 1994–1995, 2001, and 2007–2008, respectively. The overall attendance rates were high: 77% in the fourth, 78% in the fifth, and 66% in the sixth survey. In total, 30 586 unique participants aged 25 to 97 years took part in at least 1 of the surveys, and of these, 21 529 subjects participated in 2 or all 3 surveys. Participants who did not consent to medical research (n=225) and participants not officially registered as inhabitants of the municipality of Tromsø at date of study enrollment (n=47) were excluded. Furthermore, participants with a history of VTE (n=78) or ischemic stroke (n=234) were excluded. Consequently, 30 002 participants were included in the study, and followed from the date of enrollment to the end of follow-up, December 31, 2010 (Figure 1). The regional committee for medical and health research ethics in North Norway approved the study, and all participants gave their informed written consent.

Baseline Measurements

Information about the study participants was collected by physical examinations, blood samples, and self-administered questionnaires at each survey. Systolic and diastolic blood pressures were measured 3 three times with 1-minute intervals with an automatic device (Dinamap Vital Signs Monitor, 1846; Critikon Inc, Tampa, FL) with participants in a sitting position after 2 minutes of rest, and defined as the mean of the last 2 readings. Nonfasting blood samples were collected from an antecubital vein, serum was prepared by centrifugation after 1-hour respite at room temperature and analyzed at the Department of Clinical Chemistry, University Hospital of North Norway, Tromsø, Norway. Serum total cholesterol was analyzed by an enzymatic colorimetric method using a commercially available kit (CHOD-PAP; Boehringer-Mannheim, Mannheim, Germany). Serum high-density lipoprotein cholesterol was measured after precipitation of lower-density lipoproteins with heparin and manganese chloride. Height and weight were measured with participants wearing light clothes and no shoes. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Obesity (BMI $\geq$30 kg/m^2) was classified according to the World Health Organization definition.[19] Hypertension was classified as mean systolic blood pressure $\geq$140 mm Hg, mean diastolic blood pressure $\geq$90 mm Hg, or self-reported use of blood pressure–lowering drugs. Hypercholesterolemia was classified as total serum cholesterol $\geq$6.5 mmol/L or self-reported use of lipid-lowering drugs. Information on family history of myocardial infarction, diabetes mellitus, physical activity, and education level was collected from a self-administered questionnaire.

Assessment of Ischemic Stroke

Ischemic stroke was defined according to the World Health Organization definition when computed tomography or magnetic resonance imaging scans or autopsy had ruled out brain hemorrhage.[20] An independent end-point committee

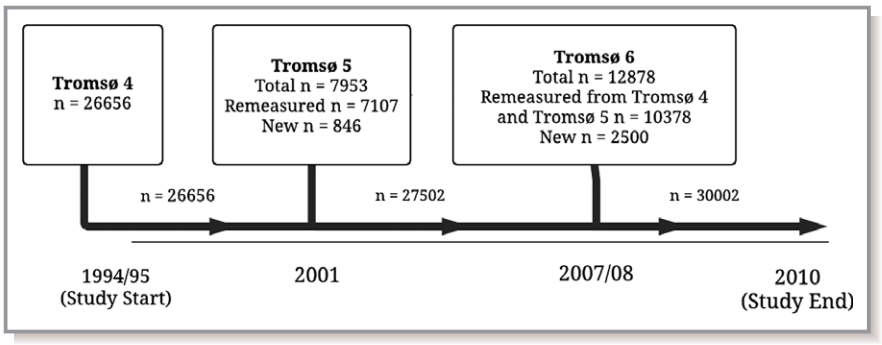

Figure 1. Inclusion of study participants from the fourth (1994–1995), fifth (2001–2002), and sixth (2007–2008) surveys of the Tromsø Study.

performed validation of hospitalized and out-of-hospital events of ischemic stroke based on data from hospital and out-of-hospital journals, autopsy records, and death certificates. The Norwegian national 11-digit identification number allowed linkage to national and local diagnosis registries. Cases of possible incident ischemic stroke were identified by linkage to the hospital discharge diagnosis registry at the University Hospital of North Norway with a broad search for the International Classification of Diseases (ICD), 9th Revision codes 430 to 438 in the period 1994 to 1998, and thereafter for the ICD, 10th Revision codes I60 to I69. Manual and/or electronic text searches were performed in paper versions (used until 2001) and digital versions of hospital records for notes on ischemic stroke in all participants with 1 or more of these diagnoses for case validation.

Assessment of VTE

All incident VTE events during follow-up were identified by searching the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry at the University Hospital of North Norway as previously described.[21] The University Hospital of North Norway is the only hospital in the region, and all diagnostic radiology and hospital care is provided exclusively by this hospital. The medical record for each potential case of VTE was reviewed by trained personnel, and a VTE event was considered verified and recorded when presence of clinical signs and symptoms of DVT or pulmonary embolism were combined with objective confirmation tests (by compression ultrasonography, venography, spiral computed tomography, perfusion–ventilation scan, pulmonary angiography, autopsy), and resulted in a VTE diagnosis that required treatment, as previously described in detail.[21] VTE cases from the autopsy registry were recorded when the death certificate indicated VTE as cause of death or a significant condition associated with death. The VTE events were classified as provoked and unprovoked, depending on the presence of provoking factors at the time of diagnosis. Provoking factors were recent surgery or trauma within the previous 8 weeks, acute medical conditions (acute myocardial infarction, ischemic stroke, or major infectious disease), active cancer, immobilization (bed rest >3 days, wheelchair use, or long-distance travel exceeding 4 hours within the last 14 days prior to the event) or any other factors described by a physician in the medical record (eg, intravascular catheter).

Statistical Analysis

Participants who developed ischemic stroke during the study period contributed with nonexposed person-time from the inclusion date to the date of a diagnosis of ischemic stroke, and then with exposed person-time from the date of ischemic stroke onwards. For each participant, nonexposed and exposed person-years were counted from the date of enrollment to the date of an incident diagnosis of VTE, the date the participant died or moved from Tromsø, or until the end of the study period, December 31, 2010, whichever came first. Participants who died or moved from the municipality during follow-up were censored at the date of death or migration.

Statistical analyses were performed using STATA version 14.0 (Stata Corporation, College Station, TX). Crude incidence rates (IR) of VTE were calculated and expressed as number of events per 1000 person-years at risk. Cox proportional hazards regression models were used to calculate hazard ratios (HR) with 95% CI of VTE, DVT, and PE after ischemic stroke. Age was used as time scale in the Cox model, with the age of the participants at study enrollment defined as entry time, and the age at the VTE event or censoring event (ie, death, migration, or the date of study end) defined as exit time. Ischemic stroke was included as a time-dependent covariate in the Cox model. Therefore, participants who developed ischemic stroke during follow-up contributed with person-years in both the unexposed and exposed group (ie, unexposed person-years from baseline inclusion to stroke and exposed person-years from stroke to end of follow-up). In those who participated in several surveys, information on potential confounders was updated at each survey. HRs for VTE were estimated with 3 different models. The first model was adjusted for age (as time scale) and sex, while the second model was additionally adjusted for BMI. Model 3 was adjusted for age (as time scale), sex, BMI, diabetes mellitus, smoking, systolic blood pressure, high-density lipoprotein cholesterol, physical activity, and education. The proportional hazard assumption was tested using Schoenfeld residuals and found not violated. Statistical interactions between ischemic stroke and sex were tested by including cross-product terms in the proportional hazards models, and no interactions were found. Finally, 1-Kaplan–Meier curves were estimated to visualize the cumulative incidence of VTE over time in subjects without and with incident ischemic stroke.

Results

During a median follow-up of 15.7 years, 1360 (4.5%) subjects developed ischemic stroke and 722 (2.4%) subjects developed VTE. Baseline characteristics of the study participants are shown in Table 1. The mean age and BMI, as well as the proportions of men and subjects with hypertension and hypercholesterolemia were higher in stroke patients than in those without stroke (Table 1).

Characteristics of the VTE events without and with ischemic stroke with regard to anatomical localization and

Table 1. Baseline Characteristics of Participants Without and With Ischemic Stroke (n=30 002)

	No Ischemic Stroke (n=28 642)	Ischemic Stroke (n=1360)
Age, y	46±14	63±13
Sex (male)	47.1 (13 497)	54.0 (734)
BMI, kg/m^2	25.3±3.9	26.6±4.1
Total cholesterol, mmol/L	5.94±1.29	6.74±1.30
HDL cholesterol, mmol/L	1.49±0.41	1.47±0.42
Triglycerides, mmol/L	1.53±1.04	1.84±1.13
Systolic blood pressure, mm Hg	133±20	153±25
Diastolic blood pressure, mm Hg	77±12	87±14
Hypertension*	32.7 (9380)	72.6 (988)
Hypercholesterolemia†	31.8 (9110)	55.2 (752)
Smoking‡	35.7 (10 247)	34.0 (463)
Physical activity§	32.9 (9411)	19.5 (265)
Education‖	28.3 (8116)	14.6 (198)
Self-reported diabetes mellitus	1.6 (468)	6.0 (82)

The Tromsø Study 1994–2010. Values are % (n) or mean±SD. BMI indicates body mass index; HDL, high-density lipoprotein.
*Mean systolic/diastolic blood pressure ≥140/≥90 mm Hg, use of antihypertensives, or self-reported hypertension.
†Total cholesterol ≥6.5 mmol/L, use of lipid-lowering drugs, or self-reported hypercholesterolemia.
‡Self-reported daily smoking, yes/no.
§≥1 hours of moderate or hard physical activity per week, yes/no.
‖>10 years of education.

Table 2. Characteristics of VTE Events (n=722)

	No Ischemic Stroke (n=665) % (n)	Ischemic Stroke (n=57) % (n)
Clinical characteristics		
Deep vein thrombosis	58.0 (386)	50.9 (29)
Pulmonary embolism	42.0 (279)	49.1 (28)
Provoked	50.0 (332)	63.2 (36)
Unprovoked	50.0 (333)	36.8 (21)
Clinical risk factors		
Estrogen*†	5.7 (38)	5.2 (3)
Pregnancy/puerperium*	0.9 (6)	—
Heredity‡	3.6 (24)	—
Provoking factors		
Surgery	15.9 (106)	7.0 (4)
Trauma	7.8 (52)	8.8 (5)
Cancer	24.5 (163)	17.5 (10)
Immobility§	16.1 (107)	43.9 (25)
Other‖	5.3 (35)	1.8 (1)

The Tromsø Study 1994–2010. DVT indicates deep vein thrombosis; PE, pulmonary embolism; VTE, venous thromboembolism.
*Only women included in the analysis.
†Current or previous use of hormone replacement therapy or oral contraceptives.
‡Venous thromboembolism in a first-degree relative before 60 years of age.
§Bed rest >3 days, journeys of >4 hours by car, boat, train, or air within the last 14 days, or other types of immobilization.
‖Other provoking factor described by a physician in the medical record (eg, intravascular catheter).

predisposing factors are shown in Table 2. In total, 57 of the 722 VTEs occurred in patients with an ischemic stroke. VTE patients with an ischemic stroke had a higher proportion of provoked events compared to those without stroke. Moreover, the proportion of patients that had been immobilized before the VTE event was substantially higher in those with stroke (51% versus 15%).

IR and HR for VTE among participants without and with incident ischemic stroke during follow-up are shown in Table 3. In participants without stroke, 665 VTE-events were identified during 361 634 person-years of follow-up, corresponding to IR of 1.8 per 1000 person-years. In subjects with incident ischemic stroke, there were 57 VTEs identified during 6482 person-years of follow-up equivalent to IR of 10.3 per 1000 person-years. Ischemic stroke was associated with a 3-times (HR 3.2; 95% CI 2.4–4.4) higher risk of VTE compared to those without ischemic stroke. The IR of VTE was highest during the first month after an ischemic stroke (IR 82.1 per 1000 person-years) with a 20-fold higher risk (HR 19.7; 95% CI, 10.1–38.5) compared to those without ischemic stroke. In the period from 1 to 3 months after the stroke, the risk of VTE was 11-fold increased in stroke patients (HR 10.6; 95% CI 5.0–22.5). The risk declined rapidly thereafter, and was in the period more than 3 months only 1.5 times increased (HR 1.5; 95% CI, 1.1–2.2). Separate analyses of DVT and PE showed that the risk of both outcomes was highest during the first 3 months after the incident ischemic stroke (Table 3). The multivariable HRs were 19.1 (95% CI, 7.8–46.9) and 10.3 (95% CI, 3.8–28.0) for DVT and 20.2 (95% CI, 7.4–55.1) and 11.0 (95% CI, 3.5–35.5) for PE during the first month and in the period 1 to 3 months after stroke, respectively. The risk estimates for both DVT (HR 1.3; 95% CI, 0.8–2.3) and PE (HR 1.8; 95% CI, 1.0–3.0) were no longer significant after the first 3 months.

The cumulative incidences of VTE in subjects without and with ischemic stroke are shown in Figure 2. There was a notable increase in the cumulative incidence of VTE during the initial 3 months following an incident stroke as displayed by the substantially steeper slope in the incidence curve for subjects with ischemic stroke compared to those without ischemic stroke. The cumulative incidence of VTE was 15% during the first 3 months in subjects with ischemic stroke, compared with 0.2% in the general population during the same time period. The incidence curves for VTE remained

Table 3. Incidence Rates and Hazard Ratios for VTE, DVT, and PE According to Ischemic Stroke Exposure

	Person-Years	VTE Events	Crude IR (95% CI)*	Model 1† HR (95% CI)	Model 2‡ HR (95% CI)	Model 3§ HR (95% CI)
Total VTE						
No stroke	361 634	665	1.8 (1.7–2.0)	Reference	Reference	Reference
<1 month	122	10	82.1 (44.2–152.5)	16.4 (8.7–30.8)	15.8 (8.4–29.8)	19.7 (10.1–38.5)
1 to 3 months	172	8	46.5 (23.2–92.9)	9.5 (4.7–19.2)	9.2 (4.5–18.5)	10.6 (5.0–22.5)
>3 months	5193	39	7.5 (5.5–10.3)	1.5 (1.1–2.1)	1.4 (1.0–2.0)	1.5 (1.1–2.2)
DVT						
No stroke	361 634	386	1.1 (1.0–1.2)	Reference	Reference	Reference
<1 month	122	6	49.2 (22.1–109.6)	17.7 (7.8–39.9)	17.4 (7.7–39.2)	19.1 (7.8–46.9)
1 to 3 months	172	4	23.2 (8.7–61.9)	8.7 (3.2–23.4)	8.5 (3.1–22.9)	10.3 (3.8–28.0)
>3 months	5193	19	3.7 (2.3–5.7)	1.3 (0.8–2.1)	1.2 (0.8–2.0)	1.3 (0.8–2.3)
PE						
No stroke	361 634	279	0.8 (0.7–0.9)	Reference	Reference	Reference
<1 month	122	4	32.8 (12.3–87.5)	14.8 (5.5–40.0)	14.0 (5.2–37.0)	20.2 (7.4–55.1)
1 to 3 months	172	4	23.2 (8.7–61.9)	10.4 (3.9–28.3)	10.0 (3.7–27.1)	11.2 (3.5–35.5)
>3 months	5193	20	3.9 (2.5–6.0)	1.7 (1.1–2.7)	1.6 (1.0–2.5)	1.8 (1.0–3.0)

The Tromsø Study 1994–2010. DVT indicates deep vein thrombosis; HR, hazard ratio; IR, incidence rates; PE, pulmonary embolism; VTE, venous thromboembolism.
*Per 1000 persons-years.
†Model 1: Age as timescale, adjusted for sex.
‡Model 2: Model 1+body mass index.
§Model 3: Model 2+systolic blood pressure, diabetes mellitus, high-density lipoprotein cholesterol, smoking, physical activity, and education level.

essentially parallel in the period more than 6 months after the incident ischemic stroke event (Figure 2).

In analyses stratified for the presence of provoking factors, ischemic stroke displayed a higher risk for provoked VTE (HR 22.6; 95% CI, 12.5–40.9) than for unprovoked VTE (HR 7.4; 95% CI, 2.7–20.1) during the first 3 months (Table 4). In subgroup analyses, ischemic stroke was associated with a 20-fold (HR 19.7; 95% CI, 9.1–42.7) higher risk of provoked DVT and a 29-fold (HR 29.0; 95% CI, 11.5–73.6) higher risk of provoked PE compared with subjects without ischemic stroke (Table 4). The risk estimates for provoked VTE (HR 1.9; 95% CI, 1.1–3.0), and provoked PE (HR 2.8; 95% CI, 1.3–5.7) remained significantly increased more than 3 months after ischemic stroke, whereas the risk estimate for provoked DVT was no longer statistically significant (HR 1.4; 95% CI, 0.7–2.8).

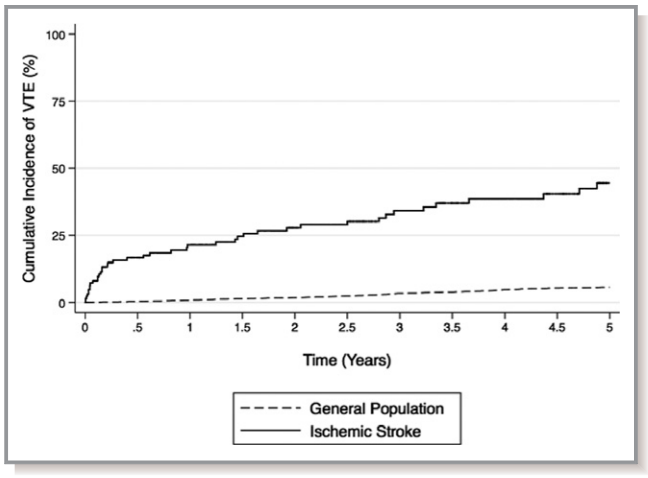

Figure 2. Overall cumulative incidence of venous thromboembolism (VTE) in subjects with and without ischemic stroke. The Tromsø Study 1994–2010.

Discussion

In our prospective cohort study, subjects who developed ischemic stroke had an increased risk of VTE, compared to those without ischemic stroke in the general population. The incidence rate and relative risk were especially high during the first 3 months after ischemic stroke and declined rapidly thereafter. Analyses stratified on predisposing factors of VTE displayed higher risk of provoked than unprovoked events, although the confidence intervals for the point estimates overlapped. As stroke was associated with a transient increased risk of both unprovoked and provoked VTE, our findings suggest that mechanisms or conditions related to the ischemic stroke itself contribute substantially to the association between ischemic stroke and VTE.

Table 4. Incidence Rates and Hazard Ratios for VTE, DVT, and PE According to Ischemic Stroke Exposure by the Presence of Predisposing Factors

	Person-Years	VTE Events	Crude IR (95% CI)*	Model 1† HR (95% CI)	Model 2‡ HR (95% CI)	Model 3§ HR (95% CI)
Provoked VTE						
No stroke	361 634	332	0.9 (0.8–1.0)	Reference	Reference	Reference
<3 months	294	14	47.6 (28.2–80.4)	19.3 (11.2–33.2)	18.8 (10.9–32.4)	22.6 (12.5–40.9)
>3 months	5193	22	4.2 (2.8–6.4)	1.1 (1.1–2.6)	1.5 (1.0–2.4)	1.9 (1.1–3.0)
Unprovoked VTE						
No stroke	361 634	333	0.9 (0.8–1.0)	Reference	Reference	Reference
<3 months	294	4	13.6 (5.1–36.2)	5.5 (2.0–14.8)	5.3 (1.9–14.2)	7.4 (2.7–20.1)
>3 months	5193	17	3.3 (2.0–5.3)	1.3 (0.8–2.2)	1.3 (0.8–2.1)	1.4 (0.8–2.5)
Provoked DVT						
No stroke	361 634	216	0.6 (0.5–0.7)	Reference	Reference	Reference
<3 months	294	8	27.2 (13.6–54.4)	17.5 (8.5–35.8)	17.2 (8.4–35.2)	19.7 (9.1–42.7)
>3 months	5193	12	2.3 (1.3–4.1)	1.4 (0.8–2.6)	1.3 (0.7–2.4)	1.4 (0.7–2.8)
Unprovoked DVT						
No stroke	361 634	170	0.5 (0.4–0.5)	Reference	Reference	Reference
<3 months	294	2	6.8 (1.7–27.2)	5.9 (1.4–23.8)	5.8 (1.4–23.4)	7.2 (1.8–29.6)
>3 months	5193	7	1.3 (0.6–2.8)	1.2 (0.5–2.5)	1.1 (0.5–2.5)	1.3 (0.6–3.0)
Provoked PE						
No stroke	361 634	116	0.3 (0.3–0.4)	Reference	Reference	Reference
<3 months	294	6	20.4 (9.2–45.4)	22.4 (9.7–51.8)	21.8 (9.4–50.1)	29.0 (11.5–73.6)
>3 months	5193	10	1.9 (1.0–3.6)	2.0 (1.0–3.9)	1.9 (1.0–3.7)	2.8 (1.3–5.7)
Unprovoked PE						
No stroke	361 634	163	0.5 (0.4–0.5)	Reference	Reference	Reference
<3 months	294	2	6.8 (1.7–27.2)	5.2 (1.3–21.0)	4.8 (1.2–19.7)	7.7 (1.9–31.5)
>3 months	5193	10	1.9 (1.0–3.6)	1.4 (0.7–2.8)	1.3 (0.7–2.6)	1.6 (0.7–3.4)

The Tromsø Study 1994–2010. DVT indicates deep vein thrombosis; HR, hazard ratio; IR, incidence rate; PE, pulmonary embolism; VTE, venous thromboembolism.
*Per 1000 persons-years.
†Model 1: Age as timescale, adjusted for sex.
‡Model 2: Model 1+body mass index.
§Model 3: Model 2+systolic blood pressure, diabetes mellitus, high-density lipoprotein cholesterol, smoking, physical activity, and education level.

Comprehensive data from clinical trials of stroke patients have consistently shown that stroke patients are at high risk of VTE, and DVT in particular.[5,6] However, only 1 previous large registry-based case–control study conducted in Denmark has investigated the risk of VTE in stroke patients compared to the general population.[18] Stroke patients had an overall 1.3-fold increased risk of VTE, but the risk was particularly high during the first 3 months after stroke with a 4.4-fold increased VTE risk compared to those without stroke. Accordingly, we found that patients with ischemic stroke had an overall 3-times higher risk of VTE compared with participants without stroke. The risk was particularly high during the first month and the subsequent 2 months with a 20- and 11-fold higher risk of VTE, respectively, and declined rapidly thereafter. The explanations for the observed association between ischemic stroke and future risk of VTE are yet unknown, but may include shared risk factors, indirect factors, or a direct relationship.[22]

Several lines of evidence argue against shared risk factors explaining the association between ischemic stroke and VTE. First, shared risk factors are expected to induce a permanent, and not a transient VTE risk as observed in our study. Second, adjustments for potentially shared cardiovascular risk factors would presumably attenuate the VTE risk by ischemic stroke. In our study, adjustments for cardiovascular risk factors had marginal impact on the risk estimates for the association between ischemic stroke and VTE. Third, ischemic stroke and VTE patients did not share the same risk profile. Cause-

specific analyses of cardiovascular risk factors in the Physician's Health Study revealed that only age and obesity were shared risk factors for ischemic stroke and VTE.[11] Our findings do not exclude, however, the possibility of joint effects between shared environmental[23–25] and inherited[26] prothrombotic risk factors that would augment the VTE risk under conditions of high thrombosis risk related to the ischemic stroke itself (eg, hospitalization, immobilization, and secondary acute infections).[2,16,17]

Several findings from our study support the concept that mechanism(s) or conditions related to the ischemic stroke itself partly explain the association between stroke and VTE. First, we observed a transient and short-term risk of VTE after ischemic stroke. Patients with ischemic stroke are hospitalized and medical complications occur frequently (eg, respiratory- and urinary tract infections).[2,16,17] These medical complications may contribute to the increased VTE risk either by themselves or via prolongation of the hospital stay.[13] Second, stratified analyses displayed a higher risk of provoked than unprovoked VTE by ischemic stroke, with a particular preponderance of immobilization and acute medical conditions as predisposing factors for VTE among patients with ischemic stroke. Similarly, data from the Worcester VTE study displayed a higher frequency of comorbid conditions and immobilization in patients with stroke-related VTE compared to VTE patients without stroke.[27] Patients with ischemic stroke are often temporarily immobilized due to bed-rest or neurological deficits of affected limbs, and are therefore more susceptible for thrombus formation secondary to venous stasis.[28] Activation of the coagulation system during the acute phase of ischemic stroke or secondary to medical complications may also contribute to the VTE risk.[29,30] Therefore, our findings suggest that transient indirect risk factors, occurring in relation to the ischemic stroke, possibly together with enhanced activity in the coagulation system, are important contributors to the transient risk of VTE after ischemic stroke.

For prevention of VTE, current guidelines recommend initiation of subcutaneous anticoagulation with low-molecular-weight heparin or unfractionated heparin within 48 hours after ischemic stroke with duration of treatment throughout the hospital stay or until the patient regains mobility.[31] Unfortunately, we do not have information on the use of preventive anticoagulant treatment during the rather long study period (1995–2010). However, it is likely that the risk estimates in our study are an underestimation of the real VTE risk (ie, risk in the absence of thromboprophylaxis), as a proportion of the stroke patients presumably have received thromboprophylaxis. Despite this potential underestimation of the VTE risk in stroke patients, we observed an absolute risk increase of 48.1 per 1000 patients for DVT and 32.0 for PE during the first month after the ischemic stroke (compared to subjects without ischemic stroke). Although some of the preventive effect may already be incorporated in our results, a recent meta-analysis of randomized clinical trials implies that preventive treatment with low-molecular-weight heparin or unfractionated heparin had the potential to reduce the incidence of symptomatic DVT by 70% and the incidence of fatal and nonfatal PE by 30%.[31] On the other hand, improved awareness and adherence to current guidelines for medical thromboprophylaxis in stroke patients may have lowered VTE rates during the last years.

Major strengths of our study include the prospective design, the large number of participants recruited from a general population, the long-term follow-up, the wide age distribution, and validated events of ischemic stroke and VTE. As many cardiovascular risk factors are modifiable, the participants' individual risk profile may change during follow-up, leading to regression dilution bias and potentially underestimation of the associations. However, an advantage of our study is the repeated measurements of subject characteristics during follow-up. Because of this, we may to a greater extent account for changes in risk factor and confounders during follow-up, resulting in more reliable risk estimates than in a traditional cohort study. Still, some potential limitations merit attention. In a cohort study, some groups are less likely to participate and nonresponse bias is therefore possible. Our estimated incidences of stroke and VTE may therefore be lower than the true incidences. Furthermore, the low number of both exposure and outcome events limits the statistical power in subgroup analyses.

In our large cohort of subjects recruited from the general population, subjects who developed ischemic stroke had a transiently increased risk of VTE that was independent of traditional cardiovascular risk factors. The transient nature of the VTE risk following an ischemic stroke implies that conditions related to the stroke itself, rather than shared risk factors, are the main contributors to the VTE risk.

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Disclosures

None.

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Ischemic Stroke and Risk of Venous Thromboembolism in the General Population: The Tromsø Study

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Paper II

Repeated Measurements of Carotid Atherosclerosis and Future Risk of Venous Thromboembolism. The Tromsø Study

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Summary

Background: Whether a relationship between atherosclerosis and subsequent venous thromboembolism (VTE) exists is controversial.

Objective: To investigate the association between carotid atherosclerosis and VTE using repeated measurements of intima media thickness (IMT) and total plaque area (TPA) in participants recruited from the general population.

Methods: Participants were recruited from the fourth (1994-1995), fifth (2001-2002) and sixth (2007-2008) surveys of the Tromsø study. In total, 10426 participants attended, for whom measurements of carotid IMT and TPA and potential confounders were updated at each available survey. Time-varying Cox-regression models were used to calculate hazard ratios (HR) of VTE across various levels of IMT and TPA adjusted for age, sex and body mass index.

Results: There were 368 incident VTE events during a median follow-up of 10.8 years. Participants with increasing IMT were on average older and had a less favorable cardiovascular risk profile. There was no association between tertiles of increasing TPA and risk of VTE in the time-varying model, and increasing IMT was not associated with increased risk of VTE (HR 0.96, 95% CI 0.86-1.07). Neither plaque formation nor plaque progression was associated with risk of VTE (HR 1.00, 95% CI 0.98-1.02 and HR 0.96, 95% CI 0.84-1.11, respectively).

Conclusion: Carotid IMT and TPA was not associated with increased risk of VTE in time-varying analyses. Furthermore, there was no association between plaque initiation or plaque progression and subsequent VTE.

Key words: Atherosclerosis – Cohort studies – Repeated measurements – Risk factors – Venous thromboembolism

Essentials

- The relationship between atherosclerosis and venous thromboembolism (VTE) is controversial
- In total, 10426 participants recruited from the general population were included
- Carotid intima media thickness and total plaque area was not associated with VTE
- There was no association between plaque initiation or plaque progression and subsequent VTE

Introduction

Although medical textbooks consider venous thromboembolism (VTE) and arterial cardiovascular disease as different disease entities [1], Virchow's triad (1856) postulates that the pathophysiology of thrombosis, either venous or arterial, is an interplay between 1) stasis of the blood, 2) hypercoagulability, and 3) vessel wall injury [2]. The vascular component of Virchow's triad has been much less studied in the etiology of VTE as compared with arterial cardiovascular disease where vessel wall injury is an established precursor of disease.

Interestingly, recent studies have shown that arterial cardiovascular diseases, such as myocardial infarction and ischemic stroke, are associated with an increased risk of VTE [3-5]. In addition, in a landmark study from 2003, Prandoni *et al.* reported that atherosclerosis, measured by total plaque area [TPA], was twice as prevalent in patients with unprovoked venous thrombosis as in age and sex matched controls [6]. These findings suggested that atherosclerosis could be a shared risk factor for arterial cardiovascular disease and VTE. Although the association between atherosclerosis and arterial cardiovascular disease is well established [7-9], the association between atherosclerosis and VTE remains controversial. For instance, case-control studies are not designed to reveal the direction of the association and does not enable interpretations on causality due to the undetermined temporal sequence between exposure and outcome. Furthermore, the association between atherosclerosis and VTE might be explained by presence of confounding risk factors, such as increasing age and obesity [10].

Previous cohort studies did not show any association between atherosclerosis and subsequent VTE [11-13]. However, these cohorts were based on a single measurement of

TPA and carotid intima media thickness (IMT) obtained at the beginning of a follow-up period that lasted for more than 10 years. Because atherosclerosis may develop over time, a long follow-up with several years between the baseline measurement and the event could introduce regression dilution bias and thereby lead to underestimation of the true association [14, 15]. Therefore, a small effect of atherosclerosis on VTE risk could be masked in traditional cohort studies with single measurements and long-term follow-up. The potential problem of regression dilution could be overcome by utilizing repeated assessments of the atherosclerosis status within the same individuals during follow-up. This will provide a more accurate estimation of the risk status at the time before the outcome occurs.

We therefore aimed to investigate the association between the presence, formation and progression of carotid atherosclerosis and VTE using a large prospective cohort with repeated measurements of IMT and TPA, in participants recruited from the general population.

Methods

Study population

Participants were recruited from the fourth, fifth and sixth surveys of The Tromsø Study, conducted in 1994-95, 2001-02 and 2007-08, respectively. In the fourth study, all inhabitants aged 55-74 years and a random 5-10% sample in the other age groups >24 years, were invited to a second, more extensive examination, including ultrasound scanning of the carotid artery [16]. Subjects who attended the second visit of Tromsø 4, in addition to random samples within different age-groups, were eligible for the second

visit of Tromsø 5 and in Tromsø 6. A detailed description of the Tromsø Study has been published elsewhere [17]. Participants with a previous history of VTE were excluded. In addition, participants attending the ultrasound examination, but with missing information on the measures of carotid atherosclerosis, were excluded. In total, 10426 participants attended an ultrasound examination of the right carotid artery in Tromsø 4, 5 and/or 6 (Figure 1). The study was approved by the regional committee for research ethics in North Norway, and all participants gave their informed, written consent.

Atherosclerotic risk factors and assessment of atherosclerosis

Information on atherosclerotic risk factors was collected by physical examination, blood samples and self-administered questionnaires, and repeated at each survey. Height, weight, blood pressure and non-fasting serum lipids were measured as previously described in detail [18]. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Questionnaires were used to obtain information on use of lipid-lowering drugs, current smoking, diabetes mellitus, physical activity and education.

Ultrasound examination of the right carotid artery was performed for assessment of TPA and IMT. A thorough description of the ultrasonographic examination has been published previously [16, 19-21]. In brief , high-resolution B-mode ultrasonography of the right carotid artery was performed by experienced examiners, with the use of an ultrasound scanner (Acuson Xp10 128 ART equipped with a 7.5 MHz linear-array transducer in Tromsø 4 and 5; and a GE Vivid 7 with a linear 12 MHz transducer in Tromsø 6). The right carotid artery was scanned longitudinally from the level of the

clavicle, through the carotid bulb (bifurcation segment) and the proximal internal carotid segment (ICA) as far downstream as possible. A plaque was defined as a localized protrusion of the vessel wall into the lumen of at least 50% compared to the adjacent IMT. Still images were reported for each plaque and digitized using the Matrox Meteor II frame grabber card and Matrox Intellicam. With the use of Adobe Photoshop 7.0, measurements of plaque area were made by outlining the perimeter of the plaque, and the plaque area was calculated as pixel values. For the resolution used in the present study, a plaque area of 167 pixels corresponded to 1 mm^2. In each subject, a maximum of six plaques were registered in the near and far walls of the distal part of the common carotid artery (CCA), bifurcation, and ICA, respectively. TPA was calculated as the sum of all plaques. IMT was defined as the average of the mean IMT values of the near and far wall of the CCA and far wall of the bifurcation. To minimize variability in IMT during the cardiac cycle, image capturing was standardized by recording images at the top of the R wave in an ECG signal. Plaque initiation was defined as development of new plaques at follow-up in vessels without plaques at the previous examination, and plaque progression as the difference in TPA two measurements. Participants with negative progression were included in the no progression group [16, 22].

Identification and validation of VTE

All incident VTE events during follow-up were identified by searching the hospital discharge diagnosis registry, the autopsy registry and the radiology procedure registry at the University Hospital of North Norway. The University Hospital of North Norway is the only hospital in the region, and all diagnostic radiology and hospital care is provided

exclusively by this hospital. The medical record for each potential case of VTE was reviewed by trained personnel, and a VTE event was considered adjudicated when presence of clinical signs and symptoms of DVT or PE were combined with objective confirmation tests (by compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, pulmonary angiography, autopsy), and resulted in a VTE diagnosis that required treatment, as previously described in detail [23]. DVTs were recorded in the upper and lower extremities including inferior vena cava, and at unusual sites (the mesenteric veins, portal veins, and in the venous sinuses). VTE cases from the autopsy registry were recorded when the death certificate indicated VTE as cause of death or a significant condition contributing to death.

Statistical analysis

Statistical analyses were performed with STATA version 14.0 (Stata Corporation, College Station, TX, USA). As the distribution of TPA was skewed to the right, TPA was square root transformed to approximate normal distribution for the analyses in which TPA was used as a continuous variable. Cox proportional hazard regression models were used to assess the association between atherosclerosis (i.e. TPA and IMT) and VTE in a time-varying analysis. In these analyses, all participants contributed with one or more observation periods, each lasting from one measurement until the next, or until a censoring event (i.e. migration, death or end of study period) occurred. The follow-up ended on December 31, 2012. Atherosclerosis measurements and other risk factors were updated at every survey, when available, and used as time-varying covariates. Of the 10426 participants included in the study, 5154 participants attended two or three surveys,

which resulted in a total number of 18154 observation periods for the time-varying analyses. For participants attending only one survey, measurements were valid from baseline to the first censoring event. Age was used as time-scale, with the participants' age at study enrolment defined as entry-time and age at the censoring event as exit-time. Hazard ratios (HRs) with 95% confidence intervals (CI) were calculated, and all analyses were adjusted for age (as time-scale), sex and BMI. The proportional hazards assumption was confirmed by the Schoenfeld's global test. Statistical interactions between the covariates and the main exposures were tested by including the cross-product terms in the proportional hazard model, and no interactions were found.

We performed two sensitivity analyses. In the first sensitivity analysis we censored participants at the next survey they did not attend. This analysis was performed to ensure that the carry-on of measurements in participants who only attended one survey did not dilute the effect in the original analyses. Statin use may potentially confound the association between atherosclerosis and VTE. Since we did not have sufficient information on statin use among the Tromsø 4 participants, the second sensitivity analysis was restricted to participants who did not use lipid-lowering drugs in Tromsø 5 or Tromsø 6.

Results

During a median follow-up of 10.8 years, 368 participants experienced an incident VTE event. Baseline characteristics of traditional atherosclerotic risk factors and TPA across quartiles of carotid IMT are shown in Table 1. In general, all traditional atherosclerotic risk factors changed for the worse across increasing quartiles of IMT. Participants in the

fourth quartile had higher blood pressure, BMI, triglycerides and total cholesterol, and lower HDL cholesterol, compared with participants in the first quartile. Participants in the highest quartile also comprised a higher proportion of males as well as participants with hypertension and self-reported diabetes, and a lower proportion of physically active and highly educated participants. Each quartile of IMT comprised approximately the same proportion of current smokers.

HRs for VTE by TPA and IMT as continuous and categorical variables are shown in Table 2. There was no association between TPA as a continuous variable and VTE (HR per standard deviation [SD] increase 0.99, 95% CI 0.90-1.11), and no linear trend of increased risk of VTE across increasing tertiles of TPA when no plaque was set as the reference group (*P* for trend=0.9). IMT was not associated with risk of VTE (HR per SD increase 0.96, 95% CI 0.86-1.07) and the *P* for trend across increasing quartiles of IMT was 0.7. Additional adjustment for total cholesterol, high-density lipoprotein cholesterol, smoking, diabetes mellitus and diastolic blood pressure had a negligible effect on the risk estimates (HR per SD increase for TPA and IMT were 0.98 [95% CI 0.88-1.09] and 0.96 [0.86-1.07], respectively). Similar results were observed when the participants were censored at the first survey they did not attend (Supplementary table 1) and when the analyses were restricted to participants not using lipid-lowering drugs in Tromsø 5 or 6 (Supplementary table 2).

HR for VTE according to formation and progression of carotid plaques are displayed in Table 3. There was no association between plaque formation and future risk of VTE (HR 1.00, 95% CI 0.98-1.02). Progression of carotid plaque size was not associated with VTE (HR 0.96, 95% CI 0.84-1.11), and there was no linear trend of VTE

risk across tertiles of plaque progression in TPA (*P* for trend=0.5). The multivariable adjusted model showed similar results for both plaque formation and plaque progression.

Discussion

Previous case-control studies have reported an association between carotid plaques and VTE [6, 24], whereas later cohort studies [11-13] have not shown any association between carotid atherosclerosis and future risk of VTE. A potential limitation of cohorts with long follow-up is that changes in atherosclerosis over time could lead to an underestimation of the true association between atherosclerosis and VTE [14, 15]. To investigate whether the apparent discrepant results in case-control and cohort studies could be explained by regression dilution bias, we conducted a study with repeated measurements of carotid atherosclerosis within the same individuals during follow-up. We found that measures of carotid atherosclerosis were not associated with future risk of VTE. Our findings suggest that atherosclerosis as measured with carotid ultrasound is not an intermediate for the association between arterial and venous thrombosis.

Our results are in accordance with previous cohort studies on the association between atherosclerosis and VTE using time-fixed analyses [11-13]. The Atherosclerosis Risk in Communities **(**ARIC) study, which included 13,000 subjects aged 45-64 years with a median follow-up time of 12.5 years, found no association between increased carotid IMT or presence of carotid plaques, and VTE risk [11]. The Cardiovascular Health Study (CHS) study followed 4100 subjects aged 65 and older over 12 years, and measured subclinical atherosclerosis by IMT, presence of carotid plaques, ankle brachial index and ECG abnormalities. In this study, subclinical atherosclerosis was not associated

with increased risk of overall or unprovoked VTE. Unexpectedly, they found an inverse relationship between high-risk carotid plaques and VTE [12]. Furthermore, a previous study from the Tromsø cohort with 15.4 years of follow-up, including more than 6200 participants, found that single measurements of IMT and TPA at baseline were associated with future myocardial infarction, but not VTE [13].

The finding of no association between atherosclerosis and VTE in cohort studies is in contrast to the results from two previous case-control studies [6, 24]. Prandoni *et al* reported a higher frequency of carotid plaques in 153 patients with unprovoked VTE compared to 146 patients with provoked VTE and 150 hospitalized controls. In this study plaques were defined as a protrusion into the vessel lumen of at least 2 mm (6). In a study including 89 cases of unprovoked VTE and 89 controls, Hong *et al* reported an association between coronary artery calcification and VTE (22). Several factors may explain the diverging results from cohorts and case-control studies conducted on this topic. Recruitment of controls that are not fully representative of the source population from which the cases were derived, may result in overestimation of the true effect in case-control studies. This problem is more likely to occur when the size of the control group is small. Moreover, the exposure is measured after the outcome in case-control studies, and therefore the temporal sequence of the events cannot be determined. In conventional cohorts, exposure may change over time and this may lead to underestimation of the true effect. However, with repeated measurements it was possible to update an individual's risk status over time, and consequently get a better estimation of an individual's atherosclerosis status in the period before the VTE diagnosis. Using this

approach, we did not find any association between carotid atherosclerosis measures and VTE risk.

Although some studies have reported associations between atherosclerotic risk factors such as diabetes, hypertension and dyslipidemia, and risk of VTE [25-27], the only atherosclerotic risk factors that have consistently been shown to increase the risk of VTE are age and obesity [18, 28, 29]. A recent meta-analysis of 9 cohorts, including almost 250,000 participants and 5000 VTEs, found no association between traditional, modifiable atherosclerotic risk factors and VTE, using traditional time-fixed Cox regression models adjusted for age, sex and BMI [30]. The only exception was cigarette smoking, which was associated with increased risk of provoked VTE, an association that was possibly mediated through other conditions such as cancer. Furthermore, in a previous report from the Tromsø study, based on repeated measurements of atherosclerotic risk factors, we showed that BMI, but not blood pressure, serum lipid levels, diabetes or smoking, were associated with increased risk of VTE [31].

Major strengths of our study include the prospective design with repeated exposure measurements and long follow-up, the large number of participants recruited from the general population, and the thorough validation and adjudication of VTE. The repeated measurements of atherosclerosis and potential confounders made it possible to update risk status over time, and thereby to reduce the chance of regression dilution bias. The study has some limitations. Unfortunately, we did not have verified baseline information on previous history of VTE among all the study-subjects. We started to identify VTE cases in January 1994, and those who were registered with a recurrent event in the study period (1994-2012), and those who had a VTE shortly before inclusion, were

identified and excluded from the analyses due to previous VTE. Subjects who had a VTE before 1994 and did not experience a recurrence in the study period would not be detected, and consequently, these would be treated as healthy participants during follow-up. As the prevalence of VTE in the general population is relatively low, this would lead to only a small change in the overall number of person-years at risk, and thus would presumably have a negligible influence on the risk estimates. Carotid ultrasonography is operator dependent and prone to measurement errors. However, a previous study found the overall reproducibility of TPA to be good, with small inter-observer mean arithmetic and mean absolute differences [16]. Although the measurement errors in carotid ultrasonography are too big to study progression of atherosclerosis at an individual level, carotid ultrasonography at a population level gives enough power to overcome the measurement variability, and makes it possible to detect even weak associations [16]. Examination of only one carotid artery may potentially introduce misclassification. However, studies comparing ultrasound IMT measurements of the left and right common carotid artery found no significant difference between the sides in the normal population [32, 33]. Furthermore, studies have shown that carotid atherosclerosis correlates well with the general extent of atherosclerotic disease in an individual [34, 35]. Statins has been shown to reduce the risk of VTE in some [36-38], but not all studies [39, 40]. Statin use reduces carotid plaque development and lowers plaque progression [41, 42], and lack of adjustment for statin use could result in underestimation of the association between atherosclerosis and VTE. However, sensitivity analysis restricted to participants who did not use statins showed no association between carotid atherosclerosis and VTE. Aspirin is often prescribed to subjects at risk of cardiovascular disease, but may also prevent venous

thrombosis. However, although aspirin use has been associated with decreased risk of recurrent VTE [43, 44], it has not been associated with reduced risk of incident VTE in population based studies [37, 45].

In conclusion, we found that formation and progression of carotid atherosclerosis, as measured with ultrasound, was not associated with future risk of VTE in time-varying analyses. Our findings suggest that atherosclerosis is not an intermediate for the association between arterial cardiovascular diseases and VTE.

Addendum

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Conceptualization: JBH, SKB, WML

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Table 1. Baseline characteristics of traditional atherosclerotic risk factors across quartiles of carotid intima media thickness (IMT). In total, 10426 participants were included in the study. The Tromsø Study, 1994-2012.

The Tromsø Study	**1st quartile** 0.36-0.73 mm	**2nd quartile** 0.73-0.83 mm	**3rd quartile** 0.83-0.95 mm	**4th quartile** 0.95-2.49 mm
Number of participants, n	2612	2618	2590	2606
VTE events, n	69	92	79	128
Age, years	53.2 ± 10.4	59.1 ± 6.7	61.5 ± 6.6	64.2 ± 6.7
Male sex, %	31.9 (832)	39.5 (1034)	51.1 (1323)	59.2 (1542)
Systolic BP, mmHg	131 ± 19	139 ± 21	144 ± 22	152 ± 23
Diastolic BP, mmHg	78 ± 11	81 ± 12	83 ± 12	85 ± 13
Hypertension, %*	34.0 (887)	50.9 (1331)	62.9 (1626)	75.1 (1956)
BMI, kg/m^2	25.2 ± 3.7	26.4 ± 4.1	26.8 ± 4.0	27.2 ± 4.2
Triglycerides, mmol/L	1.47 ± 0.98	1.61 ± 0.99	1.67 ± 1.01	1.80 ± 1.02
Total cholesterol, mmol/L	6.19 ± 1.23	6.40 ± 1.26	6.43 ± 1.27	6.61 ± 1.35
HDL cholesterol, mmol/L	1.61 ± 0.45	1.59 ± 0.44	1.53 ± 0.42	1.46 ± 0.43
Self-reported diabetes, %	1.6 (42)	2.8 (72)	3.7 (95)	6.0 (155)
Smoking, %	31.6 (823)	26.9 (703)	27.0 (699)	29.2 (761)
Physical activity, % †	32.8 (817)	33.9 (844)	31.6 (776)	25.6 (632)
Education, % ‡	26.1 (653)	23.8 (584)	21.7 (522)	17.4 (426)
Total plaque area, mm^2	0.55 ± 1.29	1.18 ± 1.79	1.98 ± 2.23	3.97 ± 2.68
No plaque, %	82.8 (2163)	65.9 (1725)	50.5 (1307)	21.9 (570)
1st tertile, %	10.7 (280)	18.2 (476)	18.1 (468)	12.7 (330)
2nd tertile, %	4.8 (126)	10.8 (282)	18.8 (488)	25.2 (658)
3rd tertile, %	1.7 (43)	5.1 (135)	12.6 (327)	40.2 (1048)

Values are % (n) or mean ± SD. BP indicates blood pressure; BMI, body mass index; HDL, high-density lipoprotein.

* Hypertension: systolic BP ≥140 or diastolic BP ≥90 or use of antihypertensive medicine

† Hard physical activity 1 hour or more every week

‡Over/equal to 15 years of education (corresponding to 3 years in university or academy)

Table 2. Hazard ratios (HR) with 95% confidence intervals (CI) of venous thromboembolism (VTE) according to total plaque area and intima media thickness using a time-varying Cox regression model. The Tromsø Study 1994-2012.

Risk factors	Events	Person-years	HR (95% CI) †
Total Plaque Area*	368		**0.99 (0.90-1.11)**
No plaque	140	54062	Ref.
1st tertile (1.018-3.506 mm^2)	64	19648	0.93 (0.69-1.29)
2nd tertile (3.506-5.031 mm^2)	78	19141	1.04 (0.79-1.38)
3rd tertile (5.031-15.696 mm^2)	86	18685	1.00 (0.75-1.32)
P for trend			*0.9*
Intima Media Thickness*	368		**0.96 (0.86-1.07)**
1st quartile (0.358-0.743 mm)	58	29229	Ref.
2nd quartile (0.744-0.849 mm)	81	28210	0.95 (0.68-1.34)
3rd quartile (0.849-0.970 mm)	101	27201	1.02 (0.73-1.43)
4th quartile (0.971-2.748 mm)	128	26896	1.07 (0.77-1.50)
P for trend			*0.5*

* Per standard deviation (SD) increase; 1 SD TPA = 2.60 mm^2; 1 SD IMT = 0.19 mm

† Adjusted for age (as time scale), sex and BMI

Table 3. Hazard ratios (HR) with 95% confidence intervals (CI) for venous thromboembolism (VTE) by initiation and progression of carotid plaques. The Tromsø Study 1994-2012.

	Model 1 **HR (95% CI) §**	**Model 2** **HR (95% CI) ¶**
Plaque formation*	1.00 (0.98-1.02)	1.00 (0.98-1.02)
Plaque progression†	0.96 (0.84-1.11)	0.96 (0.83-1.11)
No progression‡	Ref.	Ref.
0.010-8.250 mm^2 increase	0.85 (0.42-1.01)	0.68 (0.44-1.05)
8.254-17.8401 mm^2 increase	0.99 (0.68-1.44)	1.00 (0.68-1.46)
17.850-131.734 mm^2 increase	0.85 (0.57-1.25)	0.84 (0.56-1.25)
P for trend	*0.5*	*0.5*

* Initiation of plaque, i.e. increase from 0. Based on TPA measurement

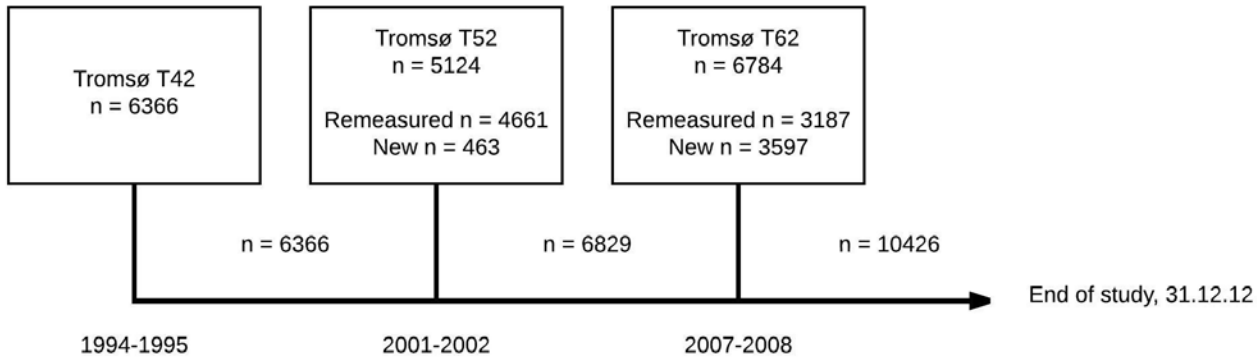
† 1 standard deviation (SD) change in plaque size based on TPA measurement. 1 SD = 13.2 mm^2 increase

‡ Participants with negative change were included in the no progression group.

§ Adjusted for age (as time scale), sex and BMI

¶ Adjusted for age (as time scale), sex, BMI, total cholesterol, high-density lipoprotein cholesterol, smoking, diabetes mellitus and diastolic blood pressure

Figure 1. Study population. Study population recruited from the second visit at the fourth, fifth and sixth surveys of The Tromsø Study, conducted in 1994-95, 2001-02 and 2007-08, respectively.



Paper III

PLOS ONE

RESEARCH ARTICLE

Atherosclerotic Risk Factors and Risk of Myocardial Infarction and Venous Thromboembolism; Time-Fixed versus Time-Varying Analyses. The Tromsø Study

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Data Availability Statement: Data are available after application and agreement with the Department of Community Medisin (UiT The Arctic University of Tromsø) according to their data application process. In addition to the application, an existing or new approval from the Regional Committee for Medical Research ethics (REK) is required. Information on each variable can be found at http://tromsoundersokelsen.uit.no/tromso/ and information on how to apply for data access at https://en.uit.no/prosjekter/prosjekt?p_document_id=7124.

Abstract

Background

Single measurements of modifiable risk factors may underestimate associations with outcomes in cohorts. We aimed to compare risk estimates of myocardial infarction (MI) and venous thromboembolism (VTE) by atherosclerotic risk factors during long follow-up using time-fixed analyses without and with correction for regression dilution and time-varying analyses.

Methods

The study included 5970 subjects enrolled in the fourth survey of the Tromsø Study (1994/95). Blood pressure, lipid levels, body mass index (BMI), diabetes and smoking status were measured at baseline, and subjects still alive at the fifth (2001/02, n = 5179) and sixth (2007/08, n = 4391) survey were re-measured. Incident events of MI (n = 714) and VTE (n = 214) were recorded until December 2010. Time-fixed and time-varying Cox regression models were used to estimate hazard ratios (HR) for MI and VTE adjusted for age and sex.

Results

Variations in BMI, blood pressure and lipid levels were small, and did not alter the risk estimates when time-varying analyses were compared to time-fixed analyses. For MI, variables that changed considerably over time yielded the greatest changes in risk estimates (HR for smoking changed from 1.80 (95% CI 1.55–2.10) to 2.08 (95% CI 1.78–2.42)). For VTE, only BMI was associated with increased risk in both time-fixed and time-varying analysis, but the

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risk estimates weakened in the time-varying analysis. Correction of time-fixed HRs with Rosner´s method tended to overestimate risk estimates compared to time-varying analysis.

Comment

For MI and VTE, risk estimates based on baseline and repeated measures corresponded well, whereas correction for regression dilution tended to overestimate risks.

Introduction

Arterial thrombotic disease (e.g. myocardial infarction [MI] and stroke) and venous thromboembolism (VTE) have traditionally been considered separate diseases with different pathophysiology, but during the last decade studies have supported a bidirectional association between them [1–4]. Whether the association between arterial and venous thrombosis is causal or mediated through shared risk factors remains uncertain. Of the traditional cardiovascular risk factors, only age, obesity and family history of MI have consistently been associated with VTE [5–10], whereas diabetes, hypertension and dyslipidemia have been associated with VTE in some [11–15] but not all [6, 16–18] studies. The majority of the studies that found an association between atherosclerotic risk factors and VTE were of a retrospective nature [11, 13–15], whereas most prospective studies reported no association [6, 16–18].

In conventional cohort studies, risk factor levels are usually assessed at the time of inclusion and related to outcomes occurring several years, or even decades, later. However, the status of a risk factor may change over time, and these changes usually become greater with time from exposure assessment. Both the effect of a risk factor (called time-dependent effect) and the value of the risk factor itself (called time-dependent covariate) can change over time. Random measurement errors, temporary fluctuations, and true changes in variables over time generally lead to regression dilution bias [19], a phenomenon that results in underestimation of the true association between exposure and outcome. As most atherosclerotic risk factors are modifiable, changes during follow-up may have influenced the risk estimates of MI and VTE in previous cohort studies. Thus, the absence of an association between atherosclerotic risk factors and VTE found in cohorts could potentially be explained by regression dilution.

Regression dilution bias is potentially a major limitation of prospective cohorts that could either be addressed by performing time-varying analysis or correct the risk estimates by a regression dilution ratio. When a variable is assessed within the same individual at different time points during the study period, time-varying analysis will allow for changes in exposure status during follow-up. If repeated measurements exists only for a subsample of individuals within a cohort, a regression dilution ratio can be calculated and used to correct the risk estimates from time-fixed analyses [20, 21]. Using this approach, a previous study reported that a single baseline measurement of cholesterol and diastolic blood pressure resulted in a respectively 47% and 76% underestimation of the association with coronary heart disease risk in the third decade of follow-up [22]. Another study reported that baseline assessment of disease risk underestimated the strength of the real associations by about one-third the first decade, about one-half the second decade, and about two-thirds the third decade [23]. However, it has been suggested that simple methods of correction for regression dilution bias may lead to overcorrection if the relationship between risk factor and disease is not short term [24].

In a prospective population-based cohort, we therefore aimed to investigate whether the use of repeated measurements of atherosclerotic risk factors influenced the risk estimates for VTE

and MI compared to using baseline measurements only, with and without correction for the regression dilution bias. Secondly, we aimed to investigate whether the lack of association between atherosclerotic risk factors and VTE in previous long-term cohorts could be explained by regression dilution bias.

Methods

Study population

Participants were recruited from the fourth, fifth and sixth surveys of the Tromsø study (conducted in 1994–1995, 2001–2002 and 2007–2008, respectively). A detailed description of the Tromsø surveys has been published elsewhere [25]. In brief, the entire population (Tromsø 4) or parts of the population (Tromsø 5 and 6) aged ≥25 years living in the municipality of Tromsø, Norway, were invited to participate in these surveys. In Tromsø 4, all men aged 55–74 and women aged 50–74, as well as smaller (5–8%) random samples of other age groups were invited to a more extensive second examination. All subjects attending the second visit in Tromsø 4 were re-invited to attend Tromsø 5 and Tromsø 6 if they were still alive and living in the municipality of Tromsø. Subjects who attended the second phase of Tromsø 4 (n = 6861), as well as subjects attending the first phase of Tromsø 4 and the two subsequent visits (n = 418), were considered eligible for the present study. Subjects with VTE (n = 22) or MI (n = 306) prior to baseline, and subjects not officially registered as inhabitants of the municipality of Tromsø at baseline (n = 7), were excluded. Moreover, subjects who were re-invited but failed to attend one or more visits were excluded from follow-up (n = 974). Subjects who died (n = 1142) or moved (n = 437) between two subsequent visits were censored at the date of death or migration during follow-up. Thus, 5970 participants were included, of which 4391 attended all three surveys (Fig 1). The study was approved by the Regional Committee of Medical Health Research Ethics North Norway, and all participants provided informed written consent.

Atherosclerotic risk factors

Information on atherosclerotic risk factors was collected by physical examinations, blood samples and self-administered questionnaires. Similar examinations, blood tests and questionnaires were repeated at each survey. Height and weight were measured with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in

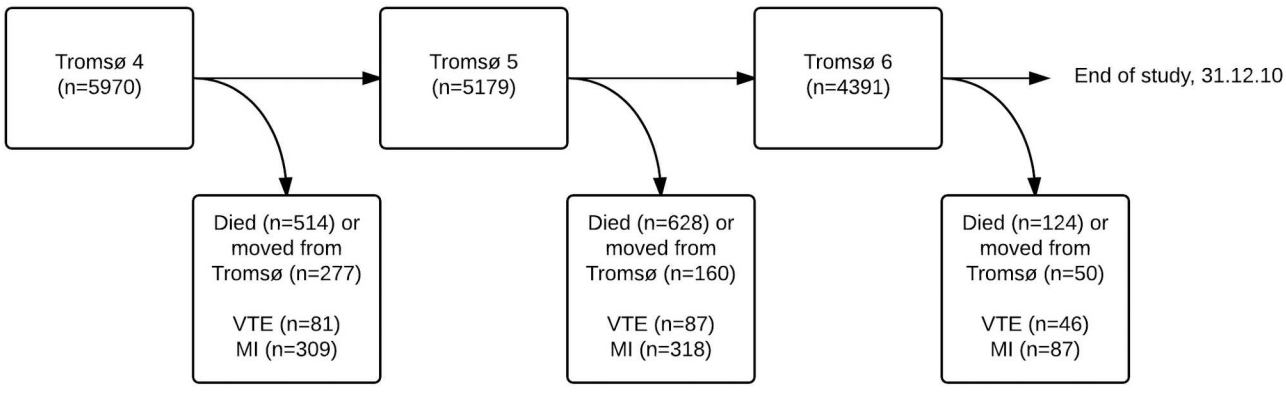

Fig 1. Study population. Study population recruited from The Tromsø Study, 1994–2010. Figure showing the common, "basic", population in each analysis, and number of incident VTE and MI events (separate analyses for each outcome)

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kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured three times with an automatic device (Dinamap Vital Signs Monitor) in a sitting position after two minutes of rest. The average of the two last readings was used in the analyses. Non-fasting blood samples were collected from an antecubital vein and total cholesterol, triglycerides and high-density lipoprotein (HDL) measured. Self-administered questionnaires were used to obtain information on diabetes, smoking (current smoker yes/no), physical activity (strenuous physical activity 1 or more hour per week) and education (over or equal to 15 years of education). Hypertension was defined as systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$90 mmHg or current antihypertensive treatment. Overweight (BMI 25–29.9 kg/m^2) and obesity (BMI $\geq$30 kg/m^2) was classified according to the World Health Organization (WHO) definition [26], and hypercholesterolemia was defined as total cholesterol $\geq$6.5 mmol/L or self-reported use of lipid-lowering drugs. Low HDL cholesterol was defined as $\leq$1.03 mmol/L in men or $\leq$1.30 mmol/L in women, according to the National Cholesterol Education Program-Adult Treatment Panel III guidelines [27], as described elsewhere [28].

Identification and validation of MI

All incident events of MI were identified by searching hospital and out-of hospital medical records, autopsy records and death certificates, and all possible events were validated by an independent end-point committee. The unique national 11-digit identification number allowed linkage to national and local diagnosis registries. Possible cases of MI were identified by linkage to the hospital discharge registry at the University Hospital of North Norway by searching for relevant International Classification of Diseases, as previously described [29] The hospital medical records were retrieved for case validation. MI events were validated according to modified World Health Organization MONICA/MORGAM criteria, including clinical signs and symptoms, findings in electrocardiograms, values of cardiac biomarkers and autopsy records when applicable [30]. Further, linkage to the National Causes of Death Registry at Statistics Norway allowed identification of fatal incident cases of MI that occurred as out-of hospital deaths, including deaths that occurred outside of Tromsø, as well as information on all-cause mortality. Information from death certificates was used to collect relevant information of the event from additional sources, including autopsy reports and records from nursing homes, ambulance services and general practitioners.

Identification and validation of venous thromboembolism

As previously described [31], all incident VTE events were identified by searching the hospital discharge diagnosis registry, the autopsy registry and the radiology procedure registry at the University Hospital of North Norway. The University Hospital of North Norway is the only hospital in the region, and all hospital care and relevant diagnostic radiology is provided exclusively by this hospital. The medical record for each potential case of VTE was reviewed by trained personnel, and an episode of VTE was confirmed and registered as a validated VTE event when all of the following four conditions were satisfied: 1) confirmation by objective diagnostic procedures, including compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, pulmonary angiography or autopsy; 2) indication in the medical records that a physician diagnosed deep vein thrombosis or pulmonary embolism; 3) presence of clinical signs and symptoms consistent with deep vein thrombosis or pulmonary embolism; and 4) treatment with anticoagulants (heparin, warfarin), thrombolytic therapy or vascular surgery was required unless contraindications were specified [31]. VTE cases from the autopsy registry were recorded when the death certificate indicated VTE as the cause of death, or a significant condition associated with death.

Statistical analysis

Statistical analyses were performed with STATA version 13.0 (Stata Corporation, College Station, TX, USA) and the figure showing intra-individual variability (Fig 2) was made using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). The significance level was set to 0.05. Follow-up time and risk estimates for VTE and MI were calculated separately. Atherosclerotic risk factors were measured at baseline (1994–1995), and subjects still living in the municipality of Tromsø at the fifth (2001–2002, n = 5179) and sixth (2007–2008, n = 4391) survey of the Tromsø study were re-measured. For each participant, person-years of follow-up were counted from the date of enrollment (1994–1995) to the date of an incident VTE or MI event (one analysis for each endpoint), the date the participant died or moved from the municipality of Tromsø, or until the end of the study period (December 31, 2010), whichever came first. Age was used as time-scale and the entry and exit time was defined as the participants' age at study enrollment and censoring event (MI, VTE, death, migration or end of study). We used three different approaches to calculate hazard ratios (HRs) of MI and VTE: (i) time-fixed analysis, (ii) correcting for time-dependent covariates using a regression dilution ratio, and (iii) time-varying analysis. In the first approach, we used a traditional Cox proportional hazard regression model that included baseline measurements from Tromsø 4. HRs with 95% confidence intervals (CI) for MI and VTE, adjusted for age as time scale and sex, were calculated. In the second approach, HRs

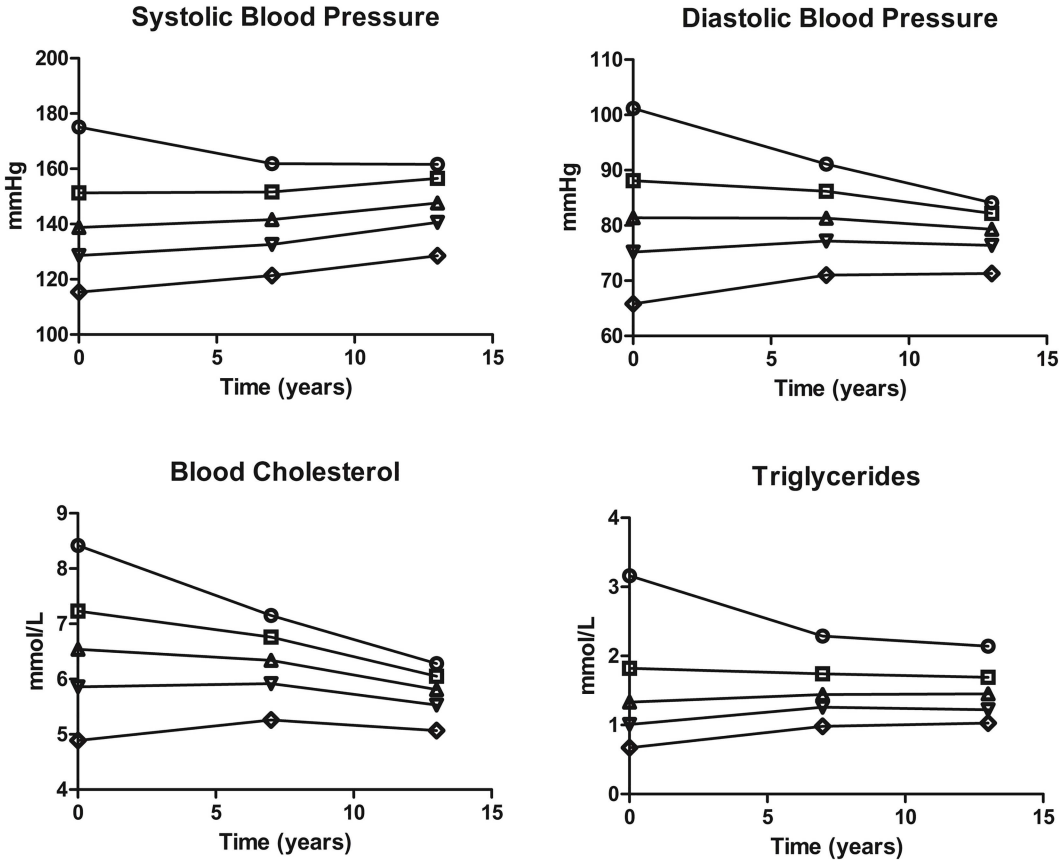

Fig 2. Intra-individual variability over time. Subjects were divided into quintiles at baseline in Tromsø 4 according to their baseline value of a certain risk factor. The mean value in each group is represented in the figure. Values were updated after approximately 7 and 13 years, in Tromsø 5 and Tromsø 6, respectively.

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calculated from traditional Cox proportional hazard regression models based on baseline measurements were corrected by a regression dilution ratio calculated by Rosner´s method for repeated measurements [32, 33]. This was done by multiplying the hazard coefficients of the normal time-fixed Cox regression model by the estimated regression dilution bias coefficient. The regression dilution bias coefficient was found as the inverse of the slope of the linear regression line of risk factor measurement of Tromsø 4 vs. Tromsø 5. The measurement time in Tromsø 5 was chosen (ignoring Tromsø 6) as the dependent value of the regression as it was approximately midway between the start and end of the study, as suggested by Clarke et al. [23]. In the last approach, a time-varying Cox proportional hazards regression analysis was used. Here, each participant contributed with one or more observation periods and each period lasted from one measurement until the next (i.e. from T4 to T5, from T5 to T6, and from T6 until the end of study the study period). The 5970 participants contributed with 15101 and 15403 observation periods in the MI and VTE analysis, respectively. The risk factors were updated at every measurement and used as time-dependent covariates in the analysis. The number of subjects included in the analyses of each risk factor varied slightly due to missing data for covariates ($< 2\%$ missing).

Joint modelling of repeated measurements and time-to-event data was considered, but found unsuitable due to convergence issues, as our dataset contained several participants with only one or two repeated measurements, particularly among those who experienced a VTE or MI event.

The proportional hazards assumption for the different risk factors was tested by evaluating the parallelism between curves of the log-log survivor function. The assumption was verified for all risk factors associated with VTE. For MI, the assumption was verified when stratifying by age (over and under 60 years old).

Results

Among the 5970 participants, 741 subjects had an incident MI and 214 subjects an incident VTE during a median follow-up time of 15.7 years. The distribution of atherosclerotic risk factors in the three different surveys is shown in Table 1. The average value of BMI increased, whereas triglycerides and total cholesterol levels decreased from baseline and throughout the study. The mean age changed less than the time intervals between the studies as a consequence of people dying or moving from the municipality of Tromsø, and the proportion of men decreased over time due to a higher mortality rate among men. The most prominent changes were observed for the proportion of subjects with self-reported diabetes (increased from 2.4% to 7.1%), the proportion of smokers (decreased from 33.0% to 16.4%), and the proportion of physically active subjects which increased from 24.2% in 94–95 to 37.9% in 07–08. In addition, the proportion of subjects with hypertension increased from 49.6% in 94–95 to 67.5% in 07–08 (Table 1). Similar patterns were observed when the analyses were restricted to those who participated in all three surveys (S1 Table).

Even though the overall changes in blood pressure and blood lipids were small, we observed large differences when subjects were divided into quintiles according to their baseline value (Fig 2). In general, the groups in the lowest and highest quintiles at baseline changed the most, and shifted towards the overall mean. This was a result of both regression towards the mean, where the most extreme values tend to normalize over time, and measurement errors.

Table 2 shows hazard ratios for MI by the different atherosclerotic risk factors. All risk factors except one subgroup of BMI (BMI 25–29.9 kg/m^2) were significantly associated with MI. For continuous variables, the differences were generally very small when the risk estimates based on baseline measurements (i.e. time-fixed analysis) were compared with those from

Table 1. Distribution of traditional atherosclerotic risk factors in the different surveys. In total 5970 were included in the study in 1994/95, and of these, 5179 and 4391 were re-measured in the 2001–02 and 2007–08, respectively.

The Tromsø Study	T4 (1994–1995)	T5 (2001–2002)	T6 (2007–2008)
Observations (n)	5970	5179	4391
Age, years	56.8 ± 11	62.9 ± 10	67.5 ± 10
Male sex	43.9	42.1	37.7
Systolic BP (mmHg)	141 ± 22	141 ± 21	145 ± 24
Diastolic BP (mmHg)	82 ± 13	81 ± 12	78 ± 11
Hypertension*	51.3 (3064)	56.8 (2941)	68.1 (3008)
Antihypertensive treatment	9.4 (560)	21.2 (1074)	35.2 (1511)
BMI (kg/m^2)	25.7 ± 3.9	26.9 ± 4.1	27.0 ± 4.3
<25 kg/m^2	46.5 (2775)	35.5 (1827)	34.3 (1503)
25–29.9 kg/m^2	40.9 (2441)	44.7 (2302)	45.3 (1984)
≥30 kg/m^2	12.5 (747)	19.9 (1023)	20.4 (894)
Triglycerides (mmol/L)	1.59 ± 1.01	1.53 ± 0.87	1.49 ± 0.81
Total cholesterol (mmol/L)	6.58 ± 1.27	6.28 ± 1.16	5.73 ± 1.13
Hypercholesterolemia †	51.9 (3097)	50.5 (2614)	46.9 (2058)
Lipid lowering drugs	1.8 (83)	12.3 (617)	23.3 (992)
HDL cholesterol (mmol/L)	1.56 ± 0.43	1.49 ± 0.40	1.57 ± 0.46
≥1.03 (♂) or ≥1.30 (♀) mmol/L	84.0 (5000)	78.6 (4051)	83.4 (3616)
<1.03 (♂) or <1.30 (♀) mmol/L	16.0 (954)	21.4 (1104)	16.6 (722)
Self-reported diabetes	2.4 (144)	4.2 (213)	7.1 (302)
Smoking	33.0 (1967)	25.6 (1328)	16.4 (719)
Physical activity |	24.2 (1492)	33.5 (1367)	37.9 (1383)
Education ⋮	20.0 (1191)	20.6 (1064)	24.1 (1037)

Values are % (n) or mean±SD. BP indicates blood pressure; BMI, body mass index; HDL, high-density lipoprotein.

*Hypertension: systolic BP ≥140 or diastolic BP ≥90 or use of antihypertensive medicine

†Hypercholesterolemia: total cholesterol ≥ 6.5 or use of lipid-lowering drugs

|Hard physical activity 1 hour or more every week

⋮Over/equal to 15 years of education (corresponding to 3 years in university or academy)

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repeated measurements (i.e. time-varying analysis). The largest differences between the two methods were observed for the categorical variables, and were particularly prominent for the variables that changed most at the population level, i.e. diabetes, smoking and physical activity. For MI, the risk estimates associated with diabetes changed from 2.94 (95% CI 2.20–3.92) to 2.11 (95% CI 1.63–2.72), smoking from 1.80 (95% CI 1.55–2.10) to 2.08 (95% CI 1.78–2.42) and physical activity from 0.74 (95% CI 0.61–0.89) to 0.61 (95% CI 0.50–0.74). Other risk estimates that changed considerably when comparing the two methods were those associated with hypertension, where the HR changed from 1.90 (95% CI 1.61–2.25) to 1.72 (95% CI 1.44–2.06) and obesity (BMI ≥30 kg/m^2), where the HR changed from 1.43 (95% CI 1.15–1.79) to 1.28 (95% CI 1.04–1.57). The regression dilution correction by Rosner´s method consistently overestimated the risk estimates compared with the time-varying analyses (Table 2).

Hazard ratios of VTE according to the different atherosclerotic risk factors are shown in Table 3. In general, there were only small differences between the risk estimates based on the time-fixed analysis and those calculated with the time-varying analysis. In the time-fixed model and in the model corrected by Rosners´s method, BMI and hypertension was significantly associated with VTE. However, the association between hypertension and VTE disappeared when adjusting for BMI in addition to age and sex with HR of 1.24 (95% CI 0.92–1.69)

Table 2. Age (as time scale)- and sex-adjusted hazard ratios (HR) with 95% confidence intervals (CI) for the risk of myocardial infarction (MI) by traditional atherosclerotic risk factors using three different approaches; time-fixed model, time-varying model and correction for regression dilution through Rosners´s method. The Tromsø Study 1994–2010.

Risk factors	Time-fixed Cox-model	Time-varying Cox-model	Time-fixed model corrected by Rosner´s method
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Male sex	2.46 (2.11–2.87)	2.46 (2.11–2.87)	2.46 (2.11–2.87)
Systolic BP (per 15 mmHg increase)	1.31 (1.23–1.37)	1.23 (1.18–1.29)	1.54 (1.43–1.67)
Diastolic BP (per 10 mmHg increase)	1.26 (1.18–1.32)	1.24 (1.18–1.32)	1.49 (1.35–1.65)
Hypertension*	1.97 (1.66–2.35)	1.73 (1.44–2.07)	3.69 (2.65–5.14)
BMI, 3 units increase (kg/m^2)	1.14 (1.08–1.21)	1.10 (1.04–1.16)	1.15 (1.08–1.21)
<25 kg/m^2	Ref.	Ref.	Ref.
25–29.9 kg/m^2	1.15 (0.98–1.35)	1.05 (0.89–1.24)	1.17 (0.96–1.43)
≥30 kg/m^2	1.43 (1.15–1.79)	1.28 (1.04–1.57)	1.25 (1.09–1.44)
Triglycerides (mmol/L)	1.21 (1.15–1.28)	1.23 (1.16–1.31)	1.49 (1.33–1.67)
Total cholesterol (mmol/L)	1.22 (1.14–1.29)	1.23 (1.16–1.31)	1.43 (1.28–1.60)
Hypercholesterolemia †	1.44 (1.24–1.68)	1.42 (1.22–1.65)	1.90 (1.45–2.50)
HDL cholesterol (mmol/L) ‡	0.78 (0.70–0.86)	0.78 (0.70–0.86)	0.71 (0.62–0.81)
≥1.03 (♂) or ≥1.30 (♀) mmol/L	Ref.	Ref.	Ref.
<1.03 (♂) or <1.30 (♀) mmol/L	1.48 (1.24–1.78)	1.34 (1.12–1.61)	2.06 (1.47–2.89)
Self-reported diabetes	2.94 (2.20–3.92)	2.11 (1.63–2.72)	3.17 (2.33–4.32)
Smoking	1.80 (1.55–2.10)	2.08 (1.78–2.42)	2.28 (1.85–2.82)
Physical activity |	0.74 (0.61–0.89)	0.61 (0.50–0.74)	0.40 (0.22–0.70)
Education ⋮	0.54 (0.43–0.69)	0.55 (0.43–0.69)	0.54 (0.43–0.69)

*Hypertension: systolic BP ≥140 or diastolic BP ≥90 or use of antihypertensive medicine

†Hypercholesterolemia: total cholesterol ≥ 6.5 or use of lipid-lowering drugs

‡HDL: per 0.5 mmol/L decrease

|Strenuous physical activity 1 hour or more every week

⋮Over/equal to 15 years of education (corresponding to 3 years in university or academy)

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and 1.52, (95% CI 0.85–2.73) in the time-fixed model and in the model corrected by Rosner´s method, respectively. When using time-varying analysis, only BMI was significantly associated with VTE (HR 1.21, 95% CI 1.10–1.33, per 3 kg/m^2 increase for BMI). For BMI as a continuous variable and for the overweight subgroup (BMI 25–29.9 kg/m^2), the Rosner correction gave slightly higher risk estimates than the time-varying analysis, whereas for obesity (BMI ≥30 kg/m^2) the Rosner correction gave a lower risk estimate than the time-varying analysis.

Discussion

In the present study, risk estimates for VTE and MI based on one baseline measurement corresponded well with risk estimates based on repeated measurements. Except for BMI, none of the atherosclerotic risk factors were associated with risk of VTE, neither in the time-fixed nor the time-varying model. These results suggest that lack of association between several atherosclerotic risk factors and VTE risk in large prospective cohorts could not be explained by regression dilution bias. For MI, the differences between risk estimates from the time-fixed and the time-varying analysis were greatest for dichotomous variables that changed much during follow-up, such as diabetes, smoking and physical activity. Correction of the time-fixed risk estimates using regression dilution ratios consistently overestimated risk of VTE and MI compared with the time-varying analysis, suggesting that this type of correction should be used with caution.

Table 3. Age (as time scale)- and sex-adjusted hazard ratios (HR) with 95% confidence intervals (CI) for the risk of venous thromboembolism (VTE) by traditional atherosclerotic risk factors using three different approaches; time-fixed model, time-varying model and correction for regression dilution through Rosners´s method. The Tromsø Study 1994–2010.

Risk factors	Time-fixed Cox-model	Time-varying Cox-model	Time-fixed model corrected by Rosner´s method
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Male sex	1.22 (0.94–1.60)	1.22 (0.94–1.60)	1.22 (0.94–1.63)
Systolic BP (per 15 mmHg increase)	1.06 (0.97–1.18)	1.00 (0.91–1.09)	1.12 (0.96–1.30)
Diastolic BP (per 10 mmHg increase)	1.09 (0.98–1.22)	0.96 (0.86–1.07)	1.17 (0.97–1.42)
Hypertension*	1.41 (1.05–1.89)	1.16 (0.86–1.58)	1.94 (1.10–3.40)
BMI, 3 units increase (kg/m^2)	1.24 (1.13–1.37)	1.21 (1.10–1.33)	1.25 (1.13–1.38)
<25 kg/m^2	Ref.	Ref.	Ref.
25–29.9 kg/m^2	1.40 (1.03–1.90)	1.10 (0.80–1.51)	1.44 (1.00–2.07)
≥30 kg/m^2	2.08 (1.42–3.05)	1.77 (1.24–2.53)	1.56 (1.23–1.98)
Triglycerides (mmol/L)	1.00 (0.87–1.15)	0.97 (0.83–1.14)	1.00 (0.75–1.33)
Total cholesterol (mmol/L)	1.07 (0.95–1.19)	0.94 (0.84–1.05)	1.13 (0.92–1.39)
Hypercholesterolemia †	1.17 (0.89–1.55)	1.01 (0.77–1.33)	1.36 (0.83–2.23)
HDL cholesterol (mmol/L) ‡	0.97 (0.82–1.14)	0.85 (0.71–1.01)	0.95 (0.76–1.20)
≥1.03 (♂) or ≥1.30 (♀) mmol/L	Ref.	Ref.	Ref.
<1.03 (♂) or <1.30 (♀) mmol/L	0.84 (0.56–1.25)	1.27 (0.91–1.76)	0.73 (0.35–1.51)
Self-reported diabetes	1.43 (0.71–2.91)	1.41 (0.82–2.42)	1.47 (0.69–3.14)
Smoking	1.20 (0.90–1.61)	1.08 (0.79–1.49)	1.30 (0.86–1.96)
Physical activity |	0.98 (0.70–1.36)	1.02 (0.74–1.42)	0.93 (0.34–2.56)
Education ⋮	1.07 (0.75–1.54)	1.10 (0.77–1.57)	1.07 (0.75–1.54)

*Hypertension: systolic BP ≥140 or diastolic BP ≥90 or use of antihypertensive medicine

†Hypercholesterolemia: total cholesterol ≥ 6.5 or use of lipid-lowering drugs

‡HDL: per 0.5 mmol/L decrease

|Strenuous physical activity 1 hour or more every week

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All the traditional atherosclerotic risk factors were significantly associated with risk of MI in both the time-fixed and time-varying analysis, and the magnitude of the risk estimates corresponded well to those of previous studies [5, 34]. For VTE, only obesity was associated with increased risk also in the time-varying approach. Moreover, the risk estimates were lower in the time-varying than in the time-fixed analyses. This was probably explained by the fact that most subjects experienced small changes in risk factor levels during follow-up, and consequently, those who changed from one risk category to another would most likely contribute to the healthiest part of their new, "unhealthy" category. For example, an individual that changed BMI from 24 to 26 during follow-up would change category from normal weight to overweight but still be under a relatively low risk of MI and VTE.

While BMI has consistently been shown to increase the risk of VTE, the impact of other atherosclerotic risk factors on VTE risk has been controversial [7, 9, 11, 12, 16, 35]. Case-control studies have shown associations between serum lipid levels, diabetes, blood pressure and VTE [11, 13, 15] whereas most cohort studies reported no association [6, 16–18]. While case-control studies may overestimate risks due to reverse causation, recall bias and selected control groups, the potential for regression dilution bias (i.e. underestimation or failure to detect a modest effect that is actually there) has been a major criticism of cohorts with a long follow-up. In the present study, we showed that the degree of regression dilution was very low for most atherosclerotic variables, and that serum lipid levels, smoking, blood pressure and diabetes were not associated with risk of VTE even in the time-varying approach.

Regression dilution was only prominent for yes/no-variables that were strongly associated with MI and had a high degree of intra-individual change during follow-up, such as smoking and physical activity. The percentage of smokers decreased from 33% in Tromsø 4 to 16% in Tromsø 6, and those who stopped smoking during follow-up were misclassified as smokers during the remaining follow-up in the time-fixed model. As these subjects had a reduced risk of MI, the association between smoking and MI was diluted. Additionally, subjects still smoking in Tromsø 6 had smoked for a longer time, and could therefore have been at greater risk of MI. The percentage of physically active subjects increased from 24% in Tromsø 4 to 38% in Tromsø 6, and consequently, the protective effect of physical activity on MI risk was underestimated in the time-fixed analysis.

In regression models with only a single risk factor, the effect of non-differential misclassification is always to reduce the magnitude of the association [19]. However, in multiple regression models, including several risk factors or confounders, non-differential misclassification can actually influence the risk estimates in both directions [36]. In fact, we observed that for some variables the use of a time-varying analysis actually reduced the risk estimates of MI and VTE compared with the time-fixed analysis, whereas Rosner´s method consistently overestimated the risk. The change in risk over time is not only a result of time-dependent covariates, but also influenced by time-dependent effects, and ageing of study participants, change in confounder status, change in environment, or improved treatment could have influenced the effect of exposures over time. For instance, many atherosclerotic risk factors are associated with a higher relative risk of MI in younger adults than in the elderly [37, 38]. In the case of diabetes and risk of MI, the risk was lower in the time varying analysis (HR 2.11) than in the time-fixed analysis (HR 2.94), whereas Rosner´s method showed a substantially higher risk estimate (HR 3.37). The effect of diabetes on the risk of MI varies not only with age and with other confounders, but also with time as the treatment has improved during the last decades. Moreover, newly diagnosed diabetic patients still has a low risk of MI [39, 40], and a higher proportion of newly diagnosed patients than patients with a long-lasting diabetes might additionally explain the lower risk in the time-varying analysis.

Our study supports previous studies finding no association between atherosclerotic risk factors, such as hypertension, serum lipids, diabetes and smoking, and future risk of VTE. The lack of association between serum lipids and VTE suggests that the possible beneficial effects of statins on VTE [41] is mediated through pleiotropic effects rather than the lipid-lowering effect, such as a potential antithrombotic effect [42, 43]. Furthermore, the unfavorable effect of smoking and favorable effect of strenuous physical exercise on the risk of MI are underestimated when single baseline measurements made several years before the event are compared to repeated measurements. Our study support the importance of simple preventive measures, such as smoking cessation, exercise and reducing cholesterol to prevent MI, as previously studied [44, 45].

The main strengths of our study includes the prospective design with participants recruited from a general population, the high attendance rate and the repeated measurements. The repeated measurements allowed us to update the participants' atherosclerotic risk factors during follow up, and thus reduce misclassification. Furthermore, the municipality of Tromsø is served by a single hospital, minimizing the chance of missing cases and loss to follow-up. Additional use of the National population registry and the National Causes of Death Registry allowed thorough validation of both MI and VTE. The study has some limitations. The study is restricted to a homogenous white population with a certain development of atherosclerotic risk factors over time. Other populations might have different tendencies, and the findings might therefore not apply to all populations. However, it is likely to assume that many of the same

trends regarding development in atherosclerotic risk factors are true for most other Western populations.

In conclusion, risk estimates for MI and VTE based on baseline measures and time-fixed analysis corresponded well with risk estimates based on repeated measurements and time-varying analyses. Of the traditional atherosclerotic risk factors, only BMI was associated with VTE in both time-fixed and time-varying analyses, suggesting that underestimation of risks by regression dilution bias is not explaining the lack of association between several atherosclerotic risk factors and VTE risk reported in most prospective cohorts. Our findings suggest that for atherosclerotic risk factors, risk estimates based on a single measurement are generally reliable in cohort studies with long follow-up, and misclassification is a problem only in situations where the association between exposure and outcome is strong and the exposure status varies greatly during follow-up. Correction of the time-fixed risk estimates using the regression dilution ratio consistently overestimated the associations compared to the time-varying analyses.

Supporting Information

S1 Table. Distribution of traditional cardiovascular risk factors in the different studies for participants who participated in all three studies (n = 4391).
(DOCX)

Author Contributions

Conceptualization: JBH SB.

Data curation: IN EBM TW MLL.

Formal analysis: BS KH.

Funding acquisition: JBH.

Methodology: JBH SB.

Project administration: JBH SB.

Supervision: JBH SB.

Visualization: JBH SB BS.

Writing – original draft: BS.

Writing – review & editing: JBH SB LBR KH EMH AV TW MLL IN EBM.

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Paper IV

Impact of prothrombotic genotypes on the association between family history of myocardial infarction and venous thromboembolism

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Essentials

- Venous thromboembolism (VTE) is associated with family history of myocardial infarction (FHMI)
- VTE cases and a sub-cohort from the Tromsø and the Nord-Trøndelag Health Studies were genotyped
- The risk of VTE by FHMI could not be explained by the prothrombotic genotypes
- The combination of FHMI and prothrombotic genotypes had an additive effect on VTE risk

Summary

Background: Family history of myocardial infarction (FHMI) is known to increase the risk of venous thromboembolism (VTE).

Objectives: To investigate the effect of prothrombotic genotypes on the association between FHMI and VTE in a case-cohort recruited from a general population.

Methods: Cases with a first VTE (n=1,493) and a sub-cohort (n=13,072) were sampled from the Tromsø study (1994-2012) and the Nord-Trøndelag health (HUNT) study (1995-2008). DNA-samples were genotyped for rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*) and rs2036914 (*F11*). Participants with missing information on risk alleles (n=175), FHMI (n=2769) and BMI (n=52) were excluded. Cox-regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CI) for VTE. To explore the role of prothrombotic genotypes for the association between FHMI and VTE, we (i) included the genotypes in the multivariable-adjusted models, and (ii) assessed the joint effects between FHMI and genotypes on VTE risk.

Results: FHMI was associated with a 1.3-fold increased risk of VTE (HR 1.32, 95% CI 1.16-1.50) and 1.5-fold increased risk of unprovoked VTE (HR 1.47, 95% CI 1.22-1.78). The risk of VTE by FHMI did not alter after adjustment for the five genotypes. The combination of FHMI and the different prothrombotic genotypes did not result in an excess VTE risk (i.e. no biological interaction).

Conclusions: Our findings suggest that the risk of VTE by FHMI is not explained by rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*) and rs2036914 (*F11*). FHMI combined with prothrombotic genotypes had an additive effect on VTE risk.

Keywords: Genotype – Myocardial infarction – Prospective studies – Risk factors – Venous thromboembolism

Introduction

Traditionally, arterial thrombotic diseases (e.g. myocardial infarction [MI] and stroke) and venous thromboembolism (VTE) (deep vein thrombosis [DVT] and pulmonary embolism [PE]) have been considered as separate disease entities with different pathophysiology and treatment. However, both registry-based studies and cohorts recruited from the general population have demonstrated bidirectional associations between arterial and venous thromboembolic diseases [1-4]. The relationship between arterial and venous thrombosis may be attributed to shared environmental or genetic risk factors, or it may be a causal association through mediating factors, such as hospitalization accompanied by immobilization and infection, or via a transient prothrombotic state following an arterial event [5]. Results from population-based cohorts have revealed that among the well-known cardiovascular risk factors, only advancing age, obesity and family history of MI (FHMI) were shared risk factors for arterial and venous thromboembolic diseases [6-11].

FHMI is a well-established risk factor for MI [11-13], and during recent years, FHMI has also been found to be associated with an increased risk of VTE [9-11, 14]. The population attributable fraction (PAF) of MI and VTE by FHMI has been estimated to be 19% and 13%, respectively [11], indicating that 19% of the total incidence of MI and 13% of the total incidence of VTE could be explained by FHMI-related risk factors. Due to the profound impact of FHMI on the risk of MI and VTE, it is important to unravel underlying mechanisms and potential common pathways. Although it is unclear how FHMI contributes to the risk of VTE, it might be due to shared environmental or genetic risk factors or mediated by an effect of a previous arterial cardiovascular disease. Previously, we reported that modifiable cardiovascular risk factors slightly attenuated the association between FHMI and MI, but had no effect on the association between FHMI and VTE. Furthermore, by applying a cause-specific model to eliminate the effect of MI on VTE, we showed that MI did not mediate the effect of FHMI on the risk of VTE [11].

As the risk of VTE by FHMI was particularly pronounced for unprovoked VTE events and increased with the number of affected relatives [11], it is plausible to assume that prothrombotic genotypes accumulating in families with arterial cardiovascular disease might explain the association between FHMI and VTE. Several genetic polymorphisms associated with an increased risk of VTE have been discovered during the last decades [15]. Some of these prothrombotic genotypes, such as rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), have also been shown to increase the risk of arterial thrombosis in some studies [16-18].

It is unknown whether prothrombotic genotypes are shared risk factors for FHMI and VTE, and how a combination of these factors influence the VTE risk. We therefore aimed to (i) investigate whether the association between FHMI and VTE was explained by the presence of prothrombotic genotypes and to (ii) explore the combined effects of FHMI and prothrombotic genotypes on the risk of VTE, using a case-cohort recruited from the general population.

Methods

Study population

Study participants were recruited from the fourth survey of the Tromsø study (Tromsø 4) conducted in 1994-1995 [19], and the second survey of the Nord-Trøndelag Health Study (HUNT 2), conducted in 1995-1997 [20]. The Tromsø Study and the HUNT Study are population-based cohorts of inhabitants in Tromsø municipality and Nord-Trøndelag County, Norway, respectively. To Tromsø 4, the entire population aged ≥25 years living in the municipality of Tromsø was invited to participate, and 27,158 participants attended (77%). To HUNT 2, all residents in Nord-Trøndelag County aged 20 years and older were invited to participate, and 66,140 participants attended (71%) [20]. More detailed descriptions of the studies have been published elsewhere [19, 20].

All participants from these surveys were followed from the date of inclusion until a verified VTE event, migration, death or end of follow-up (December 31, 2008 in the HUNT Study, and December

31, 2012 in the Tromsø Study). In the Tromsø Study, all incident VTE events were identified by searching the hospital discharge diagnosis registry, the autopsy registry and the radiology procedure registry at the University Hospital of North Norway. The medical record for each potential case of VTE was reviewed by trained personnel, and adjudication criteria for VTE were the presence of clinical signs and symptoms of DVT or PE combined with objective confirmation by radiologic procedures that resulted in treatment initiation (unless contraindications were specified). In the HUNT study, VTE events were identified by searching the hospital discharge diagnosis registry and the radiology procedure registry at the two local hospitals in the county (Levanger Hospital and Namsos Hospital) and by searching the discharge diagnosis registry of the tertiary-care center of the region, St. Olav's Hospital in Trondheim (Sør-Trøndelag County). The medical records for potential VTE cases were reviewed and validated by two physicians, and the VTE diagnosis required positive objective confirmation by radiologic procedures. Detailed descriptions of identification and validation of VTE events in the two studies have been published elsewhere [21, 22].

All cases with an incident VTE (n=1,493) and a randomly selected sub-cohort (n=13,072) of participants without previous VTE were included in our study (Figure 1). Due to the study design, in which all participants in the original cohort has an equal chance of being included in the sub-cohort, 217 cases were included in the sub-cohort. Participants not officially registered as inhabitants in Tromsø or Nord-Trøndelag at baseline (n=3) were excluded. Further, we excluded participants with missing values for at least one of the single nucleotide polymorphisms (SNPs) studied (n=175), and participants with missing data on FHMI (n=2,769) and BMI (n=52). Consequently, 1,164 incident VTEs and 10,402 sub-cohort participants were included in the study. The study was approved by the Regional Committees of Research Medical and Health Ethics, and all study participants provided informed written consent.

Classification of VTE events

VTE events were classified as provoked and unprovoked, depending on the presence of provoking factors at the time of diagnosis. In the Tromsø Study, provoking factors were active cancer, acute medical conditions (including acute MI, ischemic stroke or major infections), recent surgery or trauma within the previous eight weeks, immobilization (bed rest over three days, wheelchair use or long distance travel exceeding four hours the last 14 days prior to the event) or any other provoking factors described by a physician in the medical record (e.g. intravascular catheter). In the HUNT study, provoking factors included active cancer at the time of the event or within six months after the event, trauma, surgery or marked immobilization (paresis, paralysis, prolonged bed rest due to an acute medical illness or travel exceeding 8 hours) within the last three months, pregnancy or puerperium at the time of the event and oral contraceptives used at the time of the event or up to one month prior to the event.

Cardiovascular risk factors

Baseline information on cardiovascular risk factors was collected by physical examinations, blood samples, and self-administered questionnaires. Height and weight were measured with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meter (kg/m^2). Blood pressure was measured three times with an automatic device (Dinamap Vital Signs Monitor in the Tromsø Study and Dinamap 845XT [Critikon] in the HUNT study) in a sitting position after two minutes of rest. The average of the two last readings was used in the analyses. Non-fasting blood samples were collected from an antecubital vein and total cholesterol, triglycerides and high-density lipoprotein (HDL) were measured, as previously described [7, 20]. Self-administered questionnaires were used to obtain information on diabetes, smoking (current daily smoking, yes/no) and FHMI. To identify FHMI, subjects were asked to report whether their mother, father, sister, brother, child or none in the family had a history of MI before the age of

60 years. A positive family history was regarded as ≥ 1 first-degree relative with a history of MI before the age of 60 years.

Prothrombotic genotypes

The following single nucleotide polymorphisms (SNPs) were genotyped and used in the present study: rs8176719 in *ABO* (non-O blood type), rs6025 in *F5* (Factor V Leiden), rs1799963 in *F2* (prothrombin G20210A), rs2066865 in *FGG* and rs2036914 in *F11*. In the Tromsø Study, rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*) and rs2036914 (*F11*) were genotyped with the Sequenom platform, and rs2066865 (*FGG*) with the TaqMan platform, as previously described [23]. The HUNT study performed genotyping using the Illumina HumanCore Exome array.

Participants were considered carriers of the prothrombotic risk gene when one or two risk alleles were present. We did not differentiate between hetero- and homozygous carriers due to the low number of homozygous carriers. For rs2036914 in *F11*, the minor allele was associated with reduced risk of VTE, and in this case, we considered the common allele as the risk allele [24]. For rs8176719 (*ABO*), zero risk alleles were classified as O blood type, whereas one or two risk alleles were classified as non-O blood type. The 5-SNP score conceived by de Haan and colleagues was created by summarizing the number of risk alleles from the five sequenced SNPs [25].

Statistical analysis

Statistical analyses were carried out using STATA version 14.0 and 15.0 (Stata Corporation, College Station, TX, USA). For each participant, person-years of follow-up were counted from the date of enrollment (1994-1995 in the Tromsø Study and 1995-1997 in the HUNT Study) to the date of an incident VTE event, the date the participant died or moved from Tromsø or Nord-Trøndelag County, or until the end of the study period (December 31, 2008 in the HUNT study and December 31, 2012 in

the Tromsø Study). Participants who died or moved from Tromsø or Nord-Trøndelag County during follow-up were censored at the date of death or migration.

Cox proportional hazards regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for VTE by FHMI and prothrombotic genotypes. Age was used as the time-scale, with the age of the participants at study enrolment defined as entry time, and the age at the VTE or censoring event defined as exit time. All analyses were adjusted for age (as time-scale), sex and BMI. We estimated the risk of total VTE, as well as unprovoked VTE and provoked VTE, by FHMI and according to ≥ 1 affected relatives or ≥ 1 affected parent, respectively. In order to assess the role of prothrombotic genotypes on the association between FHMI and VTE, the five SNPs were entered into the regression models. Furthermore, to investigate combined effects, we calculated HRs of VTE according to categories of FHMI and the individual SNPs as well as categories of the 5-SNP score (0-1, 2, 3-4 and ≥ 5 risk alleles). The presence of biological interaction between the two exposures was assessed by calculating the relative excess risk due to interaction (RERI), the attributable proportion due to interaction (AP) and the synergy index (SI) with corresponding 95% CIs [26]. Briefly, the RERI can be interpreted as part of the total effect on the outcome that is due to interaction, and the AP as the proportion of cases in the combined group that is due to an interaction between the two exposures. RERI and AP > 0 and SI > 1.0 suggest positive interaction, i.e., the effect of the joint exposures to two risk factors is greater than the sum of the separate effects [26].

Because of the size of the sub-cohort, we did not make adjustments to the partial likelihood in the Cox regression analyses [27]. The proportional hazard assumption was tested using Schoenfeld residuals. Lastly, we performed a sensitivity analysis in which participants with missing information on FHMI were included in the study and categorized as not having FHMI (i.e. assuming that all missings would be due to no FHMI).

Results

The baseline characteristics of the cases and the sub-cohort are shown in Table 1. Participants who experienced a VTE were older and had slightly higher systolic blood pressure, BMI, triglycerides, and cholesterol levels compared with participants in the sub-cohort. Serum levels of triglycerides and total cholesterol along with the proportion of smokers and participants with self-reported diabetes were higher in the group with a FHMI. Compared to the sub-cohort, VTE patients had a higher proportion of participants with ≥ 1 risk allele(s) in all SNPs, and rs8176719 (*ABO*), rs6025 (*F5*), and rs1799963 (*F2*) in particular. The prevalence of the SNPs did not differ according to FHMI.

The distribution of individuals (%) across numbers of risk alleles for subjects with and without FHMI is shown in Figure 2, panel A. The number of risk alleles ranged from zero to seven with a median of two for both groups, and participants with and without FHMI had a similar distribution of the number of risk alleles. The risk of VTE (Figure 2, panel B) increased with increasing number of risk alleles when compared to zero risk alleles (p for trend<0.001). Subjects with ≥ 5 risk alleles had a 2.4-fold higher risk of VTE (HR 2.43, 95% CI 1.64-3.59) compared with those without risk alleles.

Table 2 shows HRs for total, unprovoked and provoked VTE by FHMI. In models adjusted for age (as time-scale), sex and BMI, subjects with ≥ 1 affected first-degree relative or ≥ 1 affected parent had increased risk of VTE with a HR of 1.32 (95% CI 1.16-1.50) and 1.40 (95% CI 1.20-1.64), respectively. The point estimate for unprovoked VTE (HR 1.47, 95% CI 1.22-1.78) was higher than for provoked VTE (HR 1.20, 95% CI 1.00-1.43). Similarly to total VTE, the risk estimates for unprovoked VTE were higher in the analysis of ≥ 1 affected parent (HR 1.52, 95% CI 1.20-1.93) in comparison with ≥ 1 affected first-degree relative (HR 1.47, 95% CI 1.22-1.78). Adjustments for the five prothrombotic genotypes had a negligible effect on the risk estimates for VTE. Sensitivity analyses where participants with missing information on FHMI were included in the no FHMI group yielded similar results (Supplementary Table 1).

Table 3 shows HRs with 95% CI for total VTE and unprovoked VTE by combinations of FHMI and prothrombotic genotypes, adjusted for age (as time-scale), sex and BMI. For each of the individual SNPs, the risk of VTE was increased in participants having a positive FHMI and no risk alleles, and in participants without FHMI and ≥ 1 risk alleles. However, the combined effect of having both FHMI and ≥ 1 risk alleles did not exceed the sum of the effects of the individual risk factors. For instance, having both FHMI and non-O blood type (rs8176719) was associated with a 1.8-fold increased risk of VTE (HR 1.78, 95% CI 1.49-2.13), which approximated the sum of having only FHMI (HR 1.35, 95% CI 1.07-1.71) or non-O blood type (HR 1.38, 95% CI 1.19-1.59). The combination of FHMI and the high-risk category of the 5-SNP score (i.e. ≥ 5 risk alleles) did not have a synergistic impact on the VTE risk (RERI 0.37, 95% CI -0.89 to 1.63 and AP 0.14, 95% CI -0.29 to 0.56). Thus, combinations of FHMI and prothrombotic genotypes had merely additive effects on the VTE risk, as suggested by the estimated measures of biological interaction (i.e. RERI, AP, and SI) described in Supplementary Tables 2 and 3.

Discussion

In this case-cohort study with participants recruited from the general population, we found that the association between FHMI and VTE could not be explained by common prothrombotic genotypes, such as rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*), and rs2036914 (*F11*). Adjustment for prothrombotic genotypes did not alter the association between FHMI and VTE, and the combination of FHMI and prothrombotic genotypes had merely an additive effect on the VTE risk.

The increased risk of VTE, and particularly unprovoked VTE, by a FHMI is in agreement with results from other observational studies [9-11, 14]. In two previous studies with data from the entire Tromsø cohort, FHMI was associated with a 1.3-fold increased risk of VTE [9, 11], and a 1.5-fold increased risk of unprovoked VTE [9, 11]. Adjustment for traditional cardiovascular risk factors had a negligible effect on the risk of VTE [9, 11], whereas the risk of MI was attenuated [11], indicating that cardiovascular risk factors were confounders for the association between FHMI and MI, but not for the

association between FHMI and VTE [11]. Similar results were reported in a case-cohort derived from the second survey of the HUNT study [10], and a case-control study derived from the Genetic Attributes and Thrombosis Epidemiology (GATE) study [14]. Sub-group analyses from the different studies revealed that the risk of VTE increased with increasing number of affected relatives [11, 14], and when a relative aged < 50 years experienced an MI [14]. Further, the reported risk estimates for DVT and PE were similar, but the risk was highest for unprovoked DVTs [11]. A FHMI is not considered to be a causal factor for thrombosis, but rather an indicator of genetic or environmental risk factors accumulating in certain families, which have the potential to affect the risk of VTE. Due to the particularly increased risk of unprovoked VTE, and because the risk increases with increasing numbers of affected relatives, it was suggested that the association between FHMI and VTE was caused by shared genetic risk factors [9-11, 14].

In the present study, rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*), and rs2036914 (*F11*) did not act as confounders in the association between FHMI and VTE, as adjustments for these prothrombotic genotypes had a negligible effect on the risk estimates. Furthermore, combinations of FHMI and the prothrombotic genotypes had an additive effect on the risk of VTE. For instance, having both FHMI and rs8176719 (*ABO*) was associated with a 1.8-fold increased risk of VTE, which was equal to the sum of having only FHMI or rs8176719 (*ABO*). Similar results were found for FHMI in combination with the other individual SNPs and the combined 5-SNP score. Our results suggest that FHMI and the five prothrombotic genotypes included in this study are unrelated risk factors of VTE and that these prothrombotic genotypes do not influence the association between FHMI and VTE.

Two risk factors acting through the same pathophysiological mechanism can have both synergistic and additive effects on an outcome. For instance, obesity and rs6025 (*F5*), which are associated with hypercoagulability, had synergistic effects on VTE risk [28]. Similarly, the risk of VTE in obese women using oral contraceptives has been shown to exceed the sum of the effects of the individual risk factors [29]. However, a cohort study of 66,000 genotyped participants found additive effects on VTE risk when different prothrombotic genotypes, all causing hypercoagulability, were

combined [30]. Consequently, our results do not allow us to determine the mechanisms behind the association between FHMI and VTE, and do not exclude the possibility that other unrecognized genetic variants can partly explain the association between FHMI and VTE.

Even though the prothrombotic genotypes included in our study are associated with both arterial and venous thrombosis [15-18], our results indicate that these genotypes do not explain the association between FHMI and VTE. However, on the basis of the present and previous findings [9, 11], it is likely to assume that genetic risk factors are one of the main contributors to the association between FHMI and VTE. Furthermore, environmental risk factors clustering within families may potentially act as contributors to this association. Although the association between FHMI and VTE is independent of traditional cardiovascular risk factors [9-11], other environmental risk factors related to both FHMI and VTE, such as stress and socioeconomic status [31-33], might partly explain the association.

The main strengths of our study include the long-term follow-up, the large number of genotyped participants, the high attendance rate in the parent cohorts, and the thorough outcome assessment. The study cohorts represent a general and homogenous Caucasian population, which limits confounding by ethnicity in the sub-cohort [34]. Some limitations of this study need to be addressed. First, analyses were restricted to subjects with information on FHMI. It is likely to assume that the majority of subjects with missing information on FHMI did not answer the question because they did not have, or did not know if they had, any first-degree relatives with a history of MI before the age of 60 years. It is noteworthy, however, that the association between FHMI and VTE, albeit slightly attenuated, remained when participants with missing data on FHMI were classified as having no FHMI (Supplementary table 1). Second, data on FHMI was self-reported and both under-reporting and over-reporting of affected relatives were possible. Validation of FHMI in the Tromsø Study showed high concurrence between reported and confirmed diagnosis [35], and a validation study by Kee et al. found high specificity (97%) and lower sensitivity (68%) of a positive FHMI [36]. Hence, underestimation of the risks associated with FHMI is more likely. Third, even though our study was

derived from large cohorts, the number of VTE events was low in some subgroups, particularly for the rare exposures, which resulted in limited statistical power. Our results on the measures that quantify interaction should therefore be interpreted with caution. Lastly, due to the observational nature of our study, unknown confounders could be present and lead to residual confounding.

In conclusion, our study provides evidence that the association between FHMI and VTE is not explained by rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*) or rs2036914 (*F11*). FHMI and the prothrombotic genotypes had an additive effect on VTE risk, indicating no biological interaction between the risk factors.

Addendum

Birgit Småbrekke analyzed the data and drafted the manuscript. Ludvig B. Rinde, Line H. Evensen, Vania M. Morelli, Inger Njølstad, Ellisiv B. Mathiesen and Frits R. Rosendaal were involved in the interpretation of the results and revision of the manuscript. Kristian Hveem and Maiken E. Gabrielsen were involved in data collection and revision of the manuscript. Sigrid K. Brækkan and John-Bjarne Hansen were involved in conception and design of the study, data collection and revision of the manuscript. The manuscript has been read and approved for submission to Journal of Thrombosis and Haemostasis by all authors.

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Disclosure of Conflict of interests

The authors state that they have no conflict of interest.

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Tables

T**able 1.** Baseline characteristics by family history of myocardial infarction (FHMI) and incident venous thromboembolism (VTE).

	No FHMI		FHMI	
	Sub-cohort	**VTE**	**Sub-cohort**	**VTE**
Participants, n	8376	851	2026	313
Age, years	49.8±17	60.7±15	52.7±15	59.9±14
Male sex	46.0 (3857)	49.6 (422)	42.9 (869)	42.5 (133)
Systolic BP, mmHg	137±21	144±24	140±21	144±23
Diastolic BP, mmHg	80±12	83±13	82±12	82±12
BMI, kg/m^2	26.1±4.1	27.5±4.6	26.5±4.2	27.4±4.2
Triglycerides, mmol/L	1.68±1.04	1.80±0.99	1.82±1.09	1.95±1.32
Cholesterol, mmol/L	5.88±1.26	6.34±1.28	6.26±1.32	6.63±1.28
HDL, mmol/L	1.41±0.39	1.43±0.41	1.41±0.40	1.42±0.41
Self-reported diabetes	2.9 (244)	3.8 (32)	3.9 (80)	4.5 (14)
Smoking	27.1 (2269)	25.7 (219)	31.9 (646)	31.6 (99)
rs8176719 (*ABO*)*	61.4 (5139)	69.1 (588)	63.4 (1284)	69.0 (216)
rs6025 (*F5*)*	6.8 (571)	15.3 (130)	7.2 (146)	16.0 (50)
rs1799963 (*F2*)*	1.2 (104)	1.8 (15)	1.5 (31)	3.2 (10)
rs2066865 (*FGG*)*	41.9 (3508)	44.1 (375)	44.6 (903)	50.2 (157)
rs2036914 (*F11*)*	78.5 (6572)	80.4 (684)	76.7 (1553)	82.1 (257)

Values are % (n) or mean±SD. BP indicating blood pressure; BMI, body mass index; HDL, high-density lipoprotein.

* Percentage of participants with ≥1 risk allele

Table 2. Hazard ratios (HR) with 95% confidence intervals (CI) for total, unprovoked and provoked venous thromboembolism (VTE) by family history of myocardial infarction (FHMI).

	N	Events	HR (95% CI)*	Adjusted HR (95% CI)†
Total VTE		1164		
No FHMI	9227	851	Ref.	Ref.
≥ 1 first-degree relative	2339	313	1.32 (1.16-1.50)	1.30 (1.14-1.48)
≥ 1 affected parent	1673	197	1.40 (1.20-1.64)	1.41 (1.20-1.65)
Unprovoked VTE		513		
No FHMI	9227	363	Ref.	Ref.
≥ 1 first-degree relative	2339	150	1.47 (1.22-1.78)	1.45 (1.20-1.76)
≥ 1 affected parent	1673	90	1.52 (1.20-1.92)	1.53 (1.21-1.93)
Provoked VTE		651		
No FHMI	9227	488	Ref.	Ref.
≥ 1 first-degree relative	2339	163	1.20 (1.00-1.43)	1.19 (0.99-1.42)
≥ 1 affected parent	1673	107	1.32 (1.07-1.63)	1.32 (1.07-1.63)

*Adjusted for age (as time-scale), sex and body mass index

†Adjusted for age (as time-scale), sex, body mass index, rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*), and rs2036914 (*F11*)

Table 3. Hazard ratios (HR) with 95% confidence intervals (CI) for venous thromboembolism (VTE) by combined categories of family history of myocardial infarction (FHMI) and prothrombotic genotypes.

			Total VTE		Unprovoked VTE	
		N	Events	HR (95% CI)*	Events	HR (95% CI)*
FHMI	**rs8176719 (*ABO*)†**					
-	-	3500	263	Ref.	107	Ref.
+	-	839	97	1.35 (1.07-1.71)	49	1.67 (1.19-2.34)
-	+	5727	588	1.38 (1.19-1.59)	256	1.47 (1.17-1.84)
+	+	1500	216	1.78 (1.49-2.13)	101	2.04 (1.55-2.68)
FHMI	**rs6025 (*F5*)†**					
-	-	8526	721	Ref.	296	Ref.
+	-	2143	263	1.31 (1.14-1.51)	129	1.56 (1.27-1.92)
-	+	701	130	2.31 (1.92-2.79)	67	2.92 (2.24-3.81)
+	+	196	50	3.09 (2.32-4.12)	21	3.16 (2.03-4.93)
FHMI	**rs1799963 (*F2*)†**					
-	-	9108	836	Ref.	355	Ref.
+	-	2298	303	1.30 (1.14-1.49)	145	1.46 (1.20-1.77)
-	+	119	15	1.41 (0.85-2.35)	8	1.77 (0.88-3.57)
+	+	41	10	2.49 (1.33-4.64)	5	2.91 (1.20-7.04)
FHMI	**rs2066865 (*FGG*)†**					
-	-	5344	476	Ref.	198	Ref.
+	-	1279	156	1.22 (1.02-1.47)	76	1.42 (1.09-1.86)
-	+	3883	375	1.10 (0.96-1.26)	165	1.17 (0.95-1.43)
+	+	1060	157	1.56 (1.30-1.87)	74	1.77 (1.35-2.31)
FHMI	**rs2036914 (*F11*)†**					
-	-	1971	167	Ref.	60	Ref.
+	-	529	56	1.16 (0.86-1.57)	20	1.15 (0.69-1.91)
-	+	7256	684	1.11 (0.94-1.31)	303	1.37 (1.04-1.80)
+	+	1810	257	1.50 (1.24-1.83)	130	2.11 (1.55-2.87)
FHMI	**5-SNP score‡**					
-	0-1	2117	154	Ref.	53	Ref.
+	0-1	484	46	1.16 (0.83-1.61)	21	1.52 (0.92-2.52)
-	2	2888	217	1.03 (0.84-1.26)	98	1.35 (0.96-1.88)
+	2	716	83	1.38 (1.06-1.80)	37	1.77 (1.16-2.69)
-	3-4	3833	420	1.49 (1.24-1.80)	186	1.92 (1.41-2.60)
+	3-4	1026	160	2.02 (1.62-2.52)	80	2.94 (2.07-4.16)
-	≥5	389	60	2.16 (1.60-2.91)	26	2.73 (1.71-4.36)
+	≥5	113	24	2.69 (1.75-4.13)	12	3.88 (2.07-7.28)

*Adjusted for age (as time-scale), sex and body mass index

†Positive indicating subjects with one or two risk alleles

‡Number of risk alleles

Figures

Figure 1. Study population. Participants were recruited from the fourth survey of the Tromsø Study (1994-2012), and from the second survey of the Nord-Trøndelag Health (HUNT) Study (1995-2008). VTE indicates venous thromboembolism; SNPs, single nucleotide polymorphisms; FHMI, family history of myocardial infarction

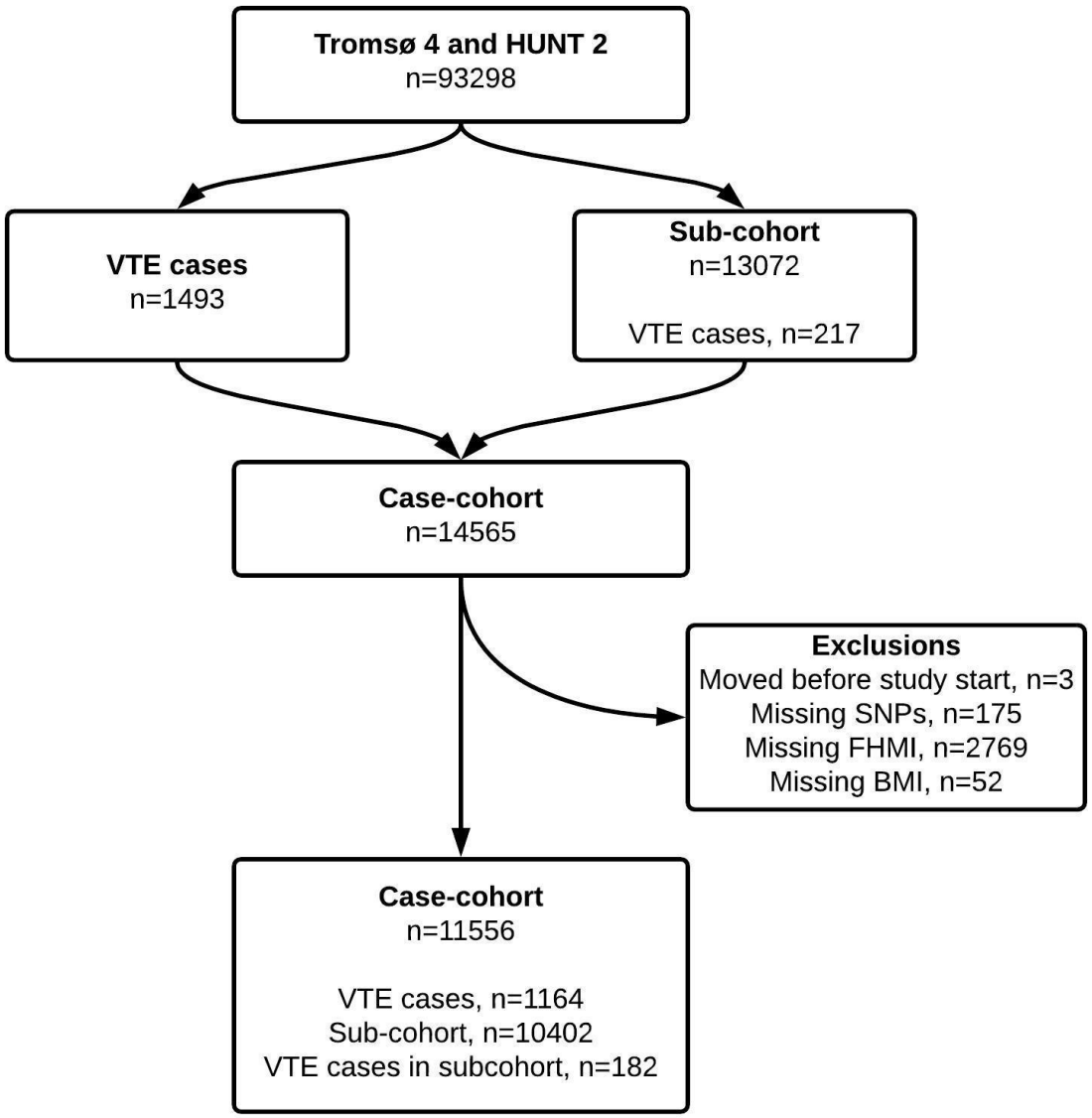


Figure 2. Panel A. Distribution (%) of individuals across number of risk alleles and family history of myocardial infarction (FHMI).

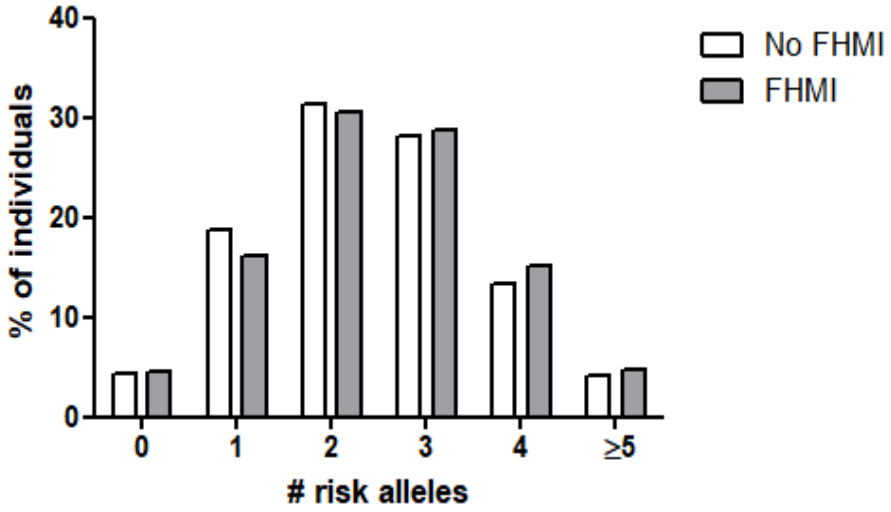
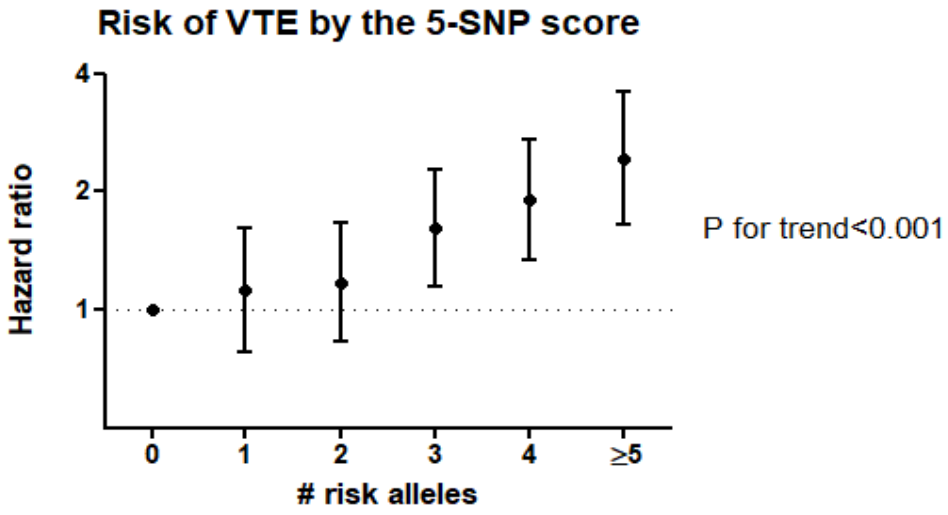


Figure 2. Panel B. Hazard ratios with 95% confidence intervals for the risk of venous thromboembolism (VTE) by number of risk alleles in the 5-SNP score.



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