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Arterial cardiovascular diseases and risk of venous thromboembolism

Ludvig Balteskard Rinde

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Faculty of Health Sciences, Department of Clinical Medicine

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Summary

Despite differences in epidemiology, pathology, and treatment, growing evidence suggests a bidirectional relationship between venous thromboembolism (VTE), a collective term for pulmonary embolism (PE) and deep vein thrombosis (DVT), and arterial cardiovascular diseases (CVD, i.e., myocardial infarction [MI] and ischemic stroke). The aim of this thesis was to investigate the impact of atherosclerosis, MI and ischemic stroke on the risk of incident VTE. Additionally, we aimed to investigate the effect of prothrombotic genotypes and ischemic stroke on the risk of VTE.

We recruited study participants from the Tromsø Study, a population-based, prospective cohort study. In all four papers, we used participants from the fourth, fifth and six survey of the Tromsø Study. In paper I, the participants consisted of a subgroup from the Tromsø Study with a more extensive examination, including ultrasonography of the carotid artery. In paper IV, a subgroup of participants with extended genetic analysis was included.

We found no association between the formation or progression of asymptomatic atherosclerosis and risk of VTE in time-varying analyses. However, MI and ischemic stroke were associated with a transient risk of VTE after adjusting for potential confounding factors. The study participants with MI had a particularly high risk of developing PE. After both MI and stroke, the risk was particularly high for provoked VTE events. The proportion of patients immobilized before the VTE event was substantially higher in those with compared in those without stroke. We also found a synergistic effect of ischemic stroke and prothrombotic genotypes on the risk of VTE. The risk increased gradually with the number of risk alleles.

Our findings imply that incident MI and ischemic stroke are associated with an increased transient risk of VTE, and that genetic risk factors are important in the development of VTE after stroke. The transient nature of the VTE risk suggests that indirect (e.g., hospitalization, immobilization) or direct (e.g. activation of the coagulation system) mechanisms related to the arterial CVD are primarily responsible for the observed association.

Sammendrag

Til tross for klare forskjeller i forekomst, sykdomsmekanisme og behandling, er det flere studier som indikerer en sammenheng mellom venøs tromboembolisme (VTE), et samlebegrep for lungeemboli (LE) og dyp venetrombose (DVT), og arterielle kardiovaskulære sykdommer som hjerteinfarkt og iskemisk hjerneslag. Målet med denne avhandlingen har vært å undersøke om aterosklerose, hjerteinfarkt og hjerneslag øker risikoen for VTE i den generelle befolkning. I tillegg har vi undersøkt om kombinasjonen av slag og trombotiske risikogener ga en samlet økt effekt på risikoen for VTE.

Studiedeltakerne ble rekruttert fra Tromsøundersøkelsen, en stor prospektiv befolkningsbasert kohortestudie. I alle fire artiklene brukte vi deltakere fra den fjerde, femte og sjette Tromsøundersøkelsen. I artikkel I besto studiedeltakerne av en undergruppe som fikk en utvidet undersøkelse som inkluderte ultralydundersøkelse av halspulsåren. Deltakerne inkludert i artikkel IV bestod av en undergruppe som fikk utført genetiske analyser.

Vi fant ingen sammenheng mellom nydannelse eller progresjon av aterosklerose og økt risiko for VTE. Derimot fant vi at både hjerteinfarkt og slag økte risikoen for VTE. For både hjerteinfarkt- og slagpasienter var risikoen for VTE høyest de første månedene etter den opprinnelige hendelsen. Særlig var risikoen for provosert VTE høy hos disse pasientene. Pasienter med hjerteinfarkt har særlig høy risiko for å utvikle LE. Slagpasientene hadde en høyere grad av immobilisering før VTE-hendelsen enn pasienter uten slag. Vi fant at kombinasjonen slag og risikogener ga en høyere risiko for VTE enn de isolerte faktorene. Risikoen ble høyere jo flere risikogener pasientene hadde.

Våre funn tyder på at pasienter som får hjerteinfarkt eller hjerneslag har en større risiko for å få VTE, men at denne risikoen er begrenset til den første tiden etter hendelsen. Videre har slagpasienter med protrombotiske gener høyere risiko for VTE enn slagpasienter uten disse genene. Den forbigående risikoen for VTE tyder på at er indirekte eller direkte mekanismer relatert til de kardiovaskulære sykdommene som er hovedgrunnen til den observerte assosiasjonen.

List of papers

I. Impact of incident myocardial infarction on the risk of venous thromboembolism. The Tromsø Study
Ludvig Balteskard Rinde, Caroline Lind, Birgit Småbrekke, Inger Njølstad, Ellisiv B. Mathiesen, Tom Wilsgaard, Maja-Lisa Løchen, Erin Mathiesen Hald, Anders Vik, Sigrid K. Brækkan, John-Bjarne Hansen
Journal of Thrombosis and Haemostasis 2016; 14: 1183-91

II. Ischemic Stroke and Risk of Venous Thromboembolism in the General Population: The Tromsø Study
Ludvig Balteskard Rinde, Birgit Småbrekke, Ellisiv B. Mathiesen, Maja-Lisa Løchen, Inger Njølstad, Erin Mathiesen Hald, Tom Wilsgaard, Sigrid K. Brækkan, John-Bjarne Hansen
Journal of American Heart Association 2016;5:e004311

III. Repeated Measurements of Carotid Atherosclerosis and Future Risk of Venous Thromboembolism. The Tromsø Study
Birgit Småbrekke, Ludvig Balteskard Rinde, Erin Mathiesen Hald, Inger Njølstad, Ellisiv B. Mathiesen, Stein Harald Johnsen, John-Bjarne Hansen, Sigrid K. Brækkan, Willem M. Lijfering
Journal of Thrombosis and Haemostasis 2017; 15: 2344–2351

IV. Effect of prothrombotic genetic variants on the risk of venous thromboembolism in patients with ischemic stroke. The Tromsø Study
Ludvig Balteskard Rinde, Vania Morelli, Birgit Småbrekke, Ellisiv B. Mathiesen, Maja-Lisa Løchen, Inger Njølstad, , Tom Wilsgaard, Erin Smith, Terry Solomon, Frits R. Rosendaal, Kelly A. Frazer, Sigrid K. Brækkan, John-Bjarne Hansen
Submitted

Abbreviations

ACCP – American College of Chest Physicians

AF – Atrial fibrillation

AR% - Attributable risk fraction (i.e., the proportion of cases among exposed that can be attributed to the exposure)

BMI – Body mass index

CI – Confidence interval

CLOTS - The Clots in Legs Or sTockings after Stroke

CVD – Cardiovascular diseases

CTEPH – Chronic thromboembolic pulmonary hypertension

DOAC – Direct oral anticoagulation

DVT- Deep vein thrombosis

FII – Factor II (prothrombin)

FIIa – Activated factor II (thrombin)

FIXa – Activated factor IX

FVII –Factor VII

FVIII – Factor VIII

FVL – Factor V Leiden

FX – Factor X

FXa – Factor Xa

HDL – High-density lipoprotein

HR – Hazard Ratio

ICD – International Classification of Diseases

IMT – Intima-Media Thickness

INVENT – International Network on VENous Thrombosis

ISTH - International Society of Thrombosis and Haemostasis

LDL – Low density lipoprotein

LMWH - Low-molecular-weight heparin

MI – Myocardial infarction

NETs - Neutrophil extracellular traps

OR – Odds ratio

PE – Pulmonary embolism

PTS – Post-thrombotic syndrome

RAM – Risk assessment model

RCT – Randomized controlled trial

RR – Relative risk

SNPs - Single nucleotide polymorphisms

STEMI – ST-elevation myocardial infarction

SSC - Scientific and Standardization Committee

TF – Tissue factor

TFPI – Tissue factor pathway inhibitor

UNN - University Hospital of North-Norway

vWF – von Willebrand factor

VTE – Venous thromboembolism

WHO – World Health Organization

1. Introduction

Venous thromboembolism (VTE) is a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE). The first recorded description of VTE is found in the ancient Indian Ayurveda medical texts from 600-900 BC written by the physician Susruta Samhita (1). Samhita described an event of DVT similar as we will describe it today: *"…the legs become extremely red, hot, soft and swollen … indescribable burning sensation"* (2, 3). After this, VTE is not described in literature until the 13th century when a French illustrated manuscript describes a man with thrombophlebitis (1, 2, 4). The famous German physician and pathologist Rudolf Virchow first made the connection between DVT and PE in the late 19th century (1, 2, 4). Today, VTE is a common disease with potentially serious short- and long-term complications, including the development of the post-thrombotic syndrome (PTS) after DVT, chronic thromboembolic pulmonary hypertension (CTEPH), and sudden death as a consequence of circulatory collapse secondary to PE (5-8). Despite the high disease burden, the public awareness of VTE is low, and increased knowledge and awareness about risk factors, symptoms and preventive treatment are needed (9).

1.1 Epidemiology - Venous thromboembolism in the general population

1.1.1 Incidence of venous thromboembolism

VTE is the third most frequent cardiovascular disease (CVD) after myocardial infarction (MI) and stroke (10), with an estimated annual incidence rate from 10.4 to 18.3 per 10 000 person-years (5, 7, 11-19). The incidence of DVT (4 to 12 per 10 000 person-years) is higher than the incidence of PE (2.9 to 7.8 per 10 000 person-years) (7, 11, 13-16). Although VTE can occur in all ages, the risk of VTE increases with age and is mainly a disease of older age (12, 18). In childhood, the incidence is 1 in 100 000, while it rises to nearly 1 in 100 in individuals over 85 years (20-23). The highest incidence exists in individuals of African-American origin, followed by individuals of Caucasian origin (11, 12, 14, 15, 19). The variation between incidence rates in different studies may depend on population characteristics including age distribution, ethnicity, available data sources, case definition and validation procedures, and study design.

The incidence trend of VTE has been studied in different populations (18, 19, 24-26). In the Tromsø Study, a 27% overall age-adjusted increase in VTE incidence was observed in the period 1996 to 2012, from 16 per 10 000 person-years in 1996 to 20 per 10 000 in 2011. The increase was mainly caused by an increased incidence of PE with and without concurrent DVT, from 4.5 per 10 000 person-years in 1996/97 to 11 per 10 000 person-years in 2010/11. The incidence of isolated DVT slightly declined from 11 to 9 per 10 000 person-years (19). Several other studies have reported a similar increase in VTE incidence, also predominantly due to an increase of PE (19, 24, 26, 27). In contrast, a study from Western France reported a 28% reduction from 1998 to 2013 (25). Notable, this study included both first and recurrent VTEs in their incidence calculations and the study was based on two cross-sectional measurements, which could make it more vulnerable to random fluctuations in the incidence rates (25).

The observed increase in PE incidence may partly reflect a higher sensitivity of diagnostic methods, particularly computed tomography pulmonary angiogram (CTPA) and magnetic resonance imaging, detecting smaller emboli of unclear clinical significance. In the Tromsø study, the proportion of PE patients examined with CTPA increased from 23.1% in 1999 to 76% in 2011 (19). Contrary, the incidence of ischemic stroke and MI have substantially decreased by 25-50% in the last decades despite the simultaneous development of high-sensitive diagnostics tools (28-30). The decrease in stroke and MI incidence is mainly attributed to the reduction of cardiovascular risk factors (28-30). Likely, improved diagnostic tools may only partly explain the increase in VTE incidence, and important risk factors are yet to be discovered.

1.1.2 Recurrent venous thromboembolism

VTE tends to recur, and patients with an incident VTE have a 50% higher risk of a recurrent VTE than individuals in the general population having a first VTE (11, 14, 31-37). The risk is highest shortly after the index VTE, despite that most patients receive anticoagulant therapy in this period. Nevertheless, the risk of recurrence never falls to baseline, and 30-40% of the VTE-patients experience a recurrent event within ten years (7, 12, 32, 38). In the Tromsø Study (39), the 1-year cumulative recurrence rate (7.2%) was found to be lower than two comparable studies from the United States (11-13%) (35, 37).

However, in long-term follow up after more than ten years, the cumulative incidence of recurrence corresponded well with 28% (39) and 30% (35), in the Tromsø Study and the Rochester Epidemiology Project respectively. The lower 1-year cumulative recurrent rate in the Tromsø Study, the most recent study, may indicate an improvement of initial short-term treatment strategies. However, similar long-term rates may reflex a catch-up phenomenon after discontinuing the initial short-term thromboprophylaxis (32, 40, 41).

Several factors, including male sex, high body mass index (BMI), and neurologic disease with leg paresis (19, 35, 42-47) increase the risk of recurrence. The highest recurrence rates are observed in patients with persisting provoking factors such as cancer, followed by patients with unprovoked VTE. Individuals with an unprovoked VTE have an estimated 11% risk of recurrence the first year after discontinuing treatment, while recurrence in individuals with persistent provoking factors depends on the presenting risk factor (33, 34, 48). Although a substantial risk factor for a first event, hereditary thrombophilia increases the risk of recurrence only 1.5-fold (47). Transient provoking risk factors (e.g., recent surgery and trauma, pregnancy, oral contraceptive and hormone therapy) present at the time of the VTE event, are associated with a lower risk of recurrence (16, 33, 35). The lowest recurrence risk is observed in VTE occurring after surgery with a 0.7% risk per patient-year, while the patients with non-surgical risk factors have a risk of recurrence of around 3% per patient-year (49).

1.1.3 Complications of venous thromboembolism

Impaired thrombus resolution after VTE may result in PTS and CTEPH, both associated with high health care expenses and substantial morbidity (8). PTS is the most common complication to DVT and develops in 25-50% of the patients. Usually, PTS develops within 1-2 years after the thrombotic event (50-52). It typically presents with pain, persistent swelling, and heaviness of the affected extremity. Around ten percent develop venous leg ulcers, a resource-demanding condition, and PTS is associated with both reduced physical functioning and work-related disability (8, 33, 50, 53, 54). Women have a higher risk than men, and obese patients have a 50% increased risk of PTS. Other important risk factors for PTS include proximal DVT location, recurrent DVTs, and varicose veins. Cancer, surgery, plaster casts or inherited thrombophilias do not influence the risk of PTS (8, 50, 53). Elastic

compression stockings were earlier recommended to prevent PTS. However, a large multicenter randomized controlled trial (RCT) found that routine use of compression stockings did not reduce PTS and did significantly increase dermatological complications (50). Consequently, the newest American College of Chest Physicians (ACCP) guideline does not recommend routine use of graduated compression stocking (55).

CTEPH is a rare, but serious complication after acute PE (8). Usually, all thrombotic material in the pulmonary vascular bed resolve. However, 2-4% of patients develop pulmonary vascular disease of the major pulmonary arteries after PE due to incomplete resolving in the pulmonary circulation (14, 56). If left untreated, CTEPH will cause increasing fibrotic occlusion of the pulmonary artery leading to increased pulmonary vascular resistance, progressive pulmonary hypertension and in the end, right ventricular dysfunction (14, 56). Although CTEPH is considered to be a chronic complication of VTE, classic thromboembolic risk factors are lacking (8). While antithrombin deficiency, protein C deficiency, protein S deficiency, factor V Leiden, and prothrombin G20210A mutation increase the risk of VTE, CTEPH patients do not commonly carry these thrombotic risk factors. Previous splenectomy, infected ventriculo-atrial shunts, indwelling venous catheters and leads, thyroid replacement therapy, cancer, and chronic inflammatory states have been identified as risk factors for both VTE and CTEPH (8). The low incidence after acute PE makes routine screening for CTEPH in asymptomatic patients after VTE unfeasible, resulting in a frequent delay of diagnosis (57). CTEPH should be considered in patients with a history of PE who develop persistent dyspnea and large persistent perfusion defects (> 15%) on ventilation/perfusion scans (8). The treatment of CTEPH is to remove the obstructive material from the pulmonary vasculature with pulmonary endarterectomy, relieving pulmonary hypertension, and significantly improving the prognosis (8).

1.1.4 Mortality after venous thromboembolism

Overall, epidemiological models estimate around 500 000 VTE-related deaths per year in Europe (17). In the United Kingdom, the annual number of deaths from VTE is fivefold higher than the combined number of deaths from breast cancer, AIDS, and road traffic incidents (58). The survival rates after an incident VTE vary, ranging from 77% to 97% at one week, and 61-75% at eight to ten years (33, 34, 59, 60). A recent study including 710 study participants with an incident VTE from 1994 until 2012, reported an all-cause mortality

rate of 9% at 30 days and 24% at one year (39). The mortality rates are almost identical to those reported in a previous Norwegian study of 740 VTE patients recruited in the period 1995-2001 (20). The occurrence of cancer may explain the high one-year mortality, as the 1-year mortality rate is 60%-80% in patients with cancer-related VTE (20, 39, 61). Nevertheless, the overall mortality rates of VTE at 30-days and 1-year are higher than the respective 30-days and 1- year mortality rates of MI. For acute coronary syndrome (ACS) in total, the 30 days cumulative mortality rate is 2-5%, and the 1-year mortality rate is 9-15% (62, 63). For ST-elevation MI (STEMI) alone, the 30 days mortality rate is 2.5-10% (62).

The survival after VTE varies with the location of the thrombus (20, 39). Almost one-quarter of PE presents as sudden death (64), and PE is associated with more than a 3-fold increase in 30-day mortality compared to isolated DVT (7, 16, 20, 37, 64). However, the increased mortality of PE compared to DVT only persists for the first three months (16, 20, 64, 65). Increasing age, male sex, lower BMI, in-hospital management, congestive heart failure, chronic lung disease, severe neurologic disease, and active cancer are all reported to be independent predictors of reduced survival after VTE (46, 64, 65). Patients with recurrent VTE does not have an increased 3-year mortality more than after an incident VTE (37).

1.2 Pathophysiology of venous thromboembolism

Hemostasis is the physiological process that stops bleeding after a vascular injury while maintaining normal blood flow elsewhere in the circulation. This is achieved by complex pro- and antithrombotic mechanisms. The bleeding is ceased by recruitment of circulating platelets that both form a temporary blockage by a platelet plug, and release chemicals (e.g., adenosine diphosphate, serotonin, von Willebrand factor (vWF), thromboxane A_2, FV, FXI). The chemicals activate additional platelets and stimulate the coagulation system, which culminates in thrombin converting fibrinogen to fibrin to stabilize the platelet plug. The coagulation cascade consists of the intrinsic and the extrinsic pathway, leading to the adjoined common pathway (66-69). The extrinsic pathway is the primary physiological activator of the coagulation cascade and is initiated by formation of the tissue factor-FVIIa complex. Tissue factor (TF) is expressed on TF-bearing cells like stromal fibroblasts, leukocytes, and microparticles released from activated cells. The formation of the TF–FVIIa complex initiates a proteolytic cascade activating the coagulation factor FX to

FXa, and culminating in the FXa-FVa prothrombinase complex (66-70). Exposure of subendothelial collagen and activation of FXII to FXIIa initiate the intrinsic pathway. FXIIa catalyzes a cascade of FXI, FIX and FVIII activation, culminating in the FXa-FVa prothrombinase complex. The path from the prothrombinase complex to the thrombin and fibrin formation, the coagulation cascade is called the common pathway (66-70). While the extrinsic pathway is the primary physiological activator of the coagulation cascade, the intrinsic pathway has a minor role in the initiation of hemostasis as illustrated by the lack of bleeding disorders in patients and animals with deficiency of FXII (71, 72).

In addition to fibrin formation, thrombin activates FVa and FVIIIa, regenerating the prothrombinase complex. As a consequence, the coagulation cascade self-perpetuates the fibrin formation, also after the inhabitation of the TF-FVIIa complex by the TF-pathway inhibitor (TFPI) (67). To confine this process, several crucial regulatory mechanisms exist. When pathologic processes overwhelm these mechanisms, increased quantities of thrombin is formed, initiating the development of pathological thrombi (67, 70).

A venous thrombus is formed under low shear stress on the surface of a mostly intact endothelium (70, 73). This is contrary to arterial thrombosis that arises under high shear stress, typically after erosion, ulceration or complete rupture of an atherosclerotic plaque with the release of constituents of the plaque into the lumen of the blood vessel (74). An undamaged endothelium is vital in maintaining an antithrombotic state by expressing various anticoagulants, such as TFPI, thrombomodulin, endothelial protein C receptor, and heparin-like proteoglycans (75). A thrombus is classified depending on the relative amount of platelets and red blood cells. White thrombi are characterized by a predominance of platelets, while red thrombi are predominated of fibrin and trapped red blood cells. Thrombosis in the arterial circulation may lead to MI and ischemic stroke, and consists of white thrombi, while DVT and PE occur in the venous circulation system and consist of red thrombi (69, 70).

Simplified, VTE occurs as a result of one or more of the following three factors; 1) hypercoagulability, 2) altering of the blood flow (stasis), or 3) endothelial dysfunction or damage of the vessel wall (76). This pathophysiological basis for VTE is known as Virchow's triad (figure 1), named after the famous pathologist and physician Rudolf Virchow, who proposed the triad in a lecture in 1855 (77). In VTE, hypercoagulability and increased stasis of the blood allow accumulation of procoagulant proteases, such as thrombin (66, 69, 70), while the direct damage of the vessel wall is less central. This is supported by a histological study with no evidence of endothelial damage in the majority of venous thrombi recovered from autopsies (78). However, endothelial damage in the understanding of dysfunction of the endothelium is probably as central as stasis and hypercoagulability in the initiation of thrombus formation (66).

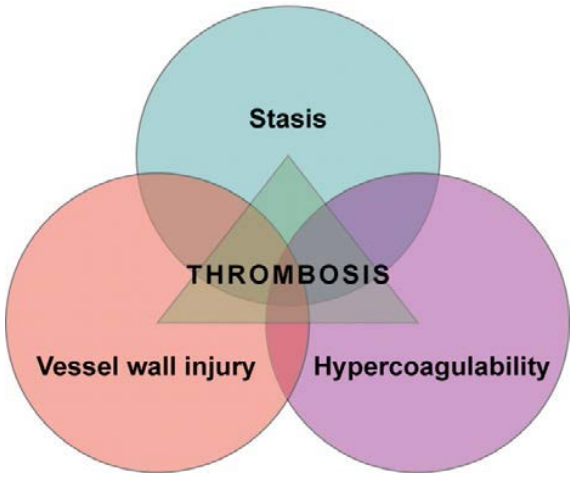

Figure 1. *Virchow's triad*

Most DVTs form in the valve pockets and soleal sinuses of the deep veins in the calves, as demonstrated by radiological and post-mortem studies (figure 2) (70, 76, 78-80). A declining oxygen gradient from the top to the bottom of the valve pocket after two hours of stasis has been demonstrated in dogs (81), indicating that hypoxia is a significant complication of stasis. Hypoxia promotes a subtle form of **endothelial injury** leading to alterations of protein expression and activation of the endothelium (82). Under this pathological condition, the endothelium is converted from an anticoagulant to a procoagulant surface (66, 82). An important mediator in the coagulation process is the relocation of P-selectin from an internal cell location to the surface of the activated endothelial cell. By P-selectin, the endothelial cells capture platelets, leukocytes, and leukocyte-derived TF-containing microvesicles (83). The leukocytes adhered to the endothelial surface become activated and express more TF. The local activation of the coagulation cascade overwhelms the protective anticoagulant pathways and triggers thrombosis (66, 70, 82). Additionally, recruited leukocytes release neutrophil extracellular traps (NETs), which is suggested to play a key role in inflammatory-mediated thrombosis

(84). However, the underlying mechanisms between microvesicles, NETs, and VTE remain to be established.

A direct correlation between DVT frequency and the number of valves exists (85). The location of the thrombi initiation have been attributed to hypoxic endothelial dysfunction and **increased stasis** followed by accumulation of coagulation factors, activation of endothelial cells, platelets and leukocytes (86). Usually, the skeletal muscle pump prevents DVT by moving blood past the venous valves and thereby inhibiting a high concentration of clotting factors in the valves pockets. Several mechanisms may overwhelm this system, including reduced mobility (e.g., bed-rest, neurological deficits or long-haul travel), hyperviscosity (e.g., polycythemia vera), congestive heart failure, and mechanic obstruction of the vessels (e.g., in pregnancy) (80, 87).

Hypercoagulability is an abnormally increased tendency toward clotting and could be inherited or acquired. In vitro studies show that plasma hypercoagulability leads to increased thrombin generation (88), which increases the risk of VTE (89). Inherited hypercoagulability is caused by prothrombotic genotypes increasing the activity or quantity of proteins promoting coagulation (i.e., Factor V Leiden and prothrombin G20210A) or genotypes decreasing the quantity of proteins that inhibit coagulation (i.e., Protein C and Protein S) (69, 90). Major surgery, cancer, obesity, chronic inflammation, antiphospholipid syndrome, and use of oral contraception may all cause acquired hypercoagulability (91, 92). These conditions increase the amount of circulating TF and other procoagulant proteases leading to hypercoagulability.

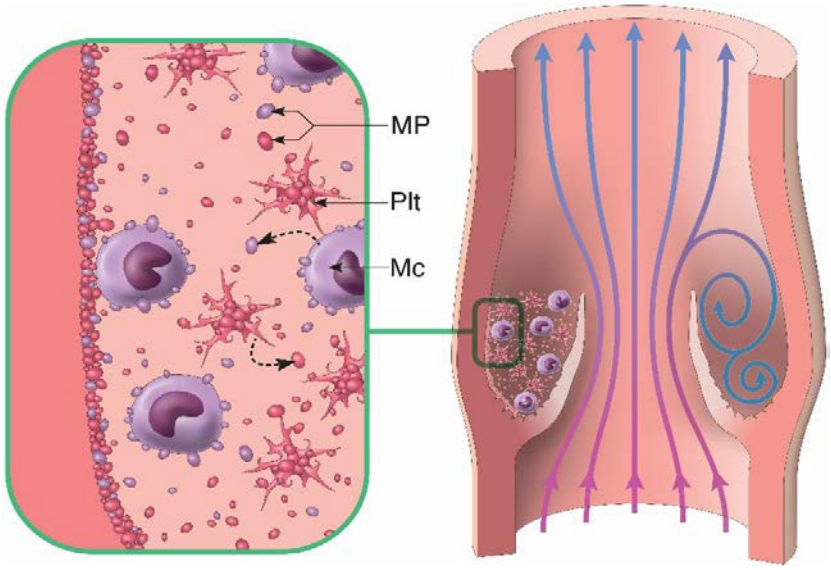

Figure 2. *The venous valvular sinus as a predilection site for DVT initiation. Blood is trapped in a vortex of the valve pockets, and the resultant hypoxia activates the venous endothelium, leading to the recruitment and binding of leukocytes, especially monocytes (Mc), platelets (Plt) and TF-positive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

PE has been considered as a complication of DVT that occurs if a part of the thrombus break away, travels to the lungs, and lodges in a pulmonary artery (69). However, studies demonstrate that the origin of the emboli remains undetected in half of the patients with PE (93-96). This could be due to a dislodging of the entire thrombus formation in the deep veins. However, recent studies support the concept that PE may arise from other sites in addition to the deep veins. Possible locations for thrombus formation include the right atrium and de novo formation in the pulmonary circulation (93, 97). Echocardiography and autopsy studies have displayed clots in the right atrium of patients with atrial fibrillation (AF) (98, 99). It is hypothesized that AF can cause right-sided cardiac thrombus formation, which could subsequently embolize, thereby leading to PE in a similar manner to systemic embolization leading to stroke (100). Supporting this hypothesis, patients diagnosed with atrial fibrillation are at a transient 6- to 10-fold increased risk of PE (97, 101). Additionally, 20% of patients with PE have a known history of AF (97, 102).

1.3 Risk factors for incident venous thromboembolism

VTE is a complex, multifactorial disease, involving interactions between acquired or inherited predispositions to thrombosis and environmental exposures (90). The risk of VTE change with age and genetic and acquired risk factors interact dynamically. To explain why thrombosis occurs in one person at a specific time, Rosendaal described the thrombosis potential model in 1999 (90). The model demonstrates how the combination of risk factors are necessary to yield a thrombosis

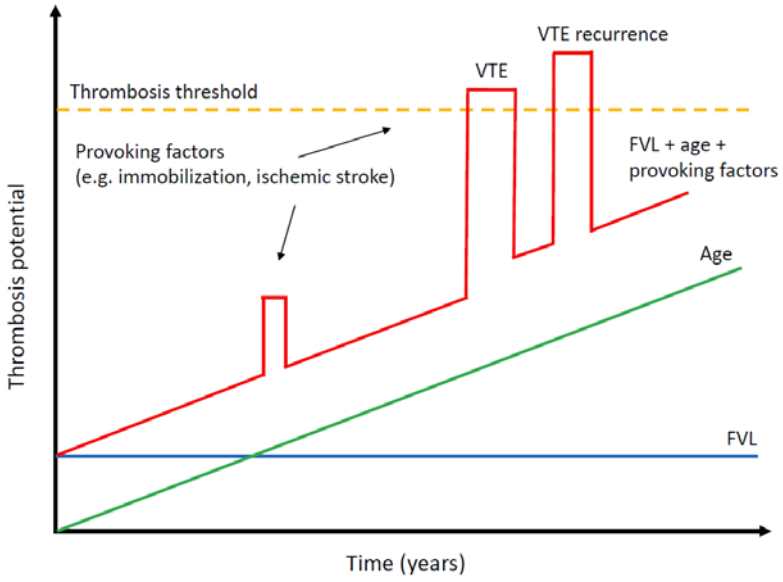

Figure 3. The thrombosis potential model. The blue line represents intrinsic factors that are stable over time such as inherited risk factors (e.g. Factor V Leiden [FVL]), and the green line represents the effect of a risk factor that increases over time, like age

potential (figure 3). When sufficient risk factors have accumulated, the thrombosis potential exceeds the 'thrombosis threshold,' and a thrombotic event occurs (47, 90).

Some risk factors for VTE are modifiable, while others, like advancing age and inherited thrombophilia, are not. Further, it is important to discriminate between transient and persistent risk factors, as this influence the risk of recurrence, and, thus, decisions on treatment duration (55). Risk factors are classified as transient if they occur up to 3 months before a VTE event and are not persistent (87). Examples of transient risk factors are surgery, pregnancy, and hospitalization, while non-modifiable risk factors as age, prothrombotic genotypes, and untreatable cancer are important persistent risk factors (15, 16, 87). Persistent risk factors are considered as clinical risk factors increasing the baseline risk of VTE. However, additionally to persistent risk factors, transient factors are often necessary to trigger a VTE event (87). VTE events are classified as unprovoked or provoked depending on the presence of provoking factors. An event is classified as unprovoked if they do not meet the criteria for provoked VTE-events (87). The amount of VTE events without provoking factors at the time of diagnosis ranges from 25 to 40% (14, 16, 103).

The degree to which risk factors are associated with thrombosis varies from very weak to very strong (87). The Scientific and Standardization Committee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH) recently stated two circumstances for when a transient risk factor should be considered major (87). First, more than half of the risk of recurrent VTE after ceasing anticoagulant therapy should be attributable to the risk factor. Secondly, the risk factor should be responsible for a 10-fold increase in the risk of a first VTE event (87). Transient risk factors are classified as minor (yet important) if it is associated with half the risk of recurrent VTE after stopping anticoagulant therapy (compared with if there was no transient risk factor), when the risk factor occurred up to 2 months before the VTE, or a 3 to 10-fold increase in the risk of having a first VTE (87). Major surgery is regarded as a major transient risk factor, whereas hospital admission for <3 days with an acute medical illness, estrogen therapy, pregnancy/puerperium and leg injury associated with immobility for >3 days are examples of minor transient risk factors (87). A recent, prospective cohort study of 646 patients with incident VTE found no difference in the recurrence rate in patients with or without exposures to risk factors more than three months before the incident VTE (104). Consequently, patients with a remote VTE risk factor

should not be managed differently from patients with unprovoked VTE. In the following sections, acquired and genetic risk factors will be elaborated.

1.3.1 Acquired risk factors

The incidence of VTE increases with **age**. In childhood, the incidence is 1 in 100 000, while it rises to nearly 1 in 100 in individuals over 85 years (20-23). The observed increased risk could be attributed to age-specific risk factors of thrombosis (i.e., endothelial dysfunction, frailty, and reduced muscle strength in the calves) or conventional risk factors that are more prevalent in the elderly than in young and middle-aged, including increased immobilization, malignant disease, and presence of co-morbidities associated with VTE risk (23). Nevertheless, a cohort study using data from the Tromsø Study showed that the increased incidence of VTE in elderly is not caused by the higher incidence of cancer in the same age group (105). Genetic risk factors are associated with increased risk of thrombosis in the elderly, although a lower relative risk is reported compared with the younger population (23).

Overweight is an important risk factor for VTE and BMI is a stronger predictor for VTE than for MI (10, 106). Results from previous prospective cohorts have consistently shown a 2- to 3-fold increase in the risk of VTE in obese individuals (13, 107, 108). Heit and colleges found that obesity accounted for about one-third of the unprovoked VTE events (18). In addition to BMI, total body fat (108), waist and hip circumference (106, 108-110), and the waist-to-height ratio (106) independently increase the risk of VTE. Of these, increased waist circumference is the preferable anthropometric measure of obesity to identify individuals at risk and to predict the risk of future VTE (106, 109). Additionally, weight gain, independent of attained BMI, is a risk factor for VTE (111). The mechanisms behind the strong association between obesity and risk of VTE are not fully understood, but recent Mendelian randomization studies imply that there may exist a causal relationship between high BMI and risk of VTE (112, 113). Chronic low-grade inflammation (114) and increased levels of procoagulant and hypofibrinolytic agents such as FVIII, fibrinogen, and PAI-1 may further affect the association (115). However, studies assessing the role of chronic low-grade inflammation in VTE are not consistent (116-118). Another possible mechanism is obesity-induced stasis caused by increased intra-abdominal pressure (119, 120).

Immobilization, whether in-hospital or in the community, increases the risk of VTE. Presumably, stasis of blood flow in the venous circulation is an important reason for the risk increase (15). In a meta-analysis including 43 observational studies of medical patients, a pooled odds ratio (OR) of more than two was reported for immobilized patients compared with non-immobilized patients (121). The most common definition of immobilization in the 43 studies was confinement to bed or bed rest lasting more than three days. In the meta-analysis, the risk of VTE was not adjusted for age. In a different study, bed rest up to 14 days was associated with an almost 6-fold risk increase of VTE in patients above 65 years (122). The risk of VTE is at its highest during the first weeks of bed-rest, but also long-term immobilization, which is most common in the elderly, increases the risk of VTE (23, 123). Even in healthy individuals, immobilization caused by for example injuries in the lower extremity treated with leg-cast or long-haul travels, may cause venous stasis and increase the risk of VTE (124, 125).

The VTE incidence is five times higher in **pregnant** women compared with non-pregnant women of similar age, and 20 times higher in the postpartum period. The risk peaks during the first six weeks postpartum and declines to rates approximating that of the general population by about 13 to 18 weeks (126, 127). The increased risk is mainly a result of hypercoagulability induced by hormonal changes present as early as the first trimester (128), increased venous stasis due to increased intra-abdominal pressure, and compression of the vena cava by the enlarging uterus (129).

About 9% of women of reproductive age worldwide use **oral contraceptives**. In Norway, as many as 70-80% of reproductive women use oral contraceptives, the highest contraceptives prevalence in the world (130). Combined oral contraceptives increase the risk of VTE 3.5-fold, and the effect size depends both on the progestogen used and the dose of ethinylestradiol (synthetic estrogen derivate) (131). Oral contraceptives containing levonorgestrel and those that contain a low-dose estrogen are associated with lower risk than preparations containing other types of progestogens and greater estrogen dose (131).

Patients in the hospital usually have several risk factors for VTE, and 40% to 60% of all cases of VTE are associated with **hospitalization** (132-135). The estimated incidence of VTE is 1 to 3 cases per 100 admissions per year (136, 137). Accordingly, the age- and sex-adjusted incidence of VTE is more than 130 times greater among hospitalized patients than among

community residents (135). Up to 20% of patients admitted to medical service, and 40% of the patients admitted to a surgical department will develop VTE (15). At discharge from the hospital, 31% of patients are at risk of VTE (138). Furthermore, almost 10% of all deaths in hospital are related to PE, a diagnosis often not suspected before death (139).

According to the Epidemiologic International Day for the Evaluation of Patients at Risk for Venous Thromboembolism in the Acute Hospital Care Setting (ENDORSE) study, less than 40% of at-risk hospitalized medical patients received the ACCP-recommended prophylaxis (140). Medical patients at-risk were less likely than surgical patients to receive appropriate prophylaxis (140). A recent study from the US showed an increase in the proportion of hospitalized patients receiving adequate VTE prophylaxis from 40% in 2005 to 90% in 2010 (134). However, the annual age- and sex-adjusted hospitalization-related VTE attack rate did not change significantly during the 5-year study period. In the study, the median duration of hospitalization and in-hospital prophylaxes were three days and 70 hours, respectively. Most VTE events (75%) occurred after hospital discharge, with an almost 20-day median time to VTE (134). Despite these findings, the latest ACCP guidelines on prevention of VTE in nonsurgical patients from 2012, recommend not to extend the duration of thromboprophylaxis beyond the period of patient immobilization or acute hospital stay, mainly due to the risk of major bleeding (141). This was supported by the Extended Prophylaxis for Venous Thromboembolism in Acutely Ill Medical Patients With Prolonged Immobilization (EXCLAIM) study, were over 6000 hospitalized patients over 40 years were randomized to receive extended-duration enoxaparin for 38 days or placebo. The risk of major bleeding was significantly increased with extended-duration enoxaparin, and there were four intracranial bleeding events in the extended enoxaparin group compared with none in the placebo group (142).

Three large trials comparing standard-duration (7-10 days) and extended-duration (25-35 days) thromboprophylaxis in acutely ill medical patients, showed that extended-prophylaxis with enoxaparin (142) and rivaroxaban (143), reduced the risk of VTE compared with standard-duration of enoxaparin, while an extended-prophylaxis with apixaban was not superior to a shorter course of enoxaparin (144). Recently, the Food and Drug Administration of the United States of America approved the direct oral anticoagulation (DOAC) betrixaban as the first DOAC for the prevention of VTE in acutely ill medical patients.

The approval of betrixaban was a result of the large APEX trial consisting of more than 7500 patients (145). In the trial, patients were randomized to either extended-duration treatment with betrixaban (35-42 days) or standard-duration treatment with enoxaparin (10±4 days). Betrixaban was superior in preventing VTE (symptomatic and asymptomatic) and major bleeding in the overall population (145, 146). These findings indicate that post-discharge prophylaxis may be more important than previously considered and that additional effort is needed to identify patients at high risk of VTE who will benefit from extended-duration prophylaxis. The recently published MARINER trial assessed post-discharge extended VTE prophylaxis with rivaroxaban in selected high-risk medical patients investigating the rate of symptomatic VTE (147). However, the trial failed to meet its primary endpoint to reduce the risk of a composite of symptomatic VTE or VTE-related death. Because the trial failed to meet its primary endpoint, any looks at secondary endpoints should be considered exploratory. Nonetheless, the authors observed lower rates of symptomatic nonfatal VTE and a composite of symptomatic VTE or all-cause death in the rivaroxaban arm (147). After the Mariner trial, the usefulness of extended thromboprophylaxis remains uncertain. The authors of the study, states that future studies should more accurately identify deaths caused by thrombotic mechanisms and focus on the patients who are at highest risk and who may benefit from anticoagulant prophylaxis (147).

There are numerous risk factors for VTE in **surgical** patients (e.g., type and extent of surgery or trauma, duration of hospital stay, and surgical complications), and up to 40% of the patients admitted to a surgical department will develop an asymptomatic VTE (15, 148). The incidence of fatal PE without proper thromboprophylaxis is around 0.1-0.8% in patients undergoing elective general surgery, 2-3% in patients having an elective total hip replacement, and 4-7% of patients undergoing surgery for a fractured hip (149). In the Million Women Study, 1 in 140 women undergoing inpatient surgery and 1 in 815 women undergoing outpatient surgery were admitted with VTE during the first 12-week after surgery compared to only 1 in 6200 women not undergoing surgery (150).

Patients with **medical illness** often have multiple comorbidities, and the VTE risk factors are diverse and probably less commonly identified than risk factors among surgical patients. PE has been recorded as the cause of death in over 5% of patients with medical illness using postmortem reports (151). Of patients with fatal PE, 80% occurred in patients

who had not received recent surgery. In more than 50% of the cases, an acute medical illness was reported within the last six weeks before death (151). Several medical conditions are associated with increased risk of VTE including cancer, infectious disease, renal impairment, acute respiratory disease (including exacerbation of chronic obstructive pulmonary disease [COPD]), heart failure, and arterial CVD. In addition, all patients admitted to intensive care units are at increased risk of VTE, even after routine anticoagulation (152).

Cancer is strongly associated with VTE (153). Already in 1861, the French physician Armand Trousseau described how thrombosis could predict occult cancer (154). A hypercoagulable state probably mediates the risk of VTE in cancer patients. The hypercoagulability is caused by the release of inflammatory cytokines and activation of the coagulation cascade (155). The high risk of VTE in cancer patients is visible as 20-25% of all VTE cases occur in cancer patients. Additionally, around 20% of all cancer patients develop VTE during the course of the disease (155, 156). Overall, cancer is associated with a 4-7-fold increased risk of VTE (156, 157). The risk of VTE is highest during the first year after the cancer diagnosis (153) and is dependent on tumor type, age, stage of cancer and cancer-treatment (158).

Several different **infectious diseases** are associated with an increased risk of VTE (159-161). Virus infections with human immunodeficiency virus and cytomegalovirus increase the risk of VTE with 30% and 70%, respectively (161). Bacterial pneumonia and urinary tract infections increase the risk of VTE considerably, with a higher risk of PE than DVT (160, 162-164).

Patients with **end-stage renal disease** have a higher incidence of VTE compared with the general population (165, 166). Stage 3 to 4 chronic renal disease is associated with an almost 2-fold risk of VTE compared with patients with normal renal function (165). Patients in dialysis are further predisposed to VTE (167). Respiratory failure and exacerbation of **COPD** are recognized as risk factors for VTE (168). Both temporary immobilization and localized hypoxia may increase the release of procoagulant factors and predispose for VTE during exacerbations. However, also patients without exacerbation with severe COPD have an increased risk of VTE compared with the general population, mainly caused by increased incidence of provoked VTE. Thus, immobilization and infections possibly increase the risk of VTE in patients with stable, severe COPD (169).

Heart failure increases the risk of DVT and PE 1.2- and 2-fold respectively (170). All three factors of Virchow's triad could explain the association between heart failure and VTE (171). First, reduced myocardial contractility, dilated cardiac chambers, and low cardiac output could all cause abnormal blood flow and stasis in the venous system. Second, heart failure increases the activity of the hemostatic system and platelets, inducing a hypercoagulable state. Third, heart failure stretches and injure the vessel wall creating endothelial dysfunction. The risk of VTE is highest in patients with right heart failure, indicating that increased stasis is of high importance in the pathogenesis (171).

1.3.2 Genetic risk factors

VTE is a highly heritable disease, and 50 to 70% of the variance in VTE incidence could be attributed to genetic risk factors (172-177). Hereditary thrombophilia follows a multifactorial, non-Mendelian inheritance model, where multiple genetic risk factors contribute to the increased risk (178, 179). Overall, the incidence of a first time VTE is 0.8% per year in carriers of a prothrombotic defect compared with 0.1% per year in non-carriers (174). However, there are considerable differences in the risk of VTE among individuals with different types of hereditary thrombophilia (174). To date, 20 to 30 genetic VTE risk factors have been identified (178, 180).

The genetic risk factors could be categorized as loss-of-function mutations and gain-of-function mutations (181). In gain-of-functions mutations, there is a gain of function of procoagulant factors, while there is a loss of function of endogenous anticoagulation in loss-of-function mutations. Gain-of-function mutations can result in the increased synthesis of a normal protein (i.e., prothrombin G20210A), impaired breakdown or down-regulation of a normal protein (i.e., Factor V Leiden), or rarely, synthesis of a functionally hyperactive protein (i.e., factor IX Padua). Loss-of-function mutations are in general rarer than gain-of-function mutations and tend to be associated with higher risk estimates for VTE.

Loss-of-function mutations

Inherited risk of VTE was first recognized by the Norwegian hematologist Olav Egeberg when he discovered a family with an increased risk of VTE due to **antithrombin**

deficiency (182). Antithrombin is a potent inhibitor of the coagulation cascade and mutations in the SERPINC1 cause antithrombin deficiency. Antithrombin deficiency is rare in the general population (0.02%) and is associated with a 10- to 50-fold increase in the risk of VTE (183, 184).

Heterozygous **deficiencies of protein C** and **protein S** are other causes of loss-of-function mutations increasing the risk of VTE (185, 186). Protein C is a natural plasma anticoagulant that, when activated, inactivates FVa and FVIIIa to down-regulate the thrombin generation. Protein S assist in the downregulation of thrombin formation by serving as a co-factor of both activated Protein C and TFPI (184, 187). Both deficiencies of protein C and protein S increase the risk of VTE by approximately 8-fold (184, 188, 189). However, deficiencies of protein C and protein S are rare, occurring in less than 1% of the general population (178, 184, 190).

Gain-of-function mutation

The most frequent prothrombotic genotype variant is **non-O blood type**, which is present in 60 to 70% of the population (191). Individuals with B and A1 blood groups are at a 1.5 to 2.0-fold higher risk of VTE compared with individuals with O and A2 blood groups, respectively (184, 191-194). The association could be caused by levels of FVIII and vWF in blood as individuals with O blood type have 25% lower levels of these factors than individuals with non-O blood (195). However, non-O blood type has been found to increase the risk of VTE independent of levels of FVIII (184, 195). Due to the high frequency of non-O blood type in the general population, over 30% of the VTE events could be partly explained by the presence of non-O blood type despite the modest relative risk increase (192). Additionally, non-O blood type has an additive effect on the risk of VTE combined with both factor V Leiden and the prothrombin mutation (193).

Activated protein C (APC) resistance was discovered in 1993 (196). One year later, Bertina *et al.* from the Leiden University Medical Center in the Netherlands, published a paper in Nature describing the **Factor V Leiden (FVL) mutation,** also known as rs6025. FVL causes the majority of the cases with APC resistance. The condition is caused by a single point mutation in the factor V gene, which predicts substitution of arginine at position 506

with glutamine (197). This substitution interferes with the normal APC cleavage site of factor V. In addition to APC-resistance, FVL is thought to be prothrombotic by the abnormal breakdown of FVIII by APC (198). In the Caucasian general population, FVL is found in 3 to 8%, while in Asian, African and indigenous Australian populations, the mutation is extremely rare (178, 198). Heterozygous carriers have a 2- to 5-fold increase in the risk of VTE compared with the general population, while homozygous carriers are under a 10- to 80-fold risk (178, 183, 184, 197-202).

In 1996, a mutation of the prothrombin gene, **prothrombin 20210A** (rs1799963), was discovered (203). The mutation causes an overproduction of prothrombin increasing the risk of VTE, and is present in 1-2% of the population (178, 181, 184). Carriers of the prothrombin 20210A allele have a 2- to 3-fold increased risk of VTE (183, 184, 203, 204). Factor V Leiden and the prothrombin 20210A mutations are relatively common and their coinheritance with other thrombophilias increase the risk of VTE (183, 193). The **Fibrinogen gamma chain (FGG)** gene encodes the fibrinogen γ chain, which is one of the three polypeptides composing the fibrinogen molecule. Fibrinogen γ is important for the antithrombin activity that develops during fibrin formation. The allele of the rs2066865 polymorphism, with frequency around 0.25, was found to reduce fibrinogen γ plasma levels and to increase the risk of VTE around 1.5-fold (205, 206).

The last decade new genotypes related to increased risk of VTE have been discovered through genome-wide association studies (GWAS). This method searches the genome for small variations called single nucleotide polymorphisms (SNPs) that occur more frequently in people with a particular disease than in people without the disease. GWAS may identify SNPs associated with VTE without a prespecified hypothesis, opposite to previous investigations of clustering of VTEs in families (182) and the candidate-gene approaches of genes coding molecules of the coagulation/fibrinolysis pathways (180) used to identify the previously mentioned risk variants. Using GWAS, tests of large samples of VTE patients may discover unknown SNPs more frequently present in VTE patients than controls (207). High plasma levels of **Factor XI** (FXI) are associated with elevated risk of VTE (180, 184, 208), and several SNPs at the FXI locus are found to be associated with a 1.3-fold increase in the risk of VTE through modulation of FXI plasma levels (190, 202, 209, 210). Recently, more SNPs associated with VTE have been identified. However, the majority of SNPs identified through

GWAS have a modest effect on VTE risk with ORs ranging from 1.10 to 1.35, and single SNPs may only have limited clinical utility in prediction and diagnostics of VTE (184).

In the future, new and more extensive studies are warranted to identify undiscovered genetic variants associated with VTE (211). To identify new genetic variants, the INVENT consortium has been created to combine several genetic studies worldwide to increase the sample size. One large meta-analysis from the INVENT consortium has already identified nine new genetic variables increasing the risk of VTE. Of these, two of the new loci identified, TSPAN15 and SLC44A2, do not belong to conventional pathways for thrombosis or have previously been associated with CVD (211). These findings suggest that unexpected actors of VTE etiology exists, and a meta-analysis of exome-wide association studies by the INVENT consortium are currently ongoing to discover new, rare genetic variants associated with VTE (178).

1.3.3 Cardiovascular risk factors, atherosclerosis, and venous thromboembolism

Atherosclerosis is a condition in which the lumen of an artery narrows as a result of a localized buildup of inflammatory cells, cholesterol and other lipids, connective tissue, and calcium deposits in the tunica intima zone of the arterial vessel wall (212). The development of atherosclerosis begins with injury to the endothelial cells lining the surface of the interior vessel wall (212, 213). Endothelial dysfunction is a consequence of many interfering factors, including hypertension, hemodynamics, hyperlipidemia, oxidative stress, and inflammation. None of them are compulsory for disease development, but all increase the risk of endothelial dysfunction. The endothelial injury eventually causes chronic endothelial dysfunction, and in turn, increased permeability through the junctions between the endothelial cells. As a consequence, low-density lipoprotein (LDL) in blood plasma invade the endothelium. Increased oxidative stress causes oxidation of the LDL, and oxidized LDL (ox-LDL) affects the migration of monocytes and lymphocytes into the subendothelial space. Once within the intima, monocytes transform into macrophages that devour lipoproteins, like ox-LDL. This process converts the macrophages into lipid-laden foam cells. The activation of these macrophages also leads to cytokine production, which recruits additional inflammatory cells and stimulates the adhesion of more monocytes and lymphocytes (212, 214). The next step on the path to a developed atherosclerotic plaque is proliferation and

migration of vascular smooth muscle cells and deposition of extracellular matrix (e.g., collagen). The final stage of the plaque consists of a raised lesion with a soft, yellow core of lipid covered by a firm, white fibrous cap (212, 215-218).

The atherosclerotic plaque may be asymptomatic for decades or could erode or rupture (219, 220). Plaque rupture exposes prothrombotic material from the core of the plaque, including tissue factor, collagen, phospholipids, and platelet-adhesive molecules, to the blood. The exposure of the prothrombotic material to the bloodstream activating the clotting cascade resulting in the generation of thrombin. Contact with collagen in the plaque's extracellular matrix can trigger platelet activation, and circulating platelets adhere to the damaged site, aggregate, activate, and release secondary aggregators like thromboxane A2, adenosine diphosphate, and serotonin. Consequently, the formation of a thrombus can occlude the lumen of the coronary vessel, causing MI, or an intracerebral artery, causing an ischemic stroke (74, 218-221). Several factors increase the risk of the development of atherosclerosis, including age, obesity, smoking, diabetes mellitus, hypertension, reduced physical activity, and hyperlipidemia. Additionally, family history of MI (FHMI) is closely associated with atherosclerotic disease (222-228).

Traditionally, arterial CVD and VTE have been classified as two separate diseases due to different epidemiology, pathophysiology, risk factors and treatment (13, 69, 229). However, in the last decade, data from studies suggests that arterial and venous thrombosis have more similarities than previously believed. In 2003, Prandoni et al. published a study in the New England Journal of Medicine where patients with unprovoked VTE had a higher prevalence of atherosclerotic plaque (230). Subsequently, several studies have investigated whether shared risk factors or causal mechanisms could explain the association between arterial and venous thrombosis. In the next section, the association between atherosclerotic diseases, traditional cardiovascular risk factors, and future risk of VTE will be discussed.

Smoking is one of the most influential risk factors for arterial CVD (231), and several studies have investigated the impact of tobacco smoking on the risk of with VTE. In 2013, a meta-analysis summarizing all published prospective and case-control studies to date regarding the risk of VTE in smokers found that both former and current smoking increased the risk of VTE slightly (232). However, the study did not differentiate between provoked and unprovoked VTE events. Data from the Tromsø study, one of the studies included in the

meta-analysis, observed that heavy smoking was a risk factor for provoked VTE in analyses with VTE as the only outcome. When participants were censored at the occurrence of cancer or MI, there was no observed association between smoking and VTE (233). In the Iowa Women's Health Study, only the incidence of secondary-, and particularly cancer-related VTE events, was higher among smoking- than never-smoking-participants (234). Recently, Mahmoodi et al. performed an individual level random-effect meta-analysis including nine prospective studies with measured baseline cardiovascular risk factors and validated VTE events (235). Different from previous meta-analyses, only prospective cohort studies with information about possible confounding factors and validated VTE events were included to reduce the risk of bias. Similar to prior findings, an association was only observed between cigarette smoking and provoked VTE (235). These findings suggest that smoking-attributable diseases (e.g., cancer, ischemic stroke or MI) or other predisposing factors are essential for smoking to convey a risk of VTE (233).

Ageno et al. found a positive association between **hypertension** and increased VTE incidence in a meta-analysis from 2008 (236). However, the authors did not adjust for confounding factors such as age and BMI, both strongly associated with hypertension as well as VTE. Further, the meta-analysis consisted mainly of retrospective, case-control studies (236). Most prospective cohort studies have shown no association between hypertension and VTE after adjusting for age, sex and obesity (13, 109, 237). In the meta-analysis by Mahmoodi et al. (235), an association was found between hypertension and VTE in the unadjusted model. The association disappeared after adjustment for age, sex, and BMI, and when modeled continuously, an inverse association was observed for systolic blood pressure (235).

Previous studies are inconsistent regarding the association between **diabetes mellitus** (DM) and risk of VTE. One prospective cohort study showed a 50% increased risk of VTE in diabetic patients (13), and two large meta-analyses identified DM as a risk factor for VTE (236, 238). However, most prospective cohort studies and the recent meta-analysis from Mahmoodi et al., did not observe an independent association between DM and VTE after adjusting for relevant confounding factors such as obesity, hospitalization, major surgery, and nursing home recidency (109, 110, 235, 239, 240). These findings suggest that

the association between DM and VTE could be caused by obesity and the other comorbidities associated with DM.

Physical activity has been found to be associated with both lower risk (237, 241, 242), and no effect on the risk of VTE (13, 110, 243) after adjustment for BMI. More surprisingly, some studies indicate that hard physical activity may increase the risk of VTE in vulnerable groups such as obese and elderly (241, 243). In elderly, this could be triggered by microtrauma activating the coagulation system.

Regarding **hyperlipidemia,** previous studies are more consistent. Despite some studies finding the levels of lipids in the bloodstream to be associated with VTE incidence (110, 237), most studies indicate that hyperlipidemia, including high levels of cholesterol and triglycerides, is not associated with risk of VTE (13, 109, 117, 235, 244). Opposite, **FHMI** is not only a significant risk factor for MI (245), but also an independent risk factor for VTE (117, 246-249).

To summarize, recent studies indicate that traditional cardiovascular risk factors, excluding age, obesity, and FHMI, are not associated with an increased risk of VTE. Smoking does increase the risk of provoked VTE, but this could be attributed to smoking-associated conditions independently increase the risk of VTE (e.g., cancer, MI or stroke).

Atherosclerosis

Atherosclerosis is a condition affecting the tunica intima zone of the arterial vessel walls (212). As the development of atherosclerosis is vital in the pathogenesis of CVD, the risk of VTE in these patients could be caused by the presence or progression of atherosclerosis. As previously mentioned, in a case-control study conducted by Prandoni et al., patients with unprovoked VTE had twice the prevalence of atherosclerotic plaque measured by carotid intima-media thickness (IMT), compared with age- and sex-matched hospitalized controls (230). Similar results were reported in two other case-control studies. Hong et al. reported a higher prevalence of coronary artery calcification in patients with unprovoked VTE compared with controls (250), and Jezovnik et al. reported a significantly thicker IMT and higher prevalence of atherosclerotic plaques (251). However, both the two latter studies and the landmark study from Prandoni have some important limitations. Most

notably, the measurement of IMT and coronary artery calcium was performed after the VTE events. Thus, the temporal relation of the association observed in the papers was not clear.

Succeeding Prandoni et al., two population-based cohort studies, using the Atherosclerosis Risk in Communities (ARIC) (250) and the Cardiovascular Health Study (252), investigated the association between atherosclerosis and risk of VTE. In both studies, measurements of IMT were done at baseline. In the ARIC study, increased carotid IMT or presence of carotid plaque was not associated with an increased incidence of VTE after adjusting for relevant confounding factors such as age and sex (253). In the Cardiovascular Health Study, an inverse association between high-risk carotid plaques and VTE was observed (252). These findings suggest that subclinical atherosclerosis in itself is not a risk factor for VTE.

IMT measured only at baseline may introduce potential bias. First, the measurement of atherosclerosis was often performed years before the VTE event. Second, both studies were unable to provide any information about the progression or prevalence of atherosclerosis later in the follow-up (253). Thus, as Prandoni himself proclaim in a review paper regarding the association between atherosclerosis and VTE, the only conclusion we may draw from the existing studies is that subclinical parameters of atherosclerosis are unlikely to predict future VTE (254). In the Tromsø study, competing risk analyses were used to eliminate the development of MI as an intermediate factor affecting the risk of VTE. In the prospective cohort, carotid atherosclerosis was associated with risk of future MI, but not VTE (252, 253).

The findings from the three large, prospective cohort studies suggest that asymptomatic atherosclerosis is unlikely to be associated with an increased risk of VTE. However, as atherosclerotic plaque progress over time, it is difficult to determine the actual association with only one measurement of plaque. The long follow-up may introduce regression dilution bias leading to a potential underestimation of the true association (254, 255).

1.3.4 Arterial cardiovascular diseases and risk of venous thromboembolism

Myocardial infarction:

Symptomatic atherosclerotic events, such as MI and ischemic stroke have been found to increase the risk of VTE in several studies. In a meta-analysis published in 1996, reviewing clinical effects of anticoagulant therapy in suspected MI, 4% of patients with MI had symptomatic PE within two weeks after hospitalization (256). Contrary, in a study of almost 24 000 autopsies, an increased risk of VTE was found in patients with ischemic stroke and peripheral artery thrombosis, but not in patients with MI. In fact, these patients had a reduced risk of VTE in comparison with the overall population (257). A high mortality rate (91%) among the patients with coronary thrombosis could explain the reduced risk. In the time setting with fewer therapeutic modalities for acute MI available than today, many of these patients did not survive long enough to be at risk of developing VTE (257). The possible association between MI and VTE was supported by a cross-sectional study where an association between coronary artery disease and PE was found in patients aged 60 years or older (258). However, the cross-sectional study design has several limitations, and prospective studies are necessary to investigate the association between MI and VTE.

In the ARIC study, no association between atherosclerosis and VTE was found (253). However, both patients with MI and patients with stroke had an increased risk of VTE (253). Similar results were found in the General Practice Research Database from the United Kingdom, using a nested case-control analysis. In this study, 6 000 VTE patients were compared with a random sample of 10 000 age- and sex-matched controls. The results showed that MI increased the risk of PE, but not DVT (259). Two large registry-based, case-control studies from Denmark showed that patients with MI and other heart diseases had an increased risk of VTE (260, 261). The earliest of the two studies, published in 2009, investigated the association between MI and VTE using almost 6 000 cases of VTE compared with 60 000 age- and sex-matched controls. In this study, MI was associated with a more than 4-fold increase in the risk of VTE the first three months after the incident event. However, after more than three months, MI was no longer significantly associated with VTE risk. The risk did not vary between provoked and unprovoked events. Similar to previous studies, the risk was higher for PE than DVT (260). The second study, using the entire population of Denmark, investigated whether heart disease increased the risk of incident PE

without apparent DVT (261). In the study, 45 000 patients had PE alone, 5000 had PE and DVT, and 60 000 had DVT alone. The control group consisted of 540 000 age- and sex-matched controls from the general population. Sørensen et al. found that MI and heart failure in the preceding three months increased the risk of isolated PE more than 40-fold, and the combined risk of PE and DVT 20-fold. The risk more than three months after the incident MI was lower, but the long-term risk was persistently increased for isolated PE compared with the general population (261). Right-sided valvular disease was associated with a higher risk than left-sided valvular disease (OR 75 vs. OR 13.5) (261). Also, the Rochester Epidemiology Project observed an association between MI and subsequent VTE using a case-control study. However, in this study, the association between MI and VTE was markedly attenuated after adjusting for hospitalization and nursing home confinement (262).

Ischemic stroke:

The increased risk of VTE in patients with ischemic stroke are well known. Already in 1972, Warlow et al. studied 30 patients with ischemic stroke causing weakness of one leg and showed that the frequency of DVT was 60% within the first ten days, detected by I-125 fibrinogen scanning (263). Of these, four patients developed PE. The study was limited by the study size and the lack of a control group. Subsequent studies have shown a high incidence of DVT in stroke patients (264-273), with DVT incidence varying from 3% (272) to 75% after stroke (264). However, the majority of these studies have actively searched for asymptomatic DVT. The incidence is, in general, lower in studies using venography and Doppler ultrasound instead of I-125 fibrinogen screening (274). Most studies report rates of asymptomatic DVT in around 30-40% of stroke patients (267-269, 273), while symptomatic DVT occurs in 1-10% of stroke patients (273, 275-279). The incidence of PE varies in studies from 1-5% of clinical PE (275, 280, 281), to around 10% in studies were the great majority of cases were asymptomatic (273). The frequency of both DVT and PE might have declined the recent years due to more intensive use of thromboprophylaxis (282).

DVT develops early after the ischemic stroke (265, 283), with a peak incidence of asymptomatic events during the first ten days (277, 284). The incidence of VTE ranges from 6% to 30% the first 14 days after stroke (270, 277, 284-286). In the large The Clots in Legs Or sTockings after Stroke (CLOTS) trial, DVT was detected within 30 days in 15% of immobile

patients with acute stroke using duplex ultrasound (277). Despite the incidence peak shortly after the stroke, the incidence of DVT and PE continue to be increased during the rehabilitation phase (287-289). Around 4% of patients entering a rehabilitation unit may develop PE (289), and bilateral venography revealed DVT in 33% of patients nine weeks after an incident stroke (287). PE events are observed as much as 4 months after the stroke (288).

In both the acute and the rehabilitation phase, the risk of DVT varies with the degree of immobilization, leg paralysis and stroke severity (269, 273, 279, 287, 290-294). Increasing age, dehydration, and pre-stroke disability are other important risk factors for VTE in stroke patients (279, 295, 296). The majority of DVTs are detected in the paretic leg (277). However, bilateral DVTs occur in 15% to 20% of the patients (269, 283).

VTE counts for a significant share of deaths after stroke (281). Stroke patients with PE had higher rates of in-hospital death and disability at 30 days and one year than stroke patients without PE (297, 298). In older studies of patients not receiving thromboprophylaxis, PE counted for up to one-quarter of early deaths after stroke (297, 298). For stroke patients with incident DVT without concurrent PE, there is not observed an increased risk of mortality (279). Fatal PE may occur as early as the first week (288). However, fatal PE-events occur most often between the second and fourth week after the stroke, and in these weeks, PE is the most common cause of death in stroke patients (298-300).

Most studies regarding the incidence of VTE after stroke are based on clinical trials with preselection of patients. Participants included in these studies have in general lower rate of comorbidities, lower age and higher motivation for rehabilitation (301). Further, the clinical trials consist solely of stroke patients. Consequently, limited data exist regarding the association between ischemic stroke and risk of VTE in the general population. A registry-based, case-control study revealed that patients with a history of stroke had a 50% overall increased risk of VTE, driven by a short-term 4.4-fold increase in risk the first three months after stroke (260). The overall risk corresponded well with an autopsy study from Sweden where cervico-cranial arterial thrombosis increased the risk of VTE with 50% after adjustment for sex, age and other possible confounding factors (257). Nevertheless, similar to the association between MI and VTE, the observation of VTE-risk after stroke is limited to registry-based studies.

2. Aims of the thesis

The aims of the thesis were:

- To investigate the association between the presence, formation, and progression of carotid atherosclerosis and risk of venous thromboembolism using a large prospective cohort with repeated measurements of intima-media thickness and total plaque area.

- To explore the association between myocardial infarction and future risk of venous thromboembolism in a population-based cohort study with validated information on exposure, endpoint, and potential confounders.

- To study the time-dependent risk of venous thromboembolism by ischemic stroke in a population-based cohort with validated information on exposure, endpoint, and potential confounders.

- To investigate the combined effect of ischemic stroke and the most influential thrombosis-associated SNPs on the risk of future venous thromboembolism using a case-cohort study with individuals recruited from the general population.

3. Study population and methods

1.4 The Tromsø Study

In 1974, the newly established University of Tromsø conducted the first Tromsø survey due to a rapid increase of cardiovascular mortality in the Northern-Norway after 1950 (302, 303). In this first survey, only young and middle-aged men were targeted. Since then, six more surveys have been conducted, including both sexes from the age of 20-89 years. In total, more than 50 000 inhabitants of Tromsø have participated in one or more of the six surveys (302-304). As time went by, more information was obtained, including more clinically oriented examinations in large subgroups. From Tromsø 4, the protocols comprised questionnaires, anthropometric and blood pressure measurements, as well as laboratory analyses of blood lipids, blood glucose, renal and liver function, hematology, hormones, and genetics (302-304).

In this thesis, we have used data from Tromsø 4, Tromsø 5 and Tromsø 6. In Tromsø 4, all inhabitants 25 years and older were invited, and 27 158 participated (77% of the eligible population). In Tromsø 5 and Tromsø 6, selected subgroups were invited, and 10 353 (79% of the invited), and 19 762 (66% of the invited) participated, respectively. In paper 1, all inhabitants in Tromsø 4 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 ultrasonography of the carotid artery (305). Study participants 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 Tromsø 6. Participants attending for the ultrasound examination were excluded if they had missing information on the measures of carotid atherosclerosis. In total, 10 426 participants attended for ultrasound examination of the right carotid artery in Tromsø 4, Tromsø 5, and Tromsø 6. The participants were followed until the follow-up (December 2012) or until a censoring event (i.e., migration, death). In paper 2 and paper 3, participants from all three surveys were followed from the date of enrollment until the outcome event occurred, the participant died or moved from the municipality of Tromsø or until the end of the study period December 31, 2010. The study population in paper 4 consisted of a subgroup of study participants from the three surveys with extended genetic analysis. In the subgroup, 692 first time VTE events from 1994 until

the end of follow-up on December 31, 2012, were included together with a subcohort consisting of 2016 age- and sex-weighted participants.

1.5 Baseline measurements

Information about study participants was collected by physical examination, blood samples and self-administrated questionnaires at each survey. 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 of ≥ 30 $kg\ m^2$) was classified according to the World Health Organization (WHO) definition (306). Systolic and diastolic blood pressures were measured three times at 1-minute intervals with an automatic device (Dinamap Vital Signs Monitor, 1846; Critikon, Tampa, FL, USA) with the participant in a sitting position after 2 min of rest, and defined as the mean of the last two readings. Hypertension was classified as a mean systolic blood pressure of ≥ 140 mmHg, a mean diastolic blood pressure of ≥ 90 mmHg, or self-reported use of blood pressure-lowering drugs. Non-fasting blood samples were collected from an antecubital vein and analyzed at the Department of Clinical Chemistry, the University Hospital of North Norway (UNN), Tromsø, Norway. Hypercholesterolemia was classified as a total serum cholesterol level of ≥ 6.5 mmol L^{-1} or self-reported use of lipid-lowering drugs.

Information on smoking status, family history of MI, diabetes mellitus, physical activity and education level was collected from a self-administered questionnaire. Smoking status was measured by self-reported daily smoking (yes/no). Physical activity and education were assessed as ≥ 1 hour of moderate or hard physical activity per week (yes/no), and more than ten years of education (yes/no).

1.6 Exposure and outcome measurements

1.6.1 Carotid atherosclerosis

Ultrasound examination of the right carotid artery was performed for assessment of total plaque area (TPA) and carotid intima-media thickness (IMT). High-resolution B-mode and color/pulsed-wave Doppler ultrasonography of the right carotid artery were performed in the extensive screenings in Tromsø 4, Tromsø 5 and Tromsø 6 by experienced examiners

who had completed a two-month pre-study training protocol to ensure similar and standardized examination techniques and measurement procedures (305, 307, 308). 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 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. For each plaque, a still image was recorded and digitized using the Matrix Meteor II frame-grabber and Matrox Intellicam. Adobe Photoshop 7.0 was subsequently used to measure plaque areas by outlining the perimeter of each plaque with a cursor, and the plaque area was calculated as pixel values. For the resolution used in paper 1 of this thesis, a plaque area of 167 pixels corresponded to one mm^2. In participants with more than one plaque, TPA was calculated as the sum of all plaque areas. IMT was defined as the average of the mean IMT values of the near and far wall of the common carotid artery and far wall of the bifurcation. Novel plaque formation was defined as development of new plaques at the second ultrasound in vessels without plaques at the first ultrasound. Plaque progression was defined as an increase in TPA between the first and second ultrasound.

1.6.2 Myocardial infarction

All first-time events of MI were identified by linkage to the hospital discharge diagnosis registry at UNN, by searching for International Classification of Diseases (ICD)-9 Revision codes 410-414, and 430-438 in the period 1994–1998, and after that ICD-10 Revision codes I20-I25 and I60-I69. The unique national 11-digit identification number in Norway allowed linkage to national and local diagnosis registries. Linkage to the National Causes of Death Registry at Statistics Norway allowed the identification of fatal incident MI cases that occurred as out-of-hospital deaths. The death certificates were used to collect relevant information on the MI events from additional sources, such as autopsy reports and records from nursing homes, ambulance services, and general practitioners.

An independent endpoint committee, using modified WHO MONICA/MORGAM criteria for MI, validated all possible hospitalized and out-of-hospital MI events. MI was defined by one of the following sets of conditions: a) typical, atypical or inadequately described symptoms + a definite new infarction in ECG recordings, b) typical symptoms +

significantly higher myocardial enzyme and/or troponin levels, c) atypical or inadequately described symptoms + significantly higher myocardial enzyme and/or troponin levels + a probable new infarction in ECG recordings, and d) post-mortem evidence of recent MI or thrombosis (309).

1.6.3 Ischemic stroke

Ischemic stroke was defined according to the WHO definition when computed tomography or magnetic resonance imaging scans or autopsy had ruled out brain hemorrhage (310). An independent end-point committee 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 UNN with a broad search for the International Classification of Diseases (ICD), 9th Revision codes 430 to 438 in the period 1994 to 1998, and after ICD, 10th Revision codes I60 to I69. Linkage to the National Causes of Death Registry allowed the identification of fatal ischemic strokes that occurred as out-of-hospital deaths.

1.6.4 Venous thromboembolism

All incident VTE events during follow-up were identified by searching the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry of UNN. All hospital care and relevant diagnostic radiology in the Tromsø municipality are provided exclusively by this hospital. The relevant discharge codes were ICD-9 codes 325, 415.1, 451, 452, 453, 671.3, 671.4, and 671.9 for the period 1994–1998 and ICD-10 codes I26, I80, I81, I82, 167.2, O22.5, O87.1 and O87.3 for the period 1999–2012. Trained personnel, blinded for the baseline variables, reviewed all medical records for each VTE case. Possible cases of VTE were confirmed and registered as a validated VTE event when all 4 of the following conditions were satisfied: 1) objectively confirmed by diagnostic procedures including compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, pulmonary angiography, or autopsy; 2) the medical record indicated that a trained physician had diagnosed DVT or PE; 3) presence of signs and

symptoms consistent with DVT or PE; and 4) the patients underwent treatment with anticoagulants (heparin, warfarin, or direct oral anticoagulation), thrombolytic therapy, or vascular surgery, unless contraindications were specified. For patients derived from the autopsy registry, a VTE-event was recorded when the autopsy record indicated PE as the cause of death or as a significant condition contributing to death.

A VTE event was classified as either a DVT or PE. When these events occurred concurrently, the event was classified as a PE. VTE events were further classified as provoked or unprovoked according to the presence of provoking risk factors at the time of diagnosis. Provoking factors included recent surgery or trauma within the previous eight weeks, active cancer, immobilization (i.e., bed rest for > 3 days, or long-distance travel exceeding 4 hours within the 14 days prior to the event), or any other factor described by a physician in the medical record (e.g., intravascular catheter). If none of these factors were present at the time of diagnosis, the event was classified as unprovoked.

In paper 4 in this thesis, the five SNPs included in the genetic risk score proposed by de Haan *et al. (311)* were investigated. Genotyping of the SNPs rs6025 (F5, FVL), rs1799963 (prothrombin G20210A), rs8176719 (ABO, non-O blood type), and rs2036914 (F11) was done using the Sequenom platform and rs2066865 (fibrinogen gamma chain [FGG]) by the TaqMan platform. Sequenom uses single-base extension followed by mass spectrometry to measure the molecular mass of the extended primers. Samples were genotyped using the Sequenom iPlex Gold Assay according to the recommended protocol, using an initial input of 10-20 ng DNA and were analyzed using the MassARRAY Analyzer 4. We only used genotypes with a high-quality score of “A. Conservative” or “B. Moderate.” When multiple attempts were made to genotype an individual, one of the highest quality genotypes across all attempts was chosen for each SNP. For TaqMan, we used an initial input of 100 ng of DNA. Samples were genotyped using the Applied Biosystems 7900HT according to the recommended protocol, processed using SDS 2.4 (Thermo Fisher). Genotypes passing a quality value threshold of 95 were used. Participants were considered carriers of the prothrombotic risk gene if one or two risk alleles were present. We did not differentiate in hetero- and homozygote due to few homozygous study participants.

4. Main results

1.7 Paper 1: Repeated Measurements of Carotid Atherosclerosis and Future Risk of Venous Thromboembolism. The Tromsø Study

The relationship between atherosclerosis and VTE is controversial, and previous prospective cohort studies have failed to find any association. However, these studies were based on a single measurement of intima-media thickness (IMT) and total plaque area (TPA). Therefore, we aimed to investigate the association between carotid atherosclerosis and VTE using repeated measurements of IMT and TPA in participants recruited from the fourth (1994-1995), fifth (2001-2002) and sixth (2007-2008) surveys of the Tromsø study. Measurements of IMT and TPA and potential confounders were updated at each available survey for the 10 426 participants attended. Time-varying Cox-regression models were used to calculate hazard ratios (HR) of VTE across various levels of TPA and carotid IMT adjusted for age, sex, and BMI. During a median follow-up of 10.8 years, 368 participants developed an incident VTE event. Participants with increasing carotid IMT were on average older and had a less favorable cardiovascular risk profile. There was no association between TPA as a continuous variable and VTE (HR per standard deviation [SD] increase 0.99, 95% confidence interval [CI] 0.90-1.11), and we found no linear trend of increased risk of VTE across increasing tertiles of TPA. Carotid 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 0.5. Additional adjustment for total cholesterol, high-density lipoprotein cholesterol, smoking, diabetes mellitus and diastolic blood pressure had a negligible effect on the risk estimates. Further, plaque formation and plaque progression showed no increased risk of VTE with HR 1.00 (95% CI 0.98-1.02) and HR 0.96 (95% CI 0.84-1.11), respectively. These findings imply that the presence of atherosclerosis and plaque progression are not associated with increased risk of VTE, even in repeated measure analysis taking regression dilution into account.

1.8 Paper 2: Impact of incident myocardial infarction on the risk of venous thromboembolism. The Tromsø Study

Previous studies have demonstrated an association between MI and VTE. However, the association has not been investigated using a prospective, population-based study with adjustment for traditional cardiovascular risk factors and validation of exposure, outcome and potential confounding factors. In total, 29 506 participants were recruited from three surveys of the Tromsø Study. During a median follow-up of 15.7 years, 1898 participants experienced a first-time MI and 699 experienced an incident VTE. In participants without MI, 652 VTE events were identified with an incidence rate of 1.8 per 1000 person-years, whereas there were 47 VTE events in participants exposed to MI with an incidence rate of 6.7 per 1000 person-years. The proportion of PEs was higher among VTE events occurring after MI compared to VTE events appearing in the absence of MI (53% versus 41%). Overall, MI was associated with a 51% increased risk of total VTE (HR 1.51; 95% CI 1.08-2.10) and a 72% increased risk of PE (HR 1.72; 95% CI 1.07-2.75). The risk of isolated DVT was not significantly increased (HR 1.36; 95% CI 0.86-2.15). Regarding both VTE and PE events, the risk estimates were higher for provoked than unprovoked events. Incidence rates and HRs of VTE were high immediately after the incident MI and declined rapidly thereafter. The highest risk estimates for PE were observed during the first six months after the MI (HR 8.49; 95% CI 4.00-18.77), and the risk was no longer increased after the first year (HR 1.45; 95% CI 0.53–3.91). MI explained 6.2% of the PEs in the population (population attributable risk) and 78.5% of the PE risk in MI patients (attributable risk). Our findings indicate that MI is associated with a transient increased risk of VTE, independently of traditional cardiovascular risk factors. The risk estimates were particularly high for PE.

1.9 Paper 3: Ischemic Stroke and Risk of Venous Thromboembolism in the General Population: The Tromsø Study

Clinical data supports a relation between ischemic stroke and VTE, but the strength and time dependence of the association remains to be settled at the population level. The purpose of this study was to investigate the association between ischemic stroke and VTE in a prospective population-based cohort adjusting for cardiovascular risk factors. Using three surveys of the Tromsø study, 30 002 participants were recruited and followed through 2010. Information about cardiovascular risk factors (age, sex, BMI, blood pressure, blood lipids, diabetes, smoking, and education) was collected at baseline, and all incident events of ischemic stroke and VTE during follow-up were recorded. In a median follow-up time of 15.7 years, 1360 participants developed ischemic stroke, and 722 participants developed incident VTE. In total, 57 of the 722 VTEs occurred in patients with ischemic stroke. VTE events in stroke patients had a higher proportion of provoked events compared to VTE events in participants without stroke. The proportion of patients immobilized before the VTE event was substantially higher in those with stroke compared in those without (44% versus 16%). The cumulative incidence of VTE was 15% during the first three months in individuals with ischemic stroke, compared with 0.2% in the general population during the same period. The incidence curves for VTE remained substantially parallel in the period more than six months after the event. The risk of VTE was highest the first month, and from one to three months after the incident stroke (HR 19.7 [95% CI 10.1-38.5] and HR 10.6 [95% CI 5.0-22.5], respectively), but declined rapidly thereafter. The risk estimates were essential for DVT (HR 19.1; 95% CI, 7.8-38.5), and PE (HR 20.2; 95% CI, 7.4-55.1). 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. Our findings suggest that additional predisposing factors, such as immobilization, potentiate the transient VTE risk in patients with ischemic stroke.

1.10 Paper 4: Effect of prothrombotic genetic variants on the risk of venous thromboembolism in patients with ischemic stroke. The Tromsø Study

Prothrombotic genotypes may augment the VTE risk under conditions of high thrombosis risk related to stroke (e.g., hospitalization, immobilization, and infections). To investigate the effect of prothrombotic genotypes in patients with ischemic stroke on the risk of VTE, we performed a case-cohort study with 660 incident VTE and a randomly selected age-weighted subcohort consisting of 1803 participants recruited from the general population. All participants were genotyped for the five SNPs in the genetic risk score proposed by de Haan *et al. (311),* including *ABO* (rs8176719), *F5* (rs6025), *F2* (rs1799963), *FGG* (rs2066865) and *F11* (rs2036914). Cox regression models were used to calculate hazard ratios (HR) for incident VTE according to individual SNPs and categories of risk alleles (the 5-SNP score; 0-1, 2, 3-4 and ≥5) in participants with and without ischemic stroke. In total, 263 patients had an incident stroke, of whom 60 developed subsequent VTE. The proportion of provoked VTE was higher in patients with ischemic stroke (75%) than in those without stroke (48%). For all SNPs, the joint exposure of stroke and risk alleles was associated with increased risk of VTE compared with stroke-free study participants with zero risk alleles. The risk of VTE increased 24% (HR 1.24, 95% CI 1.05-1.46) per increase of genetic risk category in participants without stroke, and 54% (HR 1.54, 95% CI 1.34-1.75) among those with stroke. Stroke patients with ≥5 risk alleles had a 12-fold (HR 11.77, 95% CI 4.17-33.25) higher risk of VTE than study participants without stroke and 0-1 risk alleles. Estimation of the attributable proportion due to interaction revealed that 84% of the total VTE events in study participants with stroke and ≥5 risk alleles were due to the interaction between the two exposures. When the 5-SNP score was applied in patients with stroke, the risk estimates for VTE across categories of risk alleles in the 5-SNP score were higher for provoked than for unprovoked events. The risk of provoked events increased by almost 80% per increase of risk category (HR 1.76, 95% CI 1.41-2.06). These findings imply that prothrombotic genotypes increases the risk of VTE in stroke patients, and the risk increases with increasing number of risk alleles.

5. General discussion

1.11 Methodological considerations

1.11.1 Study design

The results in the present thesis are based on data from a population-based cohort study, the Tromsø Study. In paper I-III we used the cohort study design. The cohort study design is along with the case-control study design the most commonly used observational design in clinical medicine (312). Cohort studies follow one group exposed to a risk factor or intervention of interest and another non-exposed group to determine the occurrence of the outcome of interest. They are designed to examine multiple outcomes of a single exposure and are useful for estimating the absolute and relative risk of diseases. In a cohort with a large number of participants from the general population, like the Tromsø Study, there will be a high degree of generalization, increasing the external validity of the study (313-315). The temporal sequence of the exposure and outcome avoids the debate as to which comes first. Additionally, collecting information in advance of the censoring event minimize the possibility of recall bias (313-315). The epidemiological challenges in cohort studies include loss-to-follow-up, change in risk of disease during follow-up, bias and confounding (316-318). Further, the design is time-consuming and require a large study population, and may be inefficient to investigate outcomes with a low incidence (315). Even though our study was derived from a large cohort, the number of VTE events was low in certain subgroups. Consequently, a potential limitation in the thesis is low statistical power.

Case-control studies compare the proportion of individuals with disease (i.e., cases) with specific exposure to the proportion of controls with the same exposure. As the cases are intentionally chosen based on the desired outcome, the case-control design is more cost-efficient than the cohort design as a smaller sample size is sufficient to generate adequate information. Case-control studies are particularly useful to investigate rare outcomes. There are some limitations to the case-control study design. Selection bias may occur with improper selection of cases and control. This is mainly due to problems selecting the controls as they should be representative of the population at risk of becoming cases. Further, the exposures of controls should be measured with similar accuracy as the

exposures of the cases. These principles are often impossible to satisfy as controls often are selected either from the general population, without comparable assessment of exposure, or from hospitalized patients with other diseases that may be unrepresentative of the study population. Controls recruited from the general population may also generate recall bias as cases with diseases are more likely to remember particular events or exaggerate or minimize what they consider to be risk factors compared with healthy controls. In the present thesis exploring the association between CVD and VTE, the case-control design is not suitable to confirm the direction of association due to the undetermined temporal sequence of exposure and outcome (254, 315, 317, 319).

Randomized controlled trials (RCTs) were first introduced to clinical medicine evaluating the treatment of tuberculosis (320), and have been presumed to be the most reliable methods of determining the effects of treatment and causal relationships (301, 312, 321-324). In large RCTs, participants are randomized and distributed evenly between the control and intervention groups, reducing the risk of potential of known and unknown confounders. The randomization minimizes allocation and selection bias, and it is easy to compare the two groups directly (301, 312, 325). Despite the many strengths, there are essential limitations of RCTs. Lack of external validity and generalizability is the most frequent criticism of RCT, as most trials include a selective group of participants, often younger and healthier than the general population. RCT is also resource-intensive regarding both costs and time, and impractical for urgent situations and rare diseases. Compared to RCTs, the cohort and case-control studies are ethically safe and more accessible to conduct due to the non-experimental design (321, 324-326).

In Paper IV, we used a case-cohort design with study participants recruited from Tromsø 4, Tromsø 5, and Tromsø 6. The case-cohort design includes all individuals developing VTE in the full cohort plus a randomly sampled subcohort from the entire cohort independent of disease status. In our study, 660 VTE cases were included, and an age-weighted subcohort was randomly selected from the same population. The subcohort is meant to reflect the occurrence of the exposure in the original cohort population (319, 327, 328). Similar to cohort studies, the case-cohort study has a definite temporal sequence of exposure and outcome, reducing the risk of recall bias. As the cases and controls are sampled from the same population, and exposures are assessed with investigators blinded

to the case status, there is a low degree of differential misclassification. Compared with cohort studies, the case-cohort study design is favorable for studying rare exposures as the full covariate data is only required for cases and controls in the subcohort. With appropriate sampling and analysis, the HR estimates are similar in the case-cohort as in the full-cohort (319, 327, 328). We chose the case-cohort design to limit the costs and time required for genotyping. There are some limitations to be aware of regarding the case-cohort study design. The cases in a case-cohort are often overrepresented in the sample (327). However, as the control-cohort included a large sample of study participants, there are limited risk of overrepresentation of cases. Additionally, if many participants are censored, the subcohort may be limited and not representative of the full cohort (328). In our study, participants were only censored at VTE-events, migration, or death, which should limit the introduction of bias.

1.11.2 Causality

Causality is derived from the Latin word *causa* and means cause or reason. Causal factors are in medicine often called risk factors and are the producer of an effect, results or consequence (229). A risk factor may induce an effect either directly or indirectly, and it is postulated that if the risk factor is removed, the disease will no longer occur (229). Epidemiological studies investigate the association between exposures and outcomes. In observational studies, positive associations are theoretical measures suggesting that a given exposure may cause the outcome of interest. However, a correlation or association between two phenomena does not necessarily equal the presence of causality (229, 329).

To evaluate a possible causality between smoking and lung cancer, Sir Austin Bradford Hill defined nine criteria during a talk to the Section of Occupational Medicine of the Royal Society of Medicine in 1965 (330). The nine criteria were 1) strength of the association, 2) consistency (reproducibility of the findings in different places with different samples), 3) specificity (when a single putative cause produces a specific effect), 4) temporality, 5) biological gradient (greater exposure should generally lead to higher incidence of the effect), 6) plausibility (association agrees with currently accepted understanding of pathological processes), 7) coherence (association should be compatible

with existing theory and knowledge), 8) experiment and 9) analogy (existence of other cause-effect relationships analogous to the one under study) (331). These criteria were meant as a guideline of issues that should be addressed when evaluating causality of an observed association. Of the nine criteria, the temporality criterion is the only absolute (331). Thus, failure to satisfy some of these criteria does not disprove a causal association, and causal inference should not be based on these criteria alone (329, 332, 333).

In our cohort study, several of the given criteria are fulfilled regarding a causal association between CVD and VTE. First, our study design assures temporality as all participants with previous VTE were excluded, and those developing VTE during follow-up were censored at the time. Further, both the strength of the observed association and the consistency with previous studies indicate that there might exist causation. Additionally, there are several plausible mechanisms after MI and ischemic stroke that may increase the risk of VTE. However, biological causality may not be declared, as experimental evidence is not available. The possible mechanisms behind the increased risk of VTE in patients with CVD are further discussed in the section *discussion of the main results (page 62 and 64).*

Mendelian randomization is an epidemiological method to assess causality within observational studies. The method was first described in a study more than 30 years ago, and the use of Mendelian randomization has increased simultaneously with increasing genetic information (334). Using Mendelian randomization studies, it is possible to assess the contribution of a risk factor to a disease by investigating the association between the disease and a genetic variant influencing the risk factor. This is possible due to the random assorting of alleles at conception, creating nature's own RCT (334). Thus, confounding by other factors than those related to the genetic variant are eliminated. As the genetic makeup exists from conception, a temporal sequence must necessarily exist. Some Mendelian randomization studies are performed investigating the risk of VTE, including studies implying a causal relationship between high BMI (113), and taller height (335) and VTE. Several genotypes are known to increase the risk of arterial CVD, including some of the prothrombotic genotypes collected in the Tromsø Study (336). However, the genetic variant in Mendelian randomization studies should only affect the outcome through the effect on the exposure, and not directly affect the outcome. As the genotypes we used in paper IV may affect both CVD and VTE, they are not suitable to assess any causal association.

1.11.3 Generalizability

All epidemiological studies attempt to generalize the results in the study population to a defined reference population (i.e., internal validity) and other populations (i.e., external validity). Results from RCTs are applied for clinical care of large populations. However, as most RCTs have strict criteria for participants included in the trial, the external validation, or the generalizability, is often questioned (326). Cohort studies with proper inclusion and exclusion criteria may have higher generalizability, as participants from a broader specter of groups are included. Nevertheless, selection bias may limit the generalizability of population-based cohort studies. High participation rate and minimal loss to follow up are indications of possible high external validity (313).

The participation rate in the Tromsø Study is high compared with other prospective cohort studies, with a total overall attendance rate in Tromsø 4, Tromsø 5 and Tromsø 6 exceeding 70%. The largest survey, Tromsø 4, had an attendance rate of 77% (303). In two comparable cohort studies, the Norwegian HUNT 2-study and the Danish Diet, Cancer, and Health study, the participation rates were 70% and 35%, respectively (337, 338). All inhabitants aged 25 years or older living in Tromsø were invited in Tromsø 4, ensuring a broad specter of age groups. Further, the incidence and prevalence of CVD, cardiovascular risk factors, and VTE reported in our studies are comparable to similar populations (20, 24). We assume that our results have high generalizability to other Western populations.

In a cohort study, non-responders tend to have lower socioeconomic status and higher mortality then attendees (339). This is likely due to the increased health-interest, and high attendance rates, in groups with higher socioeconomic status. Consequently, an underestimation of the actual risk may occur, as the participants in a cohort study may be healthier than the general population (339, 340). Additionally, participation in most cohort studies require physical attendance at the study site, and this may cause selection bias due to lower attendance rate of severely ill or disable participants. In the Tromsø study, there is a relatively low attendance rate in the age groups under 40 years and over 80 years, and men have a lower attendance rate than women (303). This is important to be aware of, since it may affect the generalizability in these age groups. Nevertheless, the bias between survey attendees and non-attendees is mainly theoretical, and it is not likely that it has a substantial

effect on our results. As we mainly report relative risk estimates rather than absolute risks, this type of bias should not considerably affect our results.

In Paper IV, we used genotyping information for the five SNPs rs8176719 (non-O blood type) in *ABO*, rs6025 (factor V Leiden) in *F5*, rs1799963 (prothrombin G20210A) in *F2*, rs2066865 in *FGG*, and rs2036914 in *F11*. The distribution of SNPs may vary from other as there are large global variations in the human genome (341). However, the allele frequencies for the SNPs in our study population is coherent with other western reference populations, with 0.32 and 0.30 for ABO, 0.04 and 0.05 for FVL, 0.01 and 0.02 for prothrombin G20210A, 0.23 and 0.25 for FGG, and 0.41 and 0.52 for F11, in our and the western reference population respectively (190).

1.11.4 Confounding

In cohort studies, the baseline characteristics of the exposed and unexposed participants may differ. If these differences have independent effects on the outcome, they may affect the incidence of outcome (i.e., VTE) in the different groups separately from those related to the investigated exposure (i.e., atherosclerosis, MI and ischemic stroke). This effect is known as confounding. A confounder is defined in epidemiology as a factor related to both the exposure and the outcome, unevenly distributed among the compared exposure groups (318). Any factor that represents a step in the causal chain between exposure and disease should not be treated as a confounding factor but requires special treatment as an intermediate factor (318, 342). Confounding may strengthen or weaken a true association, and may lead both to type I and type II errors. In RCTs, all potential confounders are expected to be evenly distributed among the groups being compared and not affect the risk estimates (342). Cohort studies have no similar protection against confounding factors and are particularly vulnerable to residual confounding. Thus, all observed associations in a cohort study must be assessed for possible confounders. The comparison groups in a cohort should be as identical as possible only separated by the exposure variable of interest to reduce the risk of confounding (316, 342, 343).

A practical approach to reducing confounding is to include only similar study participants (344). For example, in a study with only women, sex cannot be a confounding

factor. However, such analyses will reduce the external validity of the study. An analytic strategy to keep a high degree of external validity and reduce confounding is stratification. The advantage of stratification is more similar subgroups than the entire diverse population, which is suitable to eliminate interaction between variables. An important limitation of stratification is the possible reduction of statistical power due to the reduced number of participants in each stratum (344). Another strategy to decrease confounding is using regression models (344). Regression models use data to estimate how confounders are related to the outcome and are used to limit the effect of confounding on the risk estimates (314). There are different types of regression analysis, including linear-, logistic- and Cox regression. With regression analysis, it is possible to simultaneously examine all exposure variables and estimate the true effect of every single exposure. The main advantage of regression compared with stratification is that data from all participants are used, and the statistical power in the study is not reduced (314).

In our studies, we used regression models to limit possible confounding. We used multivariable regression analysis where potential confounders were included as covariates. A large meta-analysis showed that high BMI and advancing age are associated with both CVD and venous thromboembolism (235). It is important to adjust for these factors as they may confound the association between CVD and VTE. Further, we adjusted for several cardiovascular risk factors despite that many of these are presumably not associated with VTE (235). As the presence of cardiovascular risk factors obviously was distinctly different between those with and without CVD, we chose to adjust for these factors to minimize the difference between the exposed and unexposed groups. Further, participants with cardiovascular risk factors are less healthy compared to the general population, which may indicate an increased rate of comorbidities confounding the risk estimates for VTE. In paper I-III we performed regression models only adjusted for age, sex and BMI and models adjusted for blood pressure, diabetes mellitus, cholesterol, smoking, physical activity, and education level in addition to age, sex and BMI. We observed only small differences in risk estimates between the models, underscoring that cardiovascular risk factors do not affect the risk of VTE.

The observed associations between MI, and ischemic stroke and VTE remained statistically significant after adjustments of potential confounding factors. This indicates that

shared risk factors alone cannot fully explain the observed relation between CVD and VTE. However, there may exist residual or unmeasured confounders that affect the risk estimate. Residual confounding factors could be unknown risk factors affecting both CVD and VTE. Unmeasured confounding is risk factors we know affect the outcome, but where we lack information about the variable. Possible unmeasured confounding factors in our study include genetic risk factors and the use of antithrombotic treatment and statins. Concerning unknown residual confounders, we assume that it is unlikely for them to have a substantial, independent effect on the outcome of interest. Thus, residual confounding should not generate considerable bias, despite a possible uneven distribution (342).

Statistical interaction describes a situation where two or more risk factors modify the effect of each other with regard to the occurrence of a given outcome (314). This phenomenon is also known as effect modification. Effect modification occurs when an exposure has a different effect among different subgroups (314). A risk factor only increases the risk of disease in female is an example of effect modification. When effect modification is present, it can be approached by stratifying the data on the effect modifying variable (314). In paper I-III, we tested statistical interactions by including cross-product terms in the proportional hazards models. No interactions were found between sex and the main exposures.

1.11.5 Information bias and misclassification

Bias is a systematic error in a study's design or procedures and may result in incorrect assessments of the association between an exposure and outcome (345). Bias may be introduced through the selection of the study participants (i.e. selection bias), measurements and classification of exposure or outcomes (i.e. information bias), and the presence of confounding factors (314). Selection bias occurs when the exposure or outcome status of an individual influence the probability of participating in the study. This is primarily a problem in case-control studies and rarely lead to erroneous associations in prospective studies (313, 317). However, as mentioned previously, participants in cohort studies are more likely to be healthier than the general population (339, 340).

Information bias occurs when study participants are placed in the wrong exposure- or outcome category. In prospective cohort studies, information bias is a more frequent problem than selection bias and is important to consider. Information bias may induce misclassification, which occurs when a positive outcome is classified as negative or vice versa, or when participants are placed in the wrong exposure group. There are two main types of misclassification, non-differential misclassification, and differential misclassification (318). Non-differential misclassification occurs when a variable is misclassified independently of the outcome or the exposure. In non-differential misclassification, all groups have the same error rate or probability of being misclassified. In differential misclassification, the variable misclassified is dependent on the outcome or the exposure. As a result, one of the groups is more often misclassified than the comparison group and this may introduce bias (314). In this thesis, participants are included before the exposure and the outcome assessment, and differential misclassification is unlikely.

Non-differential misclassification is more common in cohorts and may occur in this thesis due to incomplete medical records or questionnaires. For example, study participants may have difficulties to remember past exposure during interviews or when completing questionnaires. Physical examination may also lead to misclassification due to technical errors, errors from the examination or random errors. A normotensive study participant with stress-induced high blood pressure at examination will be misclassified as hypertensive. Self-administrated questionnaires increase the risk of misclassification as questions could be misunderstood or skipped. An example is diabetes mellitus, which is defined based on self-reporting in the Tromsø Study. This may explain why the prevalence of diabetes mellitus type 2 is markedly reduced in the Tromsø Study compared with the general population (346). The reduced prevalence may generate an underestimation of the true impact of diabetes on diseases. However, misclassification in a cohort study is usually non-differential. An equal distribution of misclassification in participants with and without the exposure will not largely affect the risk estimate in the study. Self-administered questionnaires are also less expensive and time-consuming than most other methods, and self-reported questionnaires are reported to have high validity and accuracy (347). For collecting data on sensitive topics (e.g., mental health, sexuality, and alcohol use), a self-administered questionnaire is showed to be more accurate compared to interviewer-administered

questionnaires (348). The repeated measurements of several study participants allowed us to update information concerning cardiovascular risk factors during follow up and correct any misclassification in the previous survey.

To ensure the correctness of the classification of exposure and outcome variables, we validated the known effect of specific exposure variables on outcomes. In our data material, traditional cardiovascular risk factors increased the risk of both MI and stroke as expected. An independent end-point committee validated both exposures (i.e., atherosclerosis, myocardial infarction and ischemic stroke) and outcome (i.e., VTE) in all the papers in the thesis. For VTE, all possible cases were confirmed as a validated VTE event when four criteria were satisfied. The criteria included radiological evidence, symptoms, clinical diagnosis and treatment of DVT or PE. WHO criteria were used to validated stroke and MI events, while trained personal assessed presence and degree of atherosclerotic plaque in the carotid artery. We therefore assume that there is limited misclassification concerning the most important variables, and information bias should not profoundly influence our risk estimates.

1.11.6 Regression dilution bias and modifiable risk factors

Regression dilution bias is a phenomenon that results in an underestimation of the true association between exposure and outcome due to measurement errors, temporary fluctuations, and changes in variables over time (349-352). In most cohort studies, information about risk factors is collected at inclusion. As the majority of cardiovascular risk factors are modifiable, changes during follow-up may influence the risk estimates of CVD and VTE (352). When the risk profile of study participants changes during follow-up, the possibility of type I errors (an observed association when there is none) decrease, whereas the risk of type II errors (no observed association when there is a true association) increases (351).

The median follow-up time in the four papers in the thesis varied between 10.8 and 15.7 years. Changes in baseline variables during the long follow-up time may lead to an underestimation of the true association. In paper I, the awareness of possible regression dilution bias is especially important. As the exposure (i.e., atherosclerosis) may develop over

time, a long follow-up with several years between the baseline measurement and the outcome (i.e., VTE) could introduce regression dilution bias. Thus, a small effect of atherosclerosis on the risk of VTE could be masked in traditional cohort studies with single measurements and long-term follow-up (254). Using time-varying analyses with repeated measures of carotid atherosclerosis, we reduced the possibility of regression dilution bias. Our findings suggest that the discrepancy in case-control and cohort studies regarding atherosclerosis and VTE are most likely not explained by formation or progression of atherosclerosis during the long follow-up (230, 250, 252, 253, 353). Other possible explanations for the differences between case-control and cohort studies include selection bias and reverse causation as the exposure is measured after the outcome in case-control studies (354).

In paper II and III, the exposure variable (i.e., MI and ischemic stroke, respectively) was included as a time-dependent covariate and updated during follow-up. Further, as the exposure of CVD does not change during the follow-up after the incident event, the risk of regression dilution bias is limited. However, most other covariates of interest, including BMI, smoking, blood pressure, and cholesterol, are modifiable. Misclassification of confounding factors may over- or underestimate the association between exposures and outcome. However, as several study participants participated in more than one of the surveys in the Tromsø Study, information on cardiovascular risk factors was obtained at different time points during the follow-up. In the time-varying analysis, each participant contributed with observation periods from the time of inclusion 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, whichever came first. Information on possible confounding risk factors was updated at each survey in those who participated in several surveys to avoid misclassification. We performed time-varying Cox proportional hazards regression analysis to limit the influence of changes in confounders during follow-up. Thus, this should not affect our risk estimates.

We did not perform time-varying analysis in paper IV, and regression dilution may influence confounding factors affecting the risk estimates of VTE in patients with ischemic stroke and prothrombotic genotypes. However, in a recent paper from Småbrekke *et al*. (352), risk estimates of MI and VTE based on a single measurement of cardiovascular risk factors in time-fixed analysis corresponded well with risk estimates based on repeated

measurements of the same risk factors in time-varying analyses (352). Of traditional cardiovascular risk factors, only BMI and age was associated with VTE in both time-fixed and time-varying analyses, suggesting that underestimation of risks due to regression dilution bias may not explain the lack of association between cardiovascular risk factors and VTE as reported in most prospective cohorts (235, 352). Thus, regarding traditional cardiovascular risk factors, risk estimates based on a single measurement are generally reliable in cohort studies with long follow-up (352).

1.11.7 Missing data

In nearly all epidemiological studies, there are some missing data observations (355-357). Missing values may occur due to malfunction of equipment, inadequate responding to questionnaires, loss of laboratory samples, and loss to follow-up before the end of the study. There are three main types of missing data; missing completely at random (MCAR), missing at random (MAR) and not missing at random (NMAR) (355-357). MCAR rarely exist, while MAR occurs when the missing data is related to a particular variable, but it is not related to the value of the variable. An example of MAR is a study participant who accidentally forgets to answer a question in a questionnaire. NMAR is data missing for a specific reason and could occur if a particular group of participants does not answer a specific question in a questionnaire. For example, older study participants may not be aware of family history of disease and, therefore, not answer questions concerning hereditary (355, 356, 358).

A large amount of missing data is a major threat to the integrity of a study as the available data could be biased. There are several methods available to handle missing data and reduce the risk of bias (355, 357). First, both variables with a large number of missing values and individuals with incomplete data can be omitted. Omitting individuals from the study who do not have complete data is termed list-wise deletion or complete case analysis (355). When only a few observations are missing, the risk of introducing bias is minor when excluding all participants with missing values for the variables of interest. However, exclusion of participants may both reduce the statistical power and introduce selection bias, unless the data are missing completely at random. Omitting whole variables may introduce confounding, and valuable variables could be removed from the statistical analyses. Another option to reduce missing data is pair-wise deletion or available case analysis (357). In pair-wise deletion, only the variable with missing value is omitted from the analysis, while other

non-missing variables in the same study participants are included in the analysis (357). Some disadvantages of using pair-wise deletion exist. As for list-wise deletion, the results are unbiased only if the data are missing at random. Additionally, pair-wise deletion may lead to mathematical problems in computing estimates of some parameters (355, 357). Imputation is a statistical method used to replace missing values by estimating the most probable values (355). Simple imputation is based on the assumption that the missing values are MAR or MCAR, and there is not given that the estimate is the correct value of the missing variable (355-357).

In our study population, we have few missing values in the relevant variables. The total amount of missing data across all variables is only 1-2% in the Tromsø Study, (303, 304). The prospective study design, collecting of information at baseline, and the thoughtful validation of events are important for reducing the amount of missing in our cohort. In the present thesis, missing data regarding exposure (i.e., carotid atherosclerosis, MI, ischemic stroke, and genetic variables) and outcome (i.e., VTE) were handled by excluding all participants with missing variables (i.e., list-wise deletion). Due to the small number of missing and a large number of participants, we assume that the statistical power was not largely reduced by using list-wise deletion and censoring participants with missing values. Still, removing study participants may introduce selection bias if the characteristics of those with missing values differ from the rest of the study population (355). In our study, most missing is likely due to MAR, and the number of missing values are probably similar between exposed and unexposed participants. Besides, the municipality of Tromsø is served by a single hospital, minimizing the chance of loss to follow-up and missing data regarding the exposing events. For missing values of possible confounding factors used in the multivariable Cox regression, we used pair-wise deletion. By using pair-wise deletion, the power in our analysis was not affected by removing participants with missing values of confounding factors.

1.12 Discussion of main results

1.12.1 Atherosclerosis and risk of venous thromboembolism

In accordance with previous cohort studies (252, 253, 353), we found no association between atherosclerosis and VTE in paper 1. In the ARIC study, 13 081 participants aged 45-65 years underwent carotid ultrasonography to measure IMT and TPA, and no association was found during a mean follow-up of 12.5 years (253). In the Cardiovascular Health Study, an inverse association was found when investigating more than 4100 participants over 65 years over a follow-up period of 12 years. Atherosclerosis was surprisingly found to protect against unprovoked VTE (RR 0.60, CI, 0-39-0.91) (252). A previous study from the Tromsø Study, including more than 6200 participants with a follow-up time of 15 years, found that atherosclerosis was associated with MI, but not VTE (353). In contrast, three different case-control studies have demonstrated that atherosclerosis is more prevalent in patients with VTE than age- and sex-matched control, suggesting that atherosclerosis could be a shared risk factor for CVD and VTE (230, 250, 251).

Several factors could explain the discrepancies between cohort and case-control studies. First, to establish causation, temporality between the exposure and the outcome is necessary (330). Due to the undetermined temporal sequence between exposure and outcome, case-control studies are not designed to reveal the direction of an association. Further, two of the three case-control studies selected controls among hospitalized patients without VTE or atherosclerosis (230, 250), and recruitment of controls that are not representative of the source population may result in overestimation of the real effect.

The previous cohort studies were based on a single measurement of TPA, and carotid IMT obtained at the beginning of long follow-up periods. Potentially, carotid ultrasonography was performed several years prior to VTE events, which could mask a small effect of atherosclerosis on VTE-risk due to regression dilution bias (254, 255). In Paper I of this thesis, the risk status of the participants was updated during follow-up using time-varying analysis with repeated measurements of TPA and IMT. This allowed us to obtain a better estimation of an individual's atherosclerotic status closer to the VTE event. Still, we did not reveal any association between carotid atherosclerosis and VTE, and neither plaque formation nor plaque progression between two different surveys was associated with increased risk of VTE.

Our study supports previous findings that atherosclerosis is unlikely to be an intermediate for the association between CVD and VTE. However, it has been suggested that cardiovascular risk factors may confound the association by increasing the risk not only for the formation of atherosclerosis but also for VTE (254). Previous studies have thoroughly investigated the associations between known cardiovascular risk factors and risk of VTE. Cohort studies using cause-specific analyses have revealed age and obesity as shared risk factors for CVD and VTE, while other traditional cardiovascular risk factors are not likely to increase the risk of VTE (235, 244, 359). According to previous studies, our findings indicate that shared cardiovascular risk factors do not affect the observed association between atherosclerosis, CVD, and VTE. Adjustments for traditional cardiovascular risk factors would substantially attenuate the association if the risk factors were actual confounders. We found that adjusting for cardiovascular risk factors had a negligible effect on the risk estimates. As many cardiovascular risk factors are modifiable, the risk profile to the participants may change during follow-up, increasing the risk of regression dilution bias and an underestimation of the associations. In paper I to III, we performed repeated measurements of the cardiovascular risk factors during follow-up. Using time-varying analysis with updated information, we could more appropriately explore the real effect of cardiovascular risk factors on VTE during follow-up. Further, as the appearance of risk factors most commonly increases with time, shared risk factors are expected to induce a permanent or progressive risk of VTE. Conversely, we observed a transient VTE risk after MI and stroke.

1.12.2 Myocardial infarction and risk of venous thromboembolism

In paper II, we found that MI was associated with an increased risk of VTE in the general population. The risk was particularly increased for developing PE and provoked VTE-events. Our findings are in line with previous studies (253, 256, 258, 260, 261). In the two largest studies, Sørensen et al. found that the risk of VTE was higher in the first months immediately after an MI compared to the VTE risk in the general population (260, 261). As in our study, the authors demonstrated a higher risk of PE than DVT after hospitalization for MI. However, the exposure, outcome, and provoking factors for VTE were all based on information from hospital registries, and the information was not validated (260, 261, 360). Thus, results from these registry-based studies should be interpreted with caution.

Use of antiplatelet treatment and statins are standard to prevent new MI-events, incident strokes and death (361). In our study, treatment with antiplatelet agents and statins in patients with MI could affect the association between MI and VTE. A recent study, investigating antiplatelet therapy of ischemic events in stable patients with symptomatic atherosclerosis, found that more intensive antiplatelet therapy reduced the risk of VTE (362). The findings support previous data, showing that aspirin and statins reduce the risk of both incident (363, 364) and recurrent (365, 366) VTE events. The effect on VTE by statins is probably mediated by decreasing levels of FVIII, as showed in a recent RCT (367). A systematic review and meta-analysis including 13 cohort studies and 23 RCTs of statins versus placebo or no treatment suggested a beneficial effect of statin use on VTE, with up to 25% reduced risk (364). Unfortunately, we do not have information regarding antiplatelet agents and statins in our study, and an underestimation of the true impact of MI on the risk of VTE may exist.

The mechanisms causing the observed association between MI and VTE are not established (229), but as discussed, shared risk factors are unlikely to play an essential role. One possible mechanism could be a transient factor related to the MI, increasing the risk of VTE (229). We found the risk of VTE to be highest the initial six months following an incident MI and declined rapidly thereafter. Similarly, the association between MI and VTE has been reported to diminish (261) or disappear (260) after more than three months in previous studies. In a study of more than 2500 postmenopausal women with coronary heart disease, the risk of VTE increased particularly the first three months after an MI diagnosis (368). The transient increase in VTE risk after MI points towards mechanisms related to the MI-event itself. Both hospitalization and temporal immobilization are common after MI and are strong predisposing factors for VTE (132). Previous studies have suggested that hospitalization after MI could partly explain the observed association between MI and VTE (260-262). The attenuation of VTE risk if the hospitalization for MI was within three months before the index admission, but not during the same hospitalization as for VTE, supports the substantial impact of hospitalization (261). This finding indicates that MI is indirectly associated with VTE through hospitalization. Further, in a study of more than 1300 patients with incident VTE events, MI was no longer associated with VTE after adjusting for hospitalization for surgery or acute medical illness, and nursing home confinement (262). Contrary to our

findings, the risk in the large Danish registry-based studies was similar for provoked and unprovoked events (260). This could be caused by limited information regarding immobilization prior to the VTE event, which may introduce misclassification of the event as unprovoked (260). The transient risk of provoked VTE in our study suggest that indirect causal factors, such as hospitalization and subsequent immobilization as well as coronary artery bypass surgery or endovascular procedures after MI, may contribute substantially to the observed association between MI and VTE.

A direct causal relationship between MI and PE may contribute to the increased VTE risk in MI patients, and is biological plausible. First, we found a higher risk of PE than DVT after MI. DVT can be identified in only 50% of patients with PE (369), which supports the concept that pulmonary thrombi may form de novo in the lungs or originate from other sources of emboli, such as in the right ventricle after MI (97). The formation of de novo thrombi in the lungs may occur secondary to left ventricular dysfunction. Abnormal flow due to low cardiac output, dilated cardiac chambers, and poor contractility occurs in patients with reduced left ventricular ejection fraction, and may cause stasis in the pulmonary circulation due to backward failure (171). Second, atrial fibrillation is a frequent complication after MI with an estimated prevalence of 5-20%, with the highest risk the first two months after the incident event (370, 371). Our group observed that atrial fibrillation is associated with an increase in the risk of VTE, particularly for PE. Of all the PE events in the general population, one study from the Tromsø Study suggests that 15-20% could be attributed to atrial fibrillation (97). This supports the theory that isolated PE in atrial fibrillation patients may originate from right-side intracardiac thrombi, which have been identified as the only source of emboli in 4% of all patients with PE (372). Third, the other components in Virchow's triad may be responsible for the observed association in addition to stasis due to left ventricular dysfunction and atrial fibrillation. Both injury of the vascular endothelium (171, 373), and activation of the coagulation system during the acute phase of MI (171, 374, 375) may increase the risk of PE. Patients with both unstable angina and acute MI exhibit increased coagulation activity in the acute phase, which persists long after clinical stabilization (374, 375).

Considering the transient nature of VTE risk and the particularly high risk of PE observed in our study, it is likely to assume that direct causal mechanism(s) secondary to

local disturbances in the cardiopulmonary circulation or electro-mechanical activity (e.g., atrial fibrillation) may convey parts of the VTE risk after MI. However, the high risk of PE rather than DVT after MI may also partly be explained by surveillance bias, as patients with previous MI are more likely to undergo further examinations for chest pain.

1.12.3 Ischemic stroke and risk of venous thromboembolism

In the third paper of this thesis, we found that patients with ischemic stroke had a distinct increased risk of VTE compared with the general population. The incidence rate and relative risk were especially high during the first three months after the stroke diagnosis and declined rapidly thereafter. Moreover, the analysis displayed a higher risk of provoked than unprovoked events. Our findings are consistent with data from clinical trials of stroke patients, showing a high risk of VTE after stroke (273-275, 284, 376). Most data regarding ischemic stroke and risk of VTE are from randomized trials, and limited data exist concerning the association in the general population. Our findings are similar to the findings in two observational studies using a medical database including approximately 20% of the total Danish population (260) and the Danish National Patient Registry (360). In a sizeable case-control by Sørensen et al. revealed that patients with a history of stroke had a 4.4-fold increased risk of VTE the first three months after the incident stroke (260). More recently, Corraini et al. found a 5-fold increase in the VTE rates the first three-months after the stroke, which remained increased compared to the general population during the follow-up period (360). However, both studies are registry-based studies where exposure, outcome, and provoking factors for VTE were based on non-validated information from hospital registries and the Danish National Patients Registry (260, 360).

The explanations for the observed association between ischemic stroke and future risk of VTE are yet unknown but may include shared risk factors, indirect risk factors, or a direct relationship (229). The transient and short-term risk of VTE after ischemic stroke indicates that mechanism(s) or conditions related to the ischemic stroke itself partly explain the association. Hospitalization accompanied by periods of immobilization due to bed-rest or neurological deficits of affected limbs are frequent in stroke patients (272). The predilection for VTE in the paralyzed leg is probably explained by a combination of loss of the calf muscle pump and repeated minor trauma (267). Indeed, measures of stroke severity have been

shown to be strongly associated with the risk of VTE (273). Presumably, these patients are more susceptible to thrombus formation secondary to venous stasis in the affected leg (229, 286). Medical complications, including infections, frequently occur among hospitalized stroke patients (282, 377-379). The risk of DVT and PE are more than two-fold increased the two first weeks after infection of either pneumonia or urinary tract infections (162, 164). Infections after stroke may either contribute to the increased VTE risk directly due to inflammation or via prolongation of the hospital stay, or both (132). The combination of immobilization and infection have a synergetic effect on the risk of VTE (160). In our study, stratified analyses displayed a higher risk of provoked compared with unprovoked VTE, with a particular preponderance of immobilization as a predisposing factor for VTE among stroke patients. 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 (291). However, Corraini et al. showed that the risk of VTE within three-months after stroke remained slightly elevated even in the absence of prolonged immobilization (360).

Direct causal mechanisms may explain some of the observed association. Studies have shown an activation of the coagulation system during the acute phase of ischemic stroke. The coagulation activation could also be secondary to medical conditions occurring after the stroke (380-383). Additionally, the sudden tissue damage after ischemic stroke may induce a temporary inflammation, which may induce systemic hypercoagulability (384). Thus, our findings suggest that transient indirect risk factors occurring in relation to ischemic stroke, possibly combined with enhanced activity in the coagulation system, are important contributors to the transient risk of VTE after ischemic stroke.

Our risk estimates are probably an underestimation of the actual risk of VTE after stroke as current guidelines recommend initiation of anticoagulation within 48 hours after ischemic stroke with duration of treatment throughout the hospital stay or until the patient regains mobility (385). Unfortunately, we do not have information on the use of prophylactic anticoagulant treatment during the study period, but the consensus at UNN and Norwegian National Guidelines (386) corresponds well with international practice (385). 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 per 1000 patients for PE during the first

month after the ischemic stroke compared to individuals in the general population. Corraini et al. found that 15-30% of VTE events could potentially be avoided with successful VTE prophylaxis in stroke patients with moderate or high comorbidity rate (360). Although some of the preventive effects may already be incorporated in our results, clinical trials imply that preventive treatment with low-molecular-weight heparin (LMWH) or unfractionated heparin has the potential to reduce the incidence of symptomatic DVT by 70% and the incidence of fatal and nonfatal PE by 30% (385). In subanalyses of the randomized controlled EXCLAIM trial, involving almost 400 patients with acute ischemic stroke, extended-duration prophylaxis with LMWH for 35-40 days was associated with a reduction in the incidence of VTE. However, an increase in major bleeding was reported (387). Our results support that VTE prophylaxis in stroke patients may be inadequate. Future studies are warranted to identify clinical triggers mediating the effect of stroke on VTE risk to improve the identification of high-risk patients.

1.12.4 Effect of prothrombotic genetic variants on the risk of venous thromboembolism in patients with ischemic stroke

In paper IV, we found a synergistic effect of ischemic stroke and prothrombotic genotypes on the risk of VTE. The combined exposure to ischemic stroke and each of the individual SNPs (i.e., factor V Leiden, prothrombin G20210A, or variations in *FGG* or *F11)* resulted in an effect on VTE risk that exceeded the sum of the separate effects. When a genetic risk score was applied (the 5-SNP score (311)), the number of prothrombotic risk alleles displayed a dose-response relationship with VTE risk in both participants with and without ischemic stroke. The dose-response was particularly pronounced in stroke patients. Stroke patients with ≥5 risk alleles were almost 12 times more likely to develop an incident VTE compared with stroke-free individuals with one or no risk allele. By assessing the interaction between ischemic stroke and the high-risk category of the genetic score, we found that more than 80% of the VTE events appeared to be attributable to the interaction between those two risk factors.

Several mechanisms could explain the higher risk of VTE after stroke observed in patients with prothrombotic genotypes. As the risk of both stroke and VTE are reported to increase with the presence of prothrombotic genotypes (336, 388-391), shared genetic risk

factors may confound the estimates. A potential association between prothrombotic genotypes and severity of the stroke may lead to more pronounced or prolonged immobilization after acute stroke in these patients. However, whether the prothrombotic genotypes investigated in this study influence the outcome of ischemic stroke is yet unclear (392-394). The activation of the coagulation system in the inflammatory acute-phase after the ischemic stroke (380, 381) may be enhanced by the hypercoagulable state associated with prothrombotic genotypes.

Our risk estimates correspond well with the findings in paper III, as there consistently was a higher risk of provoked VTE events in participants jointly exposed to stroke and prothrombotic genotypes. The high risk of provoked VTE events indicates that stroke-related factors are the main contributor to the development of thrombotic events. This is consistent with previous findings where stroke severity, infections, immobilization, and hospitalization are suggested as potential reasons for the increased VTE risk after stroke (260, 273, 293, 360). Both prolonged immobilization (395, 396) and pneumonia (162) are found to increase the risk of VTE in carriers of prothrombotic genotypes that exceeds the sum of their separate effects. To the best of our knowledge, no other study has explored the joint effect of ischemic stroke and prothrombotic genotypes on the VTE risk.

The international guidelines for VTE prevention are mainly based on the CLOTS trial, in which no information regarding the prevalence of prothrombotic genotypes in the study population was provided (376). Despite the recommendation of anticoagulant treatment with LMWH in patients with acute ischemic stroke and restricted mobility, the high incidence of VTE observed after stroke indicates that VTE prophylaxis in stroke patients is inadequate (278). Indeed, data from clinical practice have shown that less than 50% of ischemic stroke patients at risk of VTE receive any form of thromboprophylaxis (278, 397, 398). Additionally, only 6% receives prophylaxis in the outpatient settings (278). This may be due to poor compliance in following guidelines, or uncertainty on how to assess patients at increased risk of developing VTE. Several risk assessment models (RAMs) exist to assess patients with acute medical conditions at increased risk of VTE, including the Padua Prediction Score (399), the Geneva Risk Score (400) and the IMPROVE-RAM (401), which are all prospectively designed and externally validated (402). In all three RAMs, known thrombophilic conditions, and ischemic stroke (399, 400) or paralysis of the lower extremity (401) is integrated to assess

the risk of VTE in patients with acute medical diseases. However, the existing RAMs do not differentiate between the numbers of risk alleles (399-401).

Despite that stroke have been established as a strong independent risk factor of VTE in previous studies (260), we found that the risk of VTE in stroke patients with one or zero prothrombotic risk allele was not increased as compared with participants without stroke. Our findings suggest that genetic risk factors play an important role in the development of VTE after stroke.

6. Conclusions

- We found that the formation and progression of carotid atherosclerosis, as assessed by TPA and IMT, was not associated with future risk of VTE in time-varying analyses. Our findings support previous studies reporting that atherosclerosis is not an intermediate for the association between arterial CVD and VTE.

- MI was associated with an increased risk of VTE after adjusting for potential confounding factors. The risk was particularly high for PE. The risk of VTE was limited to the first months after the MI diagnosis. The transient risk of VTE indicates that indirect or direct mechanisms related to the MI event itself are primarily responsible for the observed association.

- Ischemic stroke was associated with a transient increased risk of VTE independent of traditional cardiovascular risk factors. Our results displayed a higher risk of provoked than unprovoked events. This suggests 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.

- Prothrombotic genotypes increased the risk of VTE in stroke patients, and the risk increased with increasing number of risk alleles. The risk estimates were highest for provoked VTE events. Our findings suggest that genetic risk factors play an important role in the development of VTE after stroke.

7. Final remarks and future perspectives

Based on previous findings in cohort studies and the findings reported in this thesis, the suggested association between atherosclerosis and VTE do not appear to exist. Nevertheless, we found that symptomatic CVD (i.e., MI and ischemic stroke) induce a transient increased risk of VTE. The transient risk is probably caused by temporary risk factors related to the MI or the stroke itself. Current guidelines recommend thromboprophylaxis during the period of immobilization or hospitalization (141, 385). However, the risk of VTE extends beyond hospital discharge (134, 360, 403).

The high incidence of VTE after MI and ischemic stroke implies that extended anticoagulation may be necessary for a selection of high-risk patients based on comorbidities, risk factors for VTE and risk of bleeding. Several trials have shown that extended-duration prophylaxis reduces the risk of VTE compared with standard-duration treatment (142, 143, 145). However, current knowledge on risk factors and triggers of VTE in MI and stroke patients, particularly in the first months after the event, is scarce. To achieve a reduction in the risk of VTE without increasing the risk of bleeding, further studies are warranted to identify patients at high risk of VTE. Although there exist RAMs for VTE in patients with acute medical conditions, these are of limited clinical use, mainly due to the diversity of patients hospitalized for acute medical illness (136, 402). In existing RAMs, patients with ischemic stroke and known thrombophilic conditions are automatically placed in the high-risk category (399, 400). Thus, the RAMs are not suitable to differentiate between high- and low-risk patients among stroke patients with prothrombotic genotypes. Development of new RAMs for VTE evaluating only patients with ischemic stroke or MI may better identify patients with a favorable benefit-to-harm ratio for thromboprophylaxis (136, 402).

Our finding of a dose-dependent increased risk of VTE with increasing number of risk alleles suggests that genetic risk factors play an important role in the development of VTE after stroke. This may imply that the number of prothrombotic risk alleles could be considered when assessing the VTE risk in patients with ischemic stroke. A RAM for VTE based on clinical risk factors, biomarkers, and genetic risk factors would allow the implementation of more efficient thromboprophylaxis, which may reduce the incidence of VTE after stroke.

8. References

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Paper I-IV

Paper I

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ORIGINAL ARTICLE

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

B. SMÅBREKKE,* L. B. RINDE,* E. M. HALD,*† I. NJØLSTAD,*‡ E. B. MATHIESEN,*§¶ S. H. JOHNSEN,§¶ J.-B. HANSEN,*† S. K. BRÆKKAN*† and W. M. LIJFERING**

**K. G. Jebsen Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, UiT The Arctic University of Norway; †Division of Internal Medicine, University Hospital of North Norway; ‡Epidemiology of Chronic Diseases Research Group, Department of Community Medicine, UiT The Arctic University of Norway; §Brain and Circulation Research Group, Department of Clinical Medicine, UiT The Arctic University of Norway; ¶Department of Neurology, University Hospital of North Norway; and **Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands*

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Essentials

- The relationship between atherosclerosis and venous thromboembolism (VTE) is controversial.
- In total, 10 426 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.

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 by 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, 10 426 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 (HRs) 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 the risk of VTE in the time-varying model, and increasing IMT was not associated with an increased risk of VTE (HR 0.96, 95% confidence interval [CI] 0.86–1.07). Neither plaque formation nor plaque progression was associated with the risk of VTE (respectively: HR 1.00, 95% CI 0.98–1.02; and HR 0.96, 95% CI 0.84–1.11). *Conclusion:* Carotid IMT and TPA were not associated with an increased risk of VTE in time-varying analyses. Furthermore, there was no association between plaque initiation or plaque progression and subsequent VTE.

Keywords: atherosclerosis; cohort studies; repeated measurements; risk factors; venous thromboembolism.

Correspondence: Birgit Småbrekke, K. G. Jebsen Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, UiT The Arctic University of Norway, N-9037, Norway
Tel.: +47 9869 3789
E-mail: birgit.smabrekke@uit.no

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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: (i) stasis of the blood; (ii) hypercoagulability; and (iii) vessel wall injury [2]. The vascular component of Virchow's triad has been much less studied in the etiology of VTE than in that of arterial cardiovascular disease, for which 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 according to total plaque area (TPA), was twice as prevalent in patients with unprovoked venous thrombosis as in age-matched 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 do not enable interpretations on causality, owing to the undetermined temporal sequence between exposure and outcome. Furthermore, the association between atherosclerosis and VTE might be explained by the 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 > 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 by 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 1994–1995, 2001–2002, and 2007–2008, 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 ultrasonography 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 for 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 for the ultrasound examination, but with missing information on the measures of carotid atherosclerosis, were excluded. In total, 10 426 participants attended for ultrasound examination of the right carotid artery in Tromsø 4, 5, and/or 6 (Fig. 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 sampling, and self-administered questionnaires, and the collection was 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 the 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 IMT and TPA. A thorough description of the ultrasound examination has been

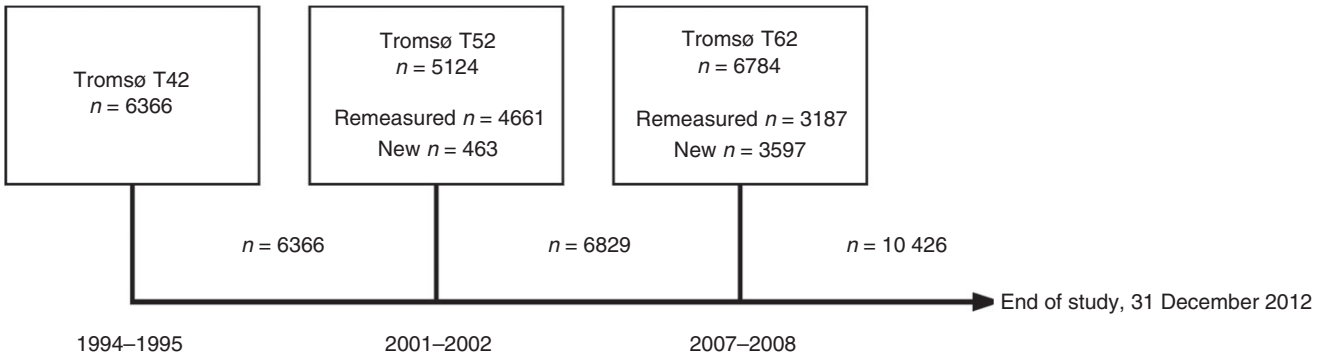

Fig. 1. Study population. The study population was recruited from the second visits at the fourth, fifth and sixth surveys of the Tromsø Study, conducted in 1994–1995, 2001–2002, and 2007–2008, respectively.

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 [Mountain View, CA, USA] equipped with a 7.5-MHz linear-array transducer in Tromsø 4 and 5; and a GE Vivid 7 [GE Vingmed Ultrasound, Horten, Norway] 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% as compared with the adjacent IMT. Still images were reported for each plaque, and digitized with 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. 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 walls of the CCA and the far wall of the bifurcation. To minimize variability in IMT during the cardiac cycle, image capture was standardized by recording images at the top of the R-wave in an electrocardiogram (ECG) signal. Plaque initiation was defined as the development of new plaques at follow-up in vessels without plaques at the previous examination, and plaque progression was defined as the difference in TPA between 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 to be adjudicated when the presence of clinical signs and symptoms of deep vein thrombosis (DVT) or pulmonary embolism was combined with objective confirmation tests (by compression ultrasonography, venography, spiral computed tomography, perfusion–ventilation scan, pulmonary angiography, and 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 the 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 the cause of death or as 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 a 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. IMT and TPA) 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 31 December 2012. Atherosclerosis measurements and other risk factors were updated at every survey, when available, and used as time-varying covariates. Of the 10 426 participants included in the study, 5154 participants attended two or three surveys, which resulted in a total number of 18 154 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 the time-scale, with the participants' age at study enrolment being defined as entry time, and age at the censoring event being defined as exit time. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated, and all analyses were adjusted for age (as time-scale), sex, and BMI. The proportional hazards assumption was confirmed with 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 that they did not attend. This analysis was performed to ensure that the carry-on of measurements in participants who attended only one survey did not dilute the effect in the original analyses. Statin use may potentially confound the association between atherosclerosis and VTE. As 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. The 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, triglyceride levels, and total cholesterol levels, and lower HDL cholesterol levels, than participants in the first quartile. Among participants in the highest quartile, there were also higher proportions of males and of participants with hypertension and self-reported diabetes, and a lower proportion of physically active and highly educated participants. The quartiles of IMT contained approximately the same proportions of current smokers.

HRs for VTE with 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 of 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 of 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, HDL cholesterol, smoking, diabetes mellitus and diastolic blood pressure had a negligible effect on the risk estimates (HRs per SD increase for TPA and IMT were 0.98 [95% CI 0.88–1.09] and 0.96 [95% CI 0.86–1.07], respectively). Similar results were obtained when the participants were censored at the first survey that they did not attend (Table S1) and when the analyses were restricted to participants not using lipid-lowering drugs in Tromsø 5 or 6 (Table S2).

HRs for VTE according to the formation and progression of carotid plaques are shownd 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 discrepancy in 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

Table 1 Baseline characteristics of traditional atherosclerotic risk factors across quartiles of carotid intima media thickness (IMT); in total, 10 426 participants were included in the study (Tromsø Study, 1994–2012)

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

BP, blood pressure; BMI, body mass index; SD, standard deviation; VTE, venous thromboembolism. *Hypertension: systolic BP of ≥ 140 mmHg, or diastolic BP of ≥ 90 mmHg, or the use of antihypertensive medicine. †Hard physical activity for ≥ 1 h every week. ‡Fifteen or more years of education (corresponding to 3 years in a university or academy).

Table 2 Hazard ratios (HRs) with 95% confidence intervals (CIs) of venous thromboembolism according to total plaque area (TPA) and intima media thickness (IMT) in a time-varying Cox regression model (Tromsø Study 1994–2012)

Risk factors	Events	Person-years	HR (95% CI)*
TPA†	368		0.99 (0.90–1.11)
No plaque	140	54 062	Ref.
First tertile (1.018–3.506 mm^2)	64	19 648	0.93 (0.69–1.29)
Second tertile (3.506–5.031 mm^2)	78	19 141	1.04 (0.79–1.38)
Third tertile (5.031–15.696 mm^2)	86	18 685	1.00 (0.75–1.32)
P for trend			0.9
IMT†	368		0.96 (0.86–1.07)
First quartile (0.358–0.743 mm)	58	29 229	Ref.
Second quartile (0.744–0.849 mm)	81	28 210	0.95 (0.68–1.34)
Third quartile (0.849–0.970 mm)	101	27 201	1.02 (0.73–1.43)
Fourth quartile (0.971–2.748 mm)	128	26 896	1.07 (0.77–1.50)
P for trend			0.5

*Adjusted for age (as time-scale), sex, and body mass index. †Per standard deviation (SD) increase: 1 SD TPA = 2.60 mm2; 1 SD IMT = 0.19 mm.

Table 3 Hazard ratios (HRs) with 95% confidence intervals (CIs) for venous thromboembolism by initiation and progression of carotid plaques (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 mm2 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.

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 ultrasonography is not an intermediate for the association between arterial and venous thrombosis.

Our results are in accordance with those of previous cohort studies on the association between atherosclerosis and VTE using time-fixed analyses [11–13]. The Atherosclerosis Risk in Communities 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 the presence of carotid plaques and VTE risk [11]. The Cardiovascular Health Study study followed 4100 subjects aged ≥ 65 years for a period of 12 years, and measured subclinical atherosclerosis according to IMT, the presence of carotid plaques, ankle brachial index, and ECG abnormalities. In this study, subclinical atherosclerosis was not associated with an 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 > 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 than in 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 divergent results between cohort 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 obtain a better estimation of an individual's atherosclerotic 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 the 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 nine 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 an 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, was associated with an 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 of 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 because of 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 interobserver mean arithmetic and mean absolute differences [16]. Although the measurement errors in carotid ultrasonography are too large to allow study of the 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 have 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 a decreased risk of recurrent VTE [43,44], it has not been associated with a reduced risk of incident VTE in population-based studies [37,45].

In conclusion, we found that the formation and progression of carotid atherosclerosis, as measured with ultrasonography, 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

J.-B. Hansen, S. K. Braekkan, and W. M. Lijfering were responsible for the concept of the study. I. Njølstad, E. B. Mathiesen, and S. H. Johnsen curated the data. B. Småbrekke and S. K. Braekkan were responsible for formal data analysis. J.-B. Hansen acquired funding. J.-B. Hansen and S. K. Braekkan were responsible for the methodology. J.-B. Hansen, S. K. Braekkan, and W. M. Lijfering were responsible for project administration. J.-B. Hansen and S. K. Braekkan supervised the study. J.-B. Hansen, S. K. Braekkan, W. M. Lijfering, and B. Småbrekke were responsible for visualization. B. Småbrekke wrote the original draft. J.-B. Hansen, B. Småbrekke, W. M. Lijfering, L. B. Rinde, E. B. Mathiesen, I. Njølstad, E. M. Hald, and S. H. Johnsen reviewed and edited the manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. HRs of VTE according to TPA and IMT. Participants were censored at the next survey they did not attend, the date of an incident VTE event, the date they died or moved from the municipality of Tromsø or at the end of follow-up, whichever came first.

Table S2. HRs of VTE according to TPA and IMT. Participants with previous or current use of lipid-lowering drugs were excluded.

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Supplementary table S1. 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. Participants were censored at the next survey they did not attend, the date of an incident VTE event, the date they died or moved from the municipality of Tromsø or at the end of follow-up, whichever came first. The Tromsø Study 1994-2012.

Risk factors	**Events**	**Person-years**	**HR (95% CI) †**
Total Plaque Area*	301		**0.98 (0.87-1.10)**
No plaque	117	50283	Ref.
1st tertile (1.018-3.506 mm^2)	54	17712	1.00 (0.72-1.39)
2nd tertile (3.506-5.031 mm^2)	67	17337	1.13 (0.83-1.54)
3rd tertile (5.031-15.696 mm^2)	63	16889	0.92 (0.67-1.28)
P for trend			*0.9*
Intima Media Thickness*	301		**0.94 (0.83-1.06)**
1st quartile (0.358-0.743 mm)	48	26683	Ref.
2nd quartile (0.744-0.849 mm)	63	25754	0.90 (0.62-1.32)
3rd quartile (0.849-0.970 mm)	87	25075	1.06 (0.73-1.53)
4th quartile (0.971-2.748 mm)	103	24708	1.05 (0.72-1.53)
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

Supplementary table S2. 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. Participants with previous or current use of lipid-lowering drugs were excluded. The Tromsø Study 1994-2012.

Risk factors	**Events**	**Person-years**	**HR (95% CI) †**
Total Plaque Area*	296		**0.99 (0.90-1.11)**
No plaque	118	45122	Ref.
1st tertile (1.183-3.506 mm^2)	52	15168	0.94 (0.67-1.31)
2nd tertile (3.506-5.031 mm^2)	62	13818	1.09 (0.79-1.49)
3rd tertile (5.033-15.696 mm^2)	64	12244	1.05 (0.76-1.45)
P for trend			*0.6*
Intima Media Thickness*	296		**0.98 (0.87-1.11)**
1st quartile (0.358-0.743 mm)	49	24993	Ref.
2nd quartile (0.744-0.849 mm)	69	22306	0.99 (0.68-1.44)
3rd quartile (0.849-0.970 mm)	80	20496	1.00 (0.69-1.45)
4th quartile (0.971-2.748 mm)	98	18557	1.09 (0.75-1.59)
P for trend			*0.6*

* 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

Paper II

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ORIGINAL ARTICLE

Impact of incident myocardial infarction on the risk of venous thromboembolism: the Tromsø Study

L. B. RINDE,* C. LIND,* B. SMÅBREKKE,* I. NJØLSTAD,*† E. B. MATHIESEN,*‡ T. WILSGAARD,† M.-L. LØCHEN,† E. M. HALD,*§ A. VIK,*§ S. K. BRÆKKAN*§ and J.-B. HANSEN*§

**K. G. Jebsen Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine; †Epidemiology of Chronic Diseases Research Group, Department of Community Medicine; ‡Brain and Circulation Research Group, Department of Clinical Medicine, University of Tromsø–The Arctic University of Norway; and §Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway*

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Essentials

- Registry-based studies indicate a link between arterial- and venous thromboembolism (VTE).
- We studied this association in a cohort with confounder information and validated outcomes.
- Myocardial infarction (MI) was associated with a 4.8-fold increased short-term risk of VTE.
- MI was associated with a transient increased risk of VTE, and pulmonary embolism in particular.

Summary. *Background:* Recent studies have demonstrated an association between venous thromboembolism (VTE) and arterial thrombotic diseases. *Objectives:* To study the association between incident myocardial infarction (MI) and VTE in a prospective population-based cohort. *Methods:* Study participants (n = 29 506) were recruited from three surveys of the Tromsø Study (conducted in 1994–1995, 2001–2002, and 2007–2008) and followed up to 2010. All incident MI and VTE events during follow-up were recorded. Cox regression models with age as the time scale and MI as a time-dependent variable were used to calculate hazard ratios (HRs) of VTE adjusted for sex, body mass index, blood pressure, diabetes mellitus, HDL cholesterol, smoking, physical activity, and education level. *Results:* During a median follow-up of 15.7 years, 1853 participants experienced an MI and 699 experienced a VTE. MI was associated with a 51% increased risk of VTE (HR 1.51; 95% confidence interval [CI] 1.08–2.10) and a 72% increased risk of pulmonary embolism (PE) (HR 1.72; 95% CI 1.07–2.75), but not significantly associated with the risk of deep vein thrombosis (DVT) (HR 1.36; 95% CI 0.86–2.15). The highest risk estimates for PE were observed during the first 6 months after the MI (HR 8.49; 95% CI 4.00–18.77). MI explained 6.2% of the PEs in the population (population attributable risk) and 78.5% of the PE risk in MI patients (attributable risk). *Conclusions:* Our findings indicate that MI is associated with a transient increased VTE risk, independently of traditional atherosclerotic risk factors. The risk estimates were particularly high for PE.

Keywords: epidemiology; myocardial infarction; pulmonary embolism; risk factors; venous thromboembolism.

Correspondence: Ludvig Balteskard Rinde, K. G. Jebsen Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, University of Tromsø–The Arctic University of Norway, N-9037 Norway.
Tel: +47 97134760; fax: +47 77646838.
E-mail: ludvig.b.rinde@uit.no

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Introduction

Despite definite differences in pathology and treatment strategies, growing evidence suggests a bidirectional relationship between venous thromboembolism (VTE) (deep vein thrombosis [DVT] and pulmonary embolism [PE]) and arterial thromboembolic diseases (ATDs) (myocardial infarction [MI] and ischemic stroke) [1–4]. The relationship between ATD and VTE could be attributable to shared risk factors, such as obesity, smoking, or a family history of MI, indirect causal factors, such as hospitalizations accompanied by periods of immobilization, or a direct causal relationship, such as a transient prothrombotic response secondary to sudden tissue damage, and venous stasis following heart failure [5].

The results from prospective cohorts, applying cause-specific regression models, have revealed that, among the traditional atherosclerotic risk factors, only age, obesity

and a familial predisposition for MI are shared risk factors for ATD and VTE [6–9]. In a case–control study, patients with unprovoked VTE were reported to have a higher frequency of carotid plaques than control participants [2]. Conversely, subsequent large population-based cohort studies have failed to confirm an association between carotid atherosclerosis and VTE [10–12], indicating that atherosclerosis is not a shared risk factor for ATD and VTE.

Population-based registry studies have shown that patients with a history of MI are at increased short-term risk of subsequent VTE [13,14]. However, results from registry-based linkage studies should be interpreted with caution, as they often lack information about confounders and have limited validation of exposure and outcomes. For instance, an evaluation of the Danish National Patient Registry revealed that the positive predictive values for VTE diagnoses from emergency departments and hospitals were only 44% and 67–77%, respectively [15]. Moreover, the lack of important clinical information, such as body mass index (BMI), has limited our ability to adjust for confounding in previous registry-based studies [13,14].

We therefore aimed to investigate the association between MI and the future risk of VTE in a population-based cohort with validated information on exposure (MI), the endpoint (VTE), and potential confounders.

Materials and 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 surveys of the Tromsø Study, conducted in 1994–1995, 2001–2002, and 2007–2008, respectively. The overall attendance rates were high: 77% in the fourth survey, 78% in the fifth survey, and 66% in the sixth survey. A detailed description of the Tromsø Study has been published elsewhere [16]. In total, 30 586 unique participants aged 25–97 years participated in at least one of the surveys, and, of these, 21 529 participants participated in two or all three surveys. Participants who did not consent to medical research (n = 225), participants not officially registered as inhabitants of the municipality of Tromsø at the date of study enrollment (n = 48) and participants with a previous history of VTE (n = 78) or MI (n = 729) before baseline were excluded. Consequently, 29 506 participants were included in the study, and followed from the date of enrollment to the end of follow-up, i.e. 31 December 2010 (Fig. 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 study participants was collected by physical examination, from blood samples and from self-administrated questionnaires at each survey. Systolic and diastolic blood pressures were measured three times at 1-min intervals with an automatic device (Dinamap Vital Signs Monitor, 1846; Critikon, Tampa, FL, USA), with the participant in a sitting position after 2 min of rest, and defined as the mean of the last two readings. Non-fasting blood samples were collected from an antecubital vein; serum was prepared by centrifugation with 3000 × g in 10 min after 1 hour in open air at room temperature, and analyzed at the Department of Clinical Chemistry, University Hospital of North Norway (UNN), Tromsø, Norway. Serum total cholesterol was analyzed by use of an enzymatic colorimetric method with a commercially available kit (CHOD-PAP; Boehringer-Mannheim, Mannheim, Germany). Serum HDL cholesterol was measured after the 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 of ≥ 30 kg m^{-2}) was classified according to the World Health Organization (WHO) definition [17]. Hypertension was classified as a mean systolic blood pressure of ≥ 140 mmHg, a mean diastolic blood

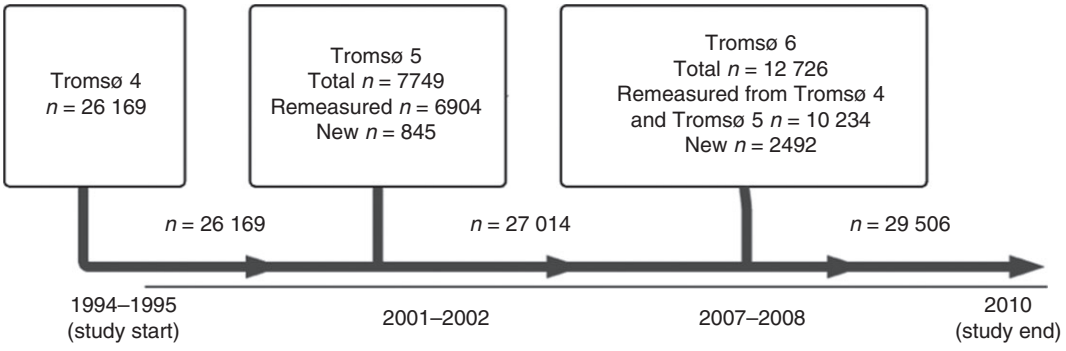

Fig. 1. Inclusion of study participants from the fourth (1994–1995), fifth (2001–2002) and sixth (2007–2008) surveys of the Tromso Study.

pressure of ≥ 90 mmHg, or self-reported use of blood pressure-lowering drugs. Hypercholesterolemia was classified as a total serum cholesterol level of ≥ 6.5 mmol L^{-1} or self-reported use of lipid-lowering drugs. Information on family history of MI, diabetes mellitus, physical activity and education level was collected from a self-administered questionnaire.

Assessment of MI

Adjudication of hospitalized and out-of-hospital MI events was performed by an independent endpoint committee, and based on data from hospital and out-of-hospital medical records, autopsy records, and death certificates. The national 11-digit identification number allowed linkage to national and local diagnosis registries. Cases of incident MI were identified by linkage to the hospital discharge diagnosis registry at the UNN, by searching for ICD-9 codes 410-414 and 430-438 in the time period 1994–1998, and thereafter ICD-10 codes I20-I25 and I60-I69. The hospital medical records were retrieved for case validation. Modified WHO MONICA/MORGAM criteria for MI [18] were used, and included clinical symptoms and signs, findings in electrocardiograms, values of cardiac biomarkers, and autopsy reports when applicable. Furthermore, linkage to the National Causes of Death Registry at Statistics Norway allowed the identification of fatal incident MI cases that occurred as out-of-hospital deaths, including deaths that occurred outside of Tromsø. The death certificates were used to collect relevant information on the MI events from additional sources, such as autopsy reports and records from nursing homes, ambulance services, and general practitioners.

Registry 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 UNN, as previously described [19]. The UNN 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 VTE case was reviewed by trained personnel, and a VTE event was considered to be verified and recorded when the presence of clinical signs and symptoms of DVT or PE were combined with objective confirmatory tests (i.e. compression ultrasonography, venography, spiral computed tomography, perfusion–ventilation scan, pulmonary angiography, or autopsy), and resulted in a VTE diagnosis that required treatment, as previously described in detail [19]. VTE cases from the autopsy registry were recorded when the death certificate indicated VTE as the cause of death or as a significant condition associated with death. The VTE events were classified as provoked or unprovoked according to the presence of provoking factors at the time of diagnosis. Provoking factors included recent surgery or trauma within the previous 8 weeks, acute medical conditions (i.e. acute MI, ischemic stroke, or major infectious diseases), active cancer, immobilization (i.e. bed rest for > 3 days, wheelchair use, or long-distance travel exceeding 4 h within the 14 days prior to the event), or any other factor described by a physician in the medical record (e.g. intravascular catheter).

Statistical analysis

Participants who developed MI during the study period contributed with non-exposed person-time from the inclusion date to the date of a diagnosis of MI, and then with exposed person-time from the date of MI onwards. For each participant, non-exposed and exposed person-years were counted from the date of enrollment to the date of an incident diagnosis of VTE or to the date on which the participant died or moved from Tromsø, or until the end of the study period, i.e. 31 December 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 with STATA version 14.0 (Stata Corporation, College Station, TX, USA). Crude incidence rates (IRs) 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 (HRs) with 95% confidence intervals (CIs) of VTE, DVT and PE after MI. Age was used as the time scale in the Cox model, with the age of the participants at study enrollment being defined as entry time, and the age at the time of the VTE event or censoring event (i.e. death, migration, or the date of study end) being defined as exit time. MI was included as a time-dependent covariate in the Cox model. Therefore, participants who developed MI during follow-up contributed with person-years in both the unexposed and exposed group. For those who participated in several surveys, information on possible potential confounders was updated at each survey. We estimated HRs with three different models. The first model was adjusted for age (as time scale) and sex, and the second model was additionally adjusted for BMI. The third model was adjusted for age (as time scale), sex, BMI, diabetes mellitus, smoking, systolic blood pressure, HDL cholesterol, physical activity, and education.

The proportional hazards assumption was tested by the use of Schoenfeld residuals, and found to be not violated. Statistical interactions between MI and sex were tested by including cross-product terms in the proportional hazards models, and no interactions were found.

Attributable risk (AR), i.e. the proportion of events among the exposed participants that could be explained by the exposure, was calculated from IRs of VTE in the MI (I_e) and non-

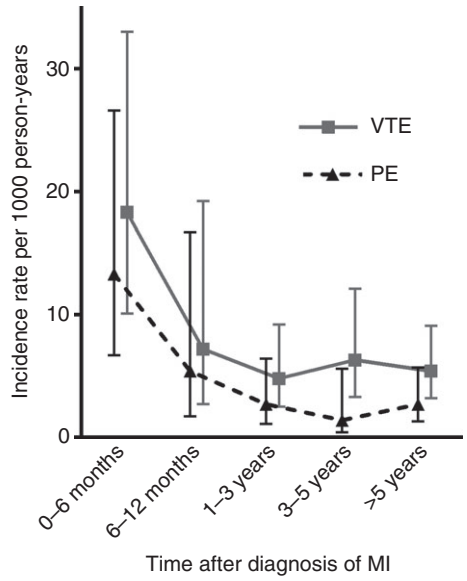

Fig. 2. Changes in crude incidence rates per 1000 person-years for venous thromboembolism (VTE) and pulmonary embolism (PE) in the periods 0–6 months, 6–12 months, 1–3 years, 3–5 years and more than 5 years after myocardial infarction (MI).

MI (I_o) populations, and expressed as a percentage (AR = 100 × [I_e – I_o]/I_e). Population AR (PAR), i.e. the proportion of events in the population that could be attributed to the exposure, was calculated on the basis of IRs of VTE in the total population (I_p) and in the non-exposed population (I_o), and expressed as a percentage (PAR = 100 × [I_p – I_o]/I_p). GRAPHPAD PRISM version 6.0 (GraphPad Software, San Diego, CA, USA) was used to generate a figure showing the change in VTE risk over time (Fig. 2).

Results

There were 1892 (6.4%) participants who developed a first-time MI, and 699 (2.4%) participants who developed a first-time VTE, during a median of 15.7 years of follow-up. The baseline characteristics of the study participants with and without MI are shown in Table 1. Participants with MI had a higher mean age and a higher BMI, and included a higher proportion of men, smokers and participants with hypertension than those without MI (Table 1).

The characteristics of the VTE events are shown in Table 2. Among the 699 VTE events, 405 (57.9%) were DVTs and 294 (42.1%) were PEs. Moreover, 358 events (51.2%) were classified as provoked, and 341 events (48.8%) were classified as unprovoked. The most frequent provoking factors were active cancer, immobilization, and surgery (Table 2). The proportion of PEs was higher among the VTE events that occurred after an MI (53% versus 41%) than among the VTE events that appeared in the absence of a previous MI. Furthermore, surgery was a more frequent provoking factor for VTE in participants with prior MI (27%) than in those without MI (15%) (data not shown).

Table 1 Baseline characteristics of participants without and with myocardial infarction (MI) (n = 29506); the Tromsø Study 1994–2010

	No MI (n = 27614)	MI (n = 1892)
Age (years), mean ± SD	45 ± 14	62 ± 13
Sex (male), % (n)	45.9 (12675)	61.0 (1154)
BMI (kg m^{-2}), mean ± SD	25.2 ± 3.9	26.6 ± 4.1
Total cholesterol (mmol L^{-1}), mean ± SD	5.90 ± 1.27	6.92 ± 1.28
HDL (mmol L^{-1}), mean ± SD	1.50 ± 0.41	1.41 ± 0.40
Triglycerides (mmol L^{-1}), mean ± SD	1.51 ± 1.02	1.95 ± 1.18
Systolic blood pressure (mmHg), mean ± SD	132 ± 19	152 ± 24
Diastolic blood pressure (mmHg), mean ± SD	77 ± 12	87 ± 14
Hypertension*, % (n)	31.5 (8700)	70.7 (1337)
Hypercholesterolemia†, % (n)	30.5 (8429)	63.0 (1192)
Smoking‡, % (n)	35.6 (9803)	41.2 (780)
Physical activity§, % (n)	33.4 (9210)	20.0 (380)
Education¶, % (n)	34.1 (9406)	12.2 (231)
Self-reported diabetes mellitus, % (n)	1.5 (401)	6.3 (120)

BMI, body mass index; SD, standard deviation. *Mean systolic/diastolic blood pressure of ≥ 140/≥ 90 mmHg, use of antihypertensives, or self-reported hypertension. †Total cholesterol level of ≥ 6.5 mmol L−1, use of lipid-lowering drugs, or self-reported hypercholesterolemia. ‡Self-reported daily smoking; yes/no. §One or more hours of moderate or hard physical activity per week; yes/no. ¶More than 10 years of education.

Table 2 Characteristics of venous thromboembolism events (n = 699); the Tromsø Study 1994–2010

	% (n)
Clinical characteristics	
Deep vein thrombosis	57.9 (405)
Pulmonary embolism	42.1 (294)
Provoked	51.2 (358)
Unprovoked	48.8 (341)
Clinical risk factors	
Oestrogen*,†	5.9 (41)
Pregnancy/puerperium*	0.9 (6)
Heredity‡	3.4 (24)
Provoking factors	
Surgery	15.5 (108)
Trauma	7.9 (55)
Cancer	24.0 (168)
Immobility§	18.3 (128)
Other¶	4.9 (34)

*Only women were included in the analysis. †Current or previous use of hormone replacement therapy or oral contraceptives. ‡Myocardial infarction in a first-degree relative before age 60 years. §Bed rest for > 3 days, journeys of > 4 h 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 (e.g. intravascular catheter).

IRs and HRs of VTE and subtypes of VTE according to MI are shown in Table 3. In participants without MI, 652 VTE events were identified during 354 865

person-years of follow-up (IR of 1.8 per 1000 person-years), whereas there were 47 VTE events during 7062 person-years of follow-up in participants exposed to MI (IR of 6.7 per 1000 person-years). Overall, participants with a previous MI had a 51% (HR 1.51; 95% CI 1.09–2.11) higher VTE risk than participants without a previous MI in a multivariable model adjusted for traditional atherosclerotic risk factors, including sex, BMI, systolic blood pressure, diabetes mellitus, HDL cholesterol, smoking, physical activity, and education level (Table 3). In a subgroup of participants with available data on family history of MI ($n = 21\ 096$), the addition of a family history of MI to the multivariable model did not change the risk estimates for the association between MI and VTE (Table S1). In separate analyses of PE and DVT after MI, PE showed higher risk estimates than DVT. The multivariable HRs were 1.72 (95% CI 1.07–2.79) for PE and 1.36 (95% CI 0.86–2.15) for DVT (Table 3).

In analyses stratified by the presence of provoking factors, MI was associated with increased risks of provoked VTE (multivariable adjusted HR 1.83; 95% CI 1.21–2.79) and provoked PE (multivariable adjusted HR 2.29; 95% CI 1.20–4.37), but not with provoked DVT or any unprovoked events (Table 4). Among MI patients, 72.4% of the VTEs could be attributed to the MI (AR), and 4.7% of the VTE events in the entire study population could be attributed to the MI (PAR). For PE, the numbers attributable to MI were higher; the AR was 78.5%, and the PAR was 6.2%.

IRs and HRs of VTE were high immediately after the MI, and declined rapidly thereafter (Table 5; Fig. 2). The IR of VTE was 18 per 1000 person-years, and the HR of VTE was five-fold higher in participants with MI than in

Table 3 Incidence rates (IRs) and hazard ratios (HRs) of venous thromboembolism (VTE), deep vein thrombosis (DVT) and pulmonary embolism (PE) according to myocardial infarction (MI) exposure; the Tromsø Study 1994–2010

	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 MI	354 865	652	1.8 (1.7–2.0)	Reference	Reference	Reference
MI	7062	47	6.7 (5.0–8.9)	1.49 (1.10–2.01)	1.41 (1.04–1.92)	1.51 (1.09–2.11)
DVT						
No MI	354 865	383	1.1 (1.0–1.2)	Reference	Reference	Reference
MI	7062	22	3.1 (2.1–4.7)	1.25 (0.81–1.93)	1.17 (0.75–1.83)	1.36 (0.86–2.15)
PE						
No MI	354 865	269	0.8 (0.7–0.9)	Reference	Reference	Reference
MI	7062	25	3.5 (2.4–5.2)	1.80 (1.18–2.73)	1.71 (1.12–2.60)	1.72 (1.07–2.79)

CI, confidence interval. *Per 1000 person-years. †Model 1: age as time scale, adjusted for sex. ‡Model 2: model 1 + body mass index. §Model 3: model 2 + systolic blood pressure, diabetes mellitus, HDL cholesterol, smoking, physical activity, and education level.

Table 4 Incidence rates (IRs) and hazard ratios (HRs) for venous thromboembolism (VTE), deep vein thrombosis (DVT) and pulmonary embolism (PE) according to myocardial infarction (MI) exposure by the presence of predisposing factors; the Tromsø Study 1994–2010

	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 MI	354 865	331	0.9 (0.8–1.0)	Reference	Reference	Reference
MI	7062	27	3.8 (2.6–5.6)	1.65 (1.11–2.46)	1.63 (1.09–2.42)	1.83 (1.21–2.79)
Unprovoked VTE						
No MI	354 865	321	0.9 (0.8–1.0)	Reference	Reference	Reference
MI	7062	20	2.8 (1.8–4.4)	1.32 (0.83–2.09)	1.19 (0.75–1.91)	1.16 (0.67–2.00)
Provoked DVT						
No MI	354 865	216	0.6 (0.5–0.7)	Reference	Reference	Reference
MI	7062	15	2.1 (1.3–3.5)	1.44 (0.85–2.47)	1.45 (0.85–2.48)	1.59 (0.91–2.76)
Unprovoked DVT						
No MI	354 865	167	0.5 (0.4–0.5)	Reference	Reference	Reference
MI	7062	7	1.0 (0.5–2.1)	0.96 (0.44–2.07)	0.79 (0.35–1.80)	1.02 (0.45–2.34)
Provoked PE						
No MI	354 865	115	0.3 (0.3–0.4)	Reference	Reference	Reference
MI	7062	12	1.7 (1.0–3.0)	2.00 (1.09–3.67)	1.91 (1.04–3.50)	2.29 (1.20–4.37)
Unprovoked PE						
No MI	354 865	154	0.4 (0.4–0.5)	Reference	Reference	Reference
MI	7062	13	1.8 (1.1–3.2)	1.65 (0.92–2.94)	1.56 (0.88–2.79)	1.29 (0.62–2.67)

CI, confidence interval. *Per 1000 person-years. †Model 1: age as time scale, adjusted for sex. ‡Model 2: model 1 + body mass index. §Model 3: model 2 + systolic blood pressure, diabetes mellitus, HDL cholesterol, smoking, physical activity, and education level.

Table 5 Incidence rates (IRs) and hazard ratios (HRs) for venous thromboembolism (VTE), deep vein thrombosis (DVT) and pulmonary embolism (PE) according to time after myocardial infarction (MI); the Tromsø Study 1994–2010

	Person-years	VTE events	Crude IR (95% CI)*	Model 1†, HR (95% CI)	Model 2‡, HR (95% CI)	Model 3§, HR (95% CI)
VTE						
No MI	354 865	652	1.8 (1.7–2.0)	Reference	Reference	Reference
< 6 months	601	11	18.3 (10.1–33.0)	4.38 (2.41–7.98)	4.26 (2.34–7.75)	4.82 (2.57–9.05)
0.5–1 year	556	4	7.2 (2.7–19.2)	1.72 (0.64–4.61)	1.68 (0.63–4.49)	2.10 (0.78–5.62)
1–3 years	1880	9	4.8 (2.5–9.2)	1.14 (0.59–2.20)	1.10 (0.57–2.13)	1.20 (0.60–2.42)
3–5 years	1428	9	6.3 (3.3–12.1)	1.45 (0.75–2.81)	1.40 (0.72–2.71)	1.56 (0.77–3.14)
>5 years	2598	14	5.4 (3.2–9.1)	1.11 (0.65–1.89)	1.00 (0.58–1.74)	0.90 (0.46–1.74)
DVT						
No MI	354 865	383	1.1 (1.0–1.2)	Reference	Reference	Reference
< 6 months	601	3	5.0 (1.6–15.5)	2.08 (0.67–6.52)	2.05 (0.66–6.42)	2.41 (0.77–7.54)
0.5–1 year	556	1	1.8 (0.3–12.8)	0.76 (0.11–5.43)	0.75 (0.11–5.37)	0.89 (0.13–6.38)
1–3 years	1880	4	2.1 (0.8–5.7)	0.89 (0.33–2.39)	0.88 (0.33–2.36)	1.03 (0.38–2.77)
3–5 years	1428	7	4.9 (2.3–10.3)	2.04 (0.96–4.32)	1.99 (0.94–4.23)	1.14 (1.14–5.15)
> 5 years	2598	7	2.7 (1.3–5.7)	1.00 (0.47–2.13)	0.85 (0.38–1.90)	0.89 (0.37–2.16)
PE						
No MI	354 865	269	0.8 (0.7–0.9)	Reference	Reference	Reference
< 6 months	601	8	13.3 (6.7–26.6)	7.46 (3.67–15.17)	7.13 (3.51–14.48)	8.49 (4.00–18.17)
0.5–1 year	556	3	5.4 (1.7–16.7)	2.98 (0.95–9.32)	2.83 (0.90–8.85)	3.78 (1.20–11.89)
1–3 years	1880	5	2.7 (1.1–6.4)	1.46 (0.60–3.54)	1.38 (0.57–3.35)	1.45 (0.53–3.91)
3–5 years	1428	2	1.4 (0.4–5.6)	0.72 (0.18–2.92)	0.68 (0.17–2.76)	0.44 (0.06–3.18)
> 5 years	2598	7	2.7 (1.3–5.7)	1.24 (0.58–2.64)	1.18 (0.56–2.52)	0.90 (0.33–2.45)

CI, confidence interval. *Per 1000 person-years. †Model 1: age as time scale, adjusted for sex. ‡Model 2: model 1 + body mass index. §Model 3: model 2 + systolic blood pressure, diabetes mellitus, HDL cholesterol, smoking, physical activity, and education level.

those without MI during the first 6 months after the incident MI diagnosis (adjusted HR 4.86; 95% CI 2.57–9.05). Following the initial 6 months after the MI, the VTE risk was not significantly increased (Table 5). Separate analysis of PE showed a similar, although augmented, risk pattern. The multivariable HR for PE during the first 6 months after MI was 8.49 (95% CI 4.00–18.17). The PE risk remained almost four-fold higher from 6 months to 1 year after the MI than the risk in those without MI (adjusted HR 3.78; 95% CI 1.20–11.89), but the association disappeared when the observation period was extended beyond 1 year (Table 5).

Discussion

In our population-based cohort, participants with MI had a higher risk of subsequent VTE, and PE in particular, than participants without MI in analyses adjusted for traditional atherosclerotic risk factors. The risk estimates for PE were highest during the first 6 months after the MI, and declined rapidly thereafter. We found that 78.5% of the PE events among MI patients could be attributed to the MI, whereas 6.2% of the PEs in the population could be attributed to MI.

Previous studies have indicated an association between MI and an increased risk of future VTE. In a meta-analysis of placebo-controlled trials evaluating the effect of antithrombotic drugs, 4% of patients with MI had a symptomatic PE during the first 2 weeks after the MI event [20]. A relationship between MI and PE was further supported by a cross-sectional study, in which an association between coronary heart disease and PE was found in patients aged ≥ 60 years [21]. In agreement with our findings, Sørensen *et al*. found that the risk of VTE, and of PE in particular, was higher in the first months immediately after an MI than the VTE risk in population-based controls in two registry-based case–control studies [13,14].

The explanations for the observed association between MI and the future VTE risk are not yet known. Potential mechanisms include shared risk factors, indirect factors, or a direct causal relationship [5]. If the association between MI and VTE is attributable to shared cardiovascular risk factors, the cardiovascular risk factors work as confounding factors by increasing the risks of both MI and VTE. In agreement with this, cohort studies conducting cause-specific analyses have revealed age, obesity and a family history of MI as shared risk factors for MI and VTE [6–9]. Conversely, our findings argue against a strong impact of shared risk factors on the association between MI and the future VTE risk. First, shared risk factors would mediate a permanent and not a transient VTE risk, as observed in our study. Second, adjustments for atherosclerotic risk factors, such as obesity and a family history of MI, would substantially attenuate the association between MI and subsequent VTE if the risk factors were actual confounders. In our study, adjustments for atherosclerotic risk factors had a marginal impact on the risk estimates for the association between

MI and VTE. However, our findings do not exclude the possibility of joint effects between shared inherited prothrombotic risk factors that would augment the VTE risk under circumstances of high thrombosis risk related to the MI itself (e.g. hospitalizations accompanied by periods of immobilization, a transient prothrombotic response secondary to sudden tissue damage, and venous stasis following heart failure) [22,23]. Furthermore, several other risk factors for VTE, including high levels of coagulation factors VIII, IX, and XI, plasminogen activator inhibitor-1, and von Willebrand factor, have also been shown to be risk factors for arterial cardiovascular disease [24,25]; the levels of these increase immediately after MI, and may therefore mediate the transient VTE risk after MI observed in our study.

The transient increase in the VTE risk after MI points towards causal mechanisms related to the MI itself. Patients with MI are hospitalized and temporarily immobilized, both of which are strong predisposing factors for VTE [26]. Previous studies have suggested that hospitalization after MI partly explains the observed association between MI and subsequent VTE [13,27]. This suggestion was supported by the short-term nature of the risk [13] and the attenuation of risk estimates after adjustment for hospitalization for surgery or acute medical illness, as well as nursing home confinement [27]. Accordingly, we observed a transient short-term VTE risk after MI, and stratified analyses revealed higher risk estimates for provoked than for unprovoked events. In agreement with previous observations [13,14,27], our findings support the notion that indirect causal factors, such as hospitalization and subsequent immobilization, as well as coronary artery bypass surgery or endovascular procedures after MI, may contribute substantially to the observed association between MI and VTE.

A direct causal relationship between MI and VTE may also contribute to the VTE risk in MI patients. Local disturbances in the cardiopulmonary circulation after MI may predispose to thrombus formation by stasis in the pulmonary circulation, owing to backward failure secondary to left ventricular dysfunction [28,29], by injury to the vascular endothelium [30], or by activation of the coagulation system during the acute phase of MI [31]. Atrial fibrillation is a frequent complication after MI [32]. Recently, we reported that atrial fibrillation was associated with an increased risk of VTE, and of PE in particular [33]. According to the transient nature of the VTE risk and the particularly high PE risk observed in our study, it is likely that direct causal mechanism(s) secondary to local disturbances in the cardiopulmonary circulation or electromechanical activity (e.g. atrial fibrillation) may be responsible for some of the VTE risk after MI. Previous studies have shown that DVT can be identified in only 50% of patients with PE [34], which supports the concept that pulmonary thrombi may form *de novo* in the lungs or originate from other sources of emboli. Alternatively, the high PE rather than DVT risk after MI may be explained by detection bias, as patients with previous MI are more likely to undergo examinations for chest pain.

Our findings may have some clinical implications. In our population-based cohort, 6.2% of PE events could be attributed to MI exposure, and 78% of the VTE events among the MI patients were attributable to the MI itself. These numbers may actually be underestimates, owing to the concomitant use of drugs (aspirin, heparins, and statins) in MI, which is known to reduce the VTE risk. The high amount of PE explained by exposure to MI may suggest that anticoagulant treatment of MI patients would prevent several subsequent PE events. Randomized clinical trials have shown that prolonged oral anticoagulant treatment with vitamin K antagonists (VKAs) is equal to (Waris II trial) [35] or superior to (ASPECT II trial) [36] antiplatelet treatment for recurrent MI. Unfortunately, none of these studies has reported VTE as a secondary endpoint. Furthermore, VKAs and non-vitamin K oral anticoagulants (NOACs) have been shown to reduce the incidence of recurrent VTE by ~ 90%. However, the impact of anticoagulant treatment together with antiplatelet treatment for prevention of PE in MI patients needs to be weighed against the expected bleeding risk resulting from combined treatment [35,37–39]. Alternatively, the transient nature of the VTE risk after MI suggests that MI patients may benefit more from extended thromboprophylaxis with low molecular weight heparins or NOACs, with an expected efficacy of 50–70% prevention of VTEs over the first 3–6 months [40,41].

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, the updated confounder information, and the validated VTE and MI events. As many cardiovascular risk factors are modifiable, some participants' individual risk profiles may change during follow-up, leading to regression dilution bias and an underestimation of the associations. However, an advantage of our study is the repeated measurements of participant characteristics during follow-up. Because of this, we can explore the real effect of cardiovascular risk factors on the outcomes during follow-up to a greater extent, resulting in more reliable risk estimates than in a traditional cohort study. However, our study has some potential limitations. In a cohort study, non-response bias is a possible limitation. Those who participate in cohort studies tend to be healthier and more interested in their health than the general population. Our estimated incidence may therefore be lower than the true incidence. Furthermore, the low numbers of both exposure and outcome events in the present cohort may lead to low statistical power for assessing the potential impact of MI on the VTE risk, particularly in subgroup analyses.

In conclusion, the present cohort study implies that first-lifetime MI is associated with an increased risk of

VTE, and particularly of PE. The transient nature of the VTE risk after MI suggests that direct or indirect causal mechanisms related to the MI event itself are primarily responsible for the observed association. We found that 6.2% of the VTE events in the population could be attributed to MI.

Addendum

L. B. Rinde was responsible for data analysis and writing of the manuscript. C. Lind was responsible for data interpretation and revision of the manuscript. B. Småbrekke was responsible for data interpretation and revision of the manuscript. I. Njølstad was responsible for data collection and revision of the manuscript. E. B. Mathiesen was responsible for data collection and revision of the manuscript. T. Wilsgaard was responsible for statistical support and revision of the manuscript. M.-L. Løchen was responsible for data collection and revision of the manuscript. E. M. Hald was responsible for data collection and interpretation, and revision of the manuscript. A. Vik was responsible for data interpretation and revision of the manuscript. S. K. Brækkan was responsible for conception and design of the study, data collection and interpretation, and writing of the manuscript. J.-B. Hansen was responsible for conception and design of the study, data collection and interpretation, and writing of the manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Incidence rates and hazard ratios for VTE, DVT and PE after acute MI, including only subjects with data on family history (n = 21 096).

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Supplemental Tables

Supplementary Table I for Online Data Supplement. Incidence Rates and Hazard Ratios for VTE, DVT and PE after AMI including only subject with data on family history (n=21 096)

	Person-years	VTE-events	Crude IR (95% CI)*	HR (95% CI)†	HR (95% CI)†!‡	HR (95% CI)†!‡
Total VTE						
No AMI	279152	472	1.7 (1.5-1.9)	Reference	Reference	Reference
AMI	5381	30	5.6 (3.9-8.0)	1.24 (0.85-1.81)	1.26 (0.83-1.92)	1.25 (0.82-1.90)
DVT						
No AMI	279152	277	1.0 (0.9-1.1)	Reference	Reference	Reference
AMI	5381	12	2.2 (1.3-3.9)	0.90 (0.50-1.62)	1.01 (0.55-1.86)	0.99 (0.54-1.83)
PE						
No AMI	279152	195	0.7 (0.6-0.8)	Reference	Reference	Reference
AMI	5381	18	3.5 (2.1-5.3)	1.66 (1.02-2.72)	1.60 (0.90-2.86)	1.59 (0.89-2.84)

AMI indicates acute myocardial infarction; CI, confidence interval; DVT, deep vein thrombosis; HR, hazard ratio; IR, incidence rate; PE, pulmonary embolism; and VTE, venous thromboembolism.

*Per 1000 persons-years

†Adjusted for age (as time scale) and sex-adjusted

!Adjusted for body mass index

‡Adjusted for systolic blood pressure, diabetes mellitus, HDL, smoking, physical activity, education level, and family history of myocardial infarction.

Paper III

ORIGINAL RESEARCH

American Heart Association | American Stroke Association

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

Ludvig B. Rinde, BSc; Birgit Småbrekke, BCs; Ellisiv B. Mathiesen, MD, PhD; Maja-Lisa Løchen, MD, PhD; Inger Njølstad, MD, PhD; Erin M. Hald, MD, PhD; Tom Wilsgaard, PhD; Sigrid K. Brækkan, PhD; John-Bjarne Hansen, MD, PhD

***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

From the K. G. Jebsen - Thrombosis Research and Expertise Center (TREC) (L.B.R., B.S., E.B.M., I.N., E.M.H., S.K.B., J.-B.H.) and Brain and Circulation Research Group (E.B.M.), Department of Clinical Medicine, and Epidemiology of Chronic Diseases Research Group, Department of Community Medicine (M.-L.L., I.N., T.W.), UiT The Arctic University of Norway, Tromsø, Norway; Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway (E.M.H., S.K.B., J.-B.H.).

Correspondence to: Ludvig B. Rinde, BSc, Department of Clinical Medicine, K. G. Jebsen - Thrombosis Research and Expertise Center (TREC), University of Tromsø, The Arctic University of Norway, Tromsø N-9037, Norway. E-mail: ludvig.b.rinde@uit.no

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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

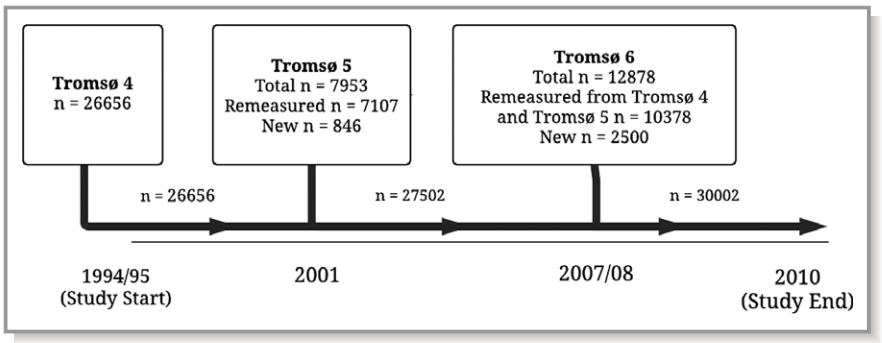

Figure 1. Inclusion of study participants from the fourth (1994–1995), fifth (2001–2002), and sixth (2007–2008) surveys of the Tromsø Study.

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).

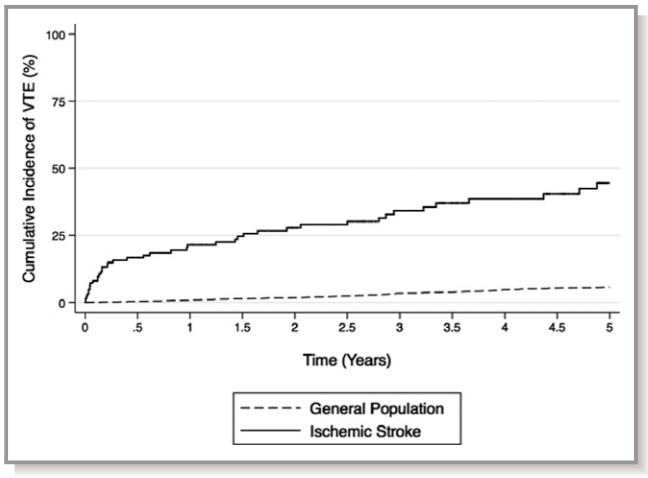

Figure 2. Overall cumulative incidence of venous thromboembolism (VTE) in subjects with and without ischemic stroke. The Tromsø Study 1994–2010.

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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Paper IV

Effect of prothrombotic genotypes on the risk of venous thromboembolism in patients with ischemic stroke. The Tromsø Study

Ludvig B. Rinde, MD[1], Vania M. Morelli, PhD[1], Birgit Småbrekke, MD[1], Ellisiv B. Mathiesen, MD, PhD[1, 2], Maja-Lisa Løchen, MD, PhD[3], Inger Njølstad, MD, PhD[1,3], Tom Wilsgaard PhD[3], Erin Smith, PhD[1, 5], Frits R. Rosendaal, MD, PhD[1,6], Kelly A. Frazer, PhD[1, 5], Sigrid K. Brækkan, PhD[1, 4], John-Bjarne Hansen, MD, PhD[1, 4]

[1]K. G. Jebsen - Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, UiT The Arctic University of Norway,
[2]Brain and Circulation Research Group, Department of Clinical Medicine, UiT The Arctic University of Norway,
[3]Epidemiology of Chronic Diseases Research Group, Department of Community Medicine, UiT The Arctic University of Norway,
[4]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway,
[5]Department of Pediatrics and Rady's Children's Hospital, University of California, San Diego, La Jolla, California, United States
[6]Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, the Netherlands

Correspondence to: Ludvig B. Rinde, MD
K. G. Jebsen - Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, University of Tromsø The Arctic University of Norway, N-9037, Norway.
E-mail ludvig.b.rinde@uit.no, telephone: +4797134760

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Abstract

Background: Patients with ischemic stroke have a transient increased risk of subsequent venous thromboembolism (VTE). Prothrombotic genotypes may augment VTE risk under conditions of high thrombosis risk related to the stroke.

Aims: To investigate the effect of prothrombotic genotypes in patients with ischemic stroke on the risk of VTE in a population-based case-cohort study.

Methods: Cases with incident VTE (n=660) and a randomly selected age-weighted sub-cohort (n=1803) were sampled from 3 surveys of the Tromsø Study (1994-2008). Participants were genotyped for *ABO* (rs8176719), *F5* (rs6025), *F2* (rs1799963), *FGG* (rs2066865) and *F11* (rs2036914) single nucleotide polymorphisms (SNPs). Cox regression models were used to calculate hazard ratios (HR) for incident VTE according to individual SNPs and categories of risk alleles (the 5-SNP score; 0-1, 2, 3-4 and ≥5) in participants with and without ischemic stroke.

Results: There were 263 patients with incident stroke, of whom 60 developed VTE during a median of 15.3 years of follow-up. The risk alleles of individual SNPs augmented the elevated risk brought about by ischemic stroke for VTE. In stroke patients, one category increase in the genetic risk score was associated with 54% higher risk of overall VTE (HR 1.54, 95% CI 1.35-1.75) and 76% higher risk of provoked VTE (HR 1.76, 95%CI 1.51-2.06). Stroke patients with ≥5 risk alleles had a 12-fold (HR 11.8, 95%CI 4.17-33.3) higher VTE risk than stroke-free participants with 0-1 risk alleles.

Conclusions: Prothrombotic genotypes increased the risk of VTE in stroke patients, and the risk increased with increasing number of risk alleles.

Introduction

Recent studies have shown that patients with arterial cardiovascular disease (i.e. myocardial infarction and ischemic stroke) have increased risk of subsequent venous thromboembolism (VTE) [1, 2]. In patients with ischemic stroke, the incidence of VTE is high, particularly of deep vein thrombosis (DVT) [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, 5], PE may account for up to 25-50% of deaths after acute stroke [5, 6]. Recently, we reported a transiently 20-fold increased risk of VTE within the first month after ischemic stroke in a population-based cohort study [7].

Simulation studies have shown that genetic profiling may be useful to discriminate between persons with high and low risk of disease [8, 9]. To identify individuals with high risk of a first VTE, de Haan *et al*. created a genetic score based on 31 single nucleotide polymorphisms (SNPs) previously reported to be associated with VTE [10]. SNPs with the highest odds ratios of VTE were added one-by-one to construct a genetic risk score using the most parsimonious model with fewer SNPs. This resulted in a score of 5 SNPs which included rs8176719 (non-O blood type) in *ABO*, rs6025 (factor V Leiden [FVL]) in *F5*, rs1799963 (prothrombin G20210A) in *F2*, rs2066865 in fibrinogen gamma gene (*FGG*), and rs2036914 in *F11*. The genetic risk score based on these 5 SNPs performed similarly to the score of all 31 SNPs [10].

Ischemic stroke is a heterogeneous multicausal disorder, and epidemiological data have provided substantial evidence for a genetic component to the disease [11, 12]. Hypercoagulability has a more pronounced effect on the risk of ischemic stroke than the risk of myocardial infarction [13]. The genes in the 5-SNP score are not only associated with increased risk of VTE but also with stroke. In a large meta-analysis, FVL and prothrombin

G20210A were found to be associated with a 33% and 44% increased risk of stroke, respectively [14]. Furthermore, non-O blood type was associated with 83% increased risk of stroke [15], and an association between *F11* variation and overall ischemic stroke was reported in individuals below 70 years of age [16]. Increased levels of plasma fibrinogen were associated with increased risk of stroke [17], but variants in *FGG* were not related to higher risk of stroke [18].

Even though ischemic stroke increases the risk of VTE and prothrombotic genotypes are associated with both stroke and VTE, the joint effect of prothrombotic genotypes and stroke on the risk of VTE has not been explored. Identification of genetic risk factors that particularly increase the risk of VTE instroke patients may guide decisions on thromboprophylaxis in stroke patients. Therefore, the aim of the present study was to investigate the combined effect of ischemic stroke and the SNPs included in the 5-SNP risk score [10] on the risk of VTE in a population-based case-cohort.

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 (n=30,371) were recruited from the fourth, fifth and sixth surveys of the Tromsø Study, conducted in 1994–1995, 2001-2002, and 2007–2008, respectively. The overall attendance rates were high with 77% in the fourth survey, 79% in the fifth survey, and 66% in the sixth survey. A detailed description of the Tromsø Study has been published elsewhere [19]. The Tromsø study was approved by the Regional Committee for Medical and Health Research Ethics in Northern Norway, and all participants provided informed written consent to participation.

All subjects (n=30,371) were followed from the date of inclusion until a verified incident VTE event, migration, death, or end of follow-up (December 31, 2012). Incident VTE events were identified by searching the hospital discharge diagnosis registry, the radiological procedure registry and the autopsy registry at the University Hospital of North Norway, and adjudicated by an end-point committee, as previously described by Brækkan *et al* [20]. A VTE event was classified as either a DVT or PE. When these events occurred concurrently, the event was classified as a PE. VTE events were further classified as provoked or unprovoked according to the presence of provoking risk factors at the time of diagnosis [20]. Major surgery or trauma within 8 weeks prior to the event, active cancer at the time of diagnosis, marked immobilization (i.e. bed rest > 3 days, confinement to wheelchair, or long-distance travel > 4 hours within the last 14 days prior to the event) or other potential provoking factors specifically described by a physician in the medical record (e.g. intravascular catheter), were considered provoking factors. Ischemic stroke, myocardial infarction, or other acute medical conditions were not included in the definition of provoked VTE.

A total of 737 individuals without previous VTE experienced a VTE during follow-up. Out of these, 45 did not have blood samples available or of sufficient quality for DNA analysis. Thus, the remaining 692 subjects were included as cases in our study. A subcohort (n=2016) was created by randomly sampling participants from the three surveys weighted for the age of the cases in 5-year age-groups. Due to the case-cohort design, 71 of the cases were sampled and included in the subcohort. Study participants with a history of ischemic stroke (n=63) or with missing values for at least one of the risk allele variants (n=182) were excluded. Thus, our final case-cohort included 2463 participants, consisting of 660 VTE cases and 1803 sub-cohort members (Figure 1).

Baseline measurements

Information about the study participants at study entry was collected by physical examinations, blood samples, and self-administered questionnaires at each survey. Blood samples were collected from an antecubital vein and analyzed at the Department of Clinical Chemistry at the University Hospital of North Norway. DNA was isolated from whole blood and stored at -70°C at the national CONOR biobank, located at the HUNT Biobank in Levanger, Norway.

Body weight and height were measured in study participants wearing light clothing and no shoes. Body mass index (BMI) was calculated by the weight in kilograms (kg) divided by the height in meters (m) squared (kg/m^2). Information regarding a history of cardiovascular disease (myocardial infarction, angina or stroke) prior to inclusion in the cohort, diabetes mellitus, smoking status (yes/no), physical activity and level of education was obtained by using self-reported questionnaires. The baseline variables have been described in detail elsewhere [19].

Genetic risk factors of VTE

We genotyped the following SNPs: rs8176719 (non-O blood type) in *ABO*, rs6025 (FVL) in *F5*, rs1799963 (prothrombin G20210A) in *F2,* and rs2036914 in *F11*, with the Sequenom platform, and rs2066865 in *FGG* with the TaqMan platform, as previously described [21]. For Sequenom, which uses single-base extension followed by mass spectrometry to measure the molecular mass of the extended primers, samples were genotyped with the Sequenom iPlex Gold Assay according to the recommended protocol, with an initial input of 10–20 ng of DNA, and were analyzed with the MassARRAY Analyzer 4. Only genotypes with a high quality score of ‘A. Conservative’ or ‘B. Moderate’ were used. When multiple attempts were made

to genotype an individual, one of the highest-quality genotypes across all attempts was chosen for each SNP. For TaqMan, an initial input of 100 ng of DNA was used. Samples were genotyped with the Applied Biosystems 7900HT (Foster City, CA, USA) according to the recommended protocol, and processed with SDS 2.4 (Thermo Fisher, Foster City, CA, USA). Genotypes passing a quality value threshold of 95 were used.

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 reduced risk of VTE was the rs2036914 in *F11*, and in this case, we considered the common allele as the risk allele. The 5-SNP score conceived by de Haan *et al*. was created by summarizing the number of risk alleles from the five sequenced SNPs [10].

Assessment of ischemic stroke

Ischemic stroke was during follow-up defined according to the World Health Organization definition when computed tomography or magnetic resonance imaging or autopsy had ruled out brain hemorrhage [22]. An end-point committee 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, as previously described [7].

Statistical methods

Statistical analyses were performed using STATA version 15.0 (Stata Corporation, College Station, TX, USA). Cox proportional hazards regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for incident VTE according to the individual SNPs or categories of risk alleles (the 5-SNP score categories; 0-1, 2, 3-4 and ≥5 risk alleles) in

study participants with and without ischemic stroke. The joint risk conferred by ischemic stroke and the individual SNP was calculated using participants without stroke and with no risk allele as the reference category. When the 5-SNP score was assessed instead of the individual SNPs, participants without stroke and with 0-1 risk allele were used as the reference category. 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 (i.e., 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. Thus, participants who developed stroke during follow-up contributed person-years in both the unexposed and exposed group. All analyses were adjusted for age (as time scale) and sex. Because of the size of the subcohort, we did not make adjustment to the partial likelihood in the Cox regression analyses [23]. Subgroup analyses were performed according to the anatomical localization of the thrombotic event (DVT and PE), and the presence of provoking risk factors at the time of diagnosis. The proportional hazards assumption was tested using Schoenfeld residuals and found not violated.

To investigate whether the effect of ischemic stroke on the risk of VTE differed across strata of prothrombotic risk alleles, the presence of interaction on an additive scale between these 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. These three measures of interaction with their corresponding 95% CIs were calculated according to Andersson *et al.* [24], using an excel sheet (epinet.se/res/xls/epinetcalculation.xls).

Results

In our cohort, 263 participants had incident ischemic stroke during a median of 15.3 years of follow-up. Baseline characteristics of the study participants are shown in Table 1. The mean age was higher in stroke patients than in those without stroke, 67 years versus 57 years, respectively. The proportion of study participants with hypertension, hypercholesterolemia and diabetes mellitus were higher in stroke patients, while mean BMI and the proportion of males and current smokers was similar in both groups. The prevalence of risk alleles for each of the SNPs studied was similar in the two groups (Table 1). In the 5-SNP score, the distribution of individuals across number of risk alleles was virtually the same in participants with and without ischemic stroke (Figure 2).

In total, 60 of the 660 VTEs in our study occurred in patients with ischemic stroke. Characteristics of the VTE events in participants with and without stroke are shown in Table 2. The distribution of DVT and PE (60% and 40% respectively) was similar in both groups. The proportion of provoked VTE was higher in patients with ischemic stroke (75%) than in those without stroke (48%). Moreover, the proportion of patients immobilized before the VTE was substantially higher in those with stroke (62% versus 16%). Ischemic stroke was associated with an age- and sex-adjusted overall 2.1-fold (HR 2.06, 95% CI 1.56-2.71) increased risk of VTE, 2.3-fold (HR 2.36, 95% CI 1.65-3.37) higher risk of DVT, and 1.7-fold (HR 1.73, 95% CI 1.12-2.65) higher risk of PE, compared with study participants without stroke.

The risk estimates of VTE according to the individual SNPs in study participants with and without ischemic stroke are shown in Table 3. In the absence of ischemic stroke, non-O blood type (rs8176719), FVL (rs6025) and prothrombin G20210A (rs1799963) all increased the risk of VTE, whereas no effect on VTE risk was observed for the SNPs in *FGG* (rs2066865) and *F11* (rs2036914). For all the individual SNPs, the combinations of ischemic stroke and ≥1

risk alleles were associated with increased risk of VTE. The highest risk of VTE was conferred by the joint exposure of ischemic stroke and FVL (HR 4.42, 95% CI 2.28-8.59) or prothrombin G20210A (HR 9.62, 95% CI 1.32-69.0). As depicted in Table 4, measures quantifying interaction on an additive scale (i.e. RERI, AP, and synergy index) suggested a positive interaction between ischemic stroke and each of the prothrombotic SNPs, with the exception of the non-O blood type.

When the 5-SNP score was used (Table 3), the risk of VTE increased gradually across categories of number of risk alleles (0-1, 2, 3-4, and ≥ 5 risk alleles) in both study participants with and without stroke compared to participants without stroke and 0-1 risk allele. Of note, the dose-response relationship was even more pronounced in stroke patients. When the score was analyzed as an ordinal variable, the risk of VTE increased 24% (HR 1.24, 95% CI 1.05-1.46) per increase of genetic risk category in study participants without stroke, and 54% (HR 1.54, 95% CI 1.34-1.75) among those with stroke. We found a synergistic effect between the number of risk alleles and ischemic stroke on the risk of VTE. Patients with ischemic stroke and ≥5 risk alleles had 12-fold (HR 11.8, 95% CI 4.17-33.3) higher risk of VTE than study participants without stroke and 0-1 risk alleles. This was higher than expected on the basis of the individual effects of ischemic stroke and ≥ 5 risk alleles. Indeed, all measures of interaction described in Table 4 suggested a positive interaction between the two exposures. For instance, the AP revealed that 84% of the total VTE events in participants with stroke and ≥5 risk alleles were due to the interaction between the two exposures. In subgroup analyses, having both stroke and ≥5 risk alleles resulted in a hazard ratio of 13.3 (95% CI 3.08-57.5) for DVT and 9.97 (95% CI 2.38-43.5) for PE (Supplementary Table 1). As in the overall analysis, measures of interaction suggested a positive interaction between stroke and ≥5 risk alleles on the risk of either DVT or PE (Supplementary Table 2).

The risk estimates of provoked and unprovoked VTE according to the individual SNPs in participants with and without stroke are shown in Supplemental Table 3. For non-O blood type, FVL, and the SNPs in *FGG* and *F11*, the risk estimates for provoked events were higher than those for unprovoked events in study participants jointly exposed to stroke and ≥1 risk alleles. In patients with stroke, the risk estimates for VTE across categories of risk alleles in the 5-SNP score were higher for provoked than for unprovoked events (Table 5). The risk of provoked events increased almost 80% per increase of risk category (HR 1.76, 95% CI 1.41-2.06), while no association was observed for unprovoked events (HR 1.17, 95% CI 0.92-1.50). Participants jointly exposed to ≥5 risk alleles and stroke had an almost 23-fold (HR 22.6 95% CI 7.71-66.2) higher risk of provoked VTE compared with study participants without stroke and 0-1 risk alleles. In participants without stroke, the risk estimates for unprovoked VTE were higher than those for provoked VTE in all risk categories (Table 5).

Discussion

In the present case-cohort study with participants recruited from the general population, we found a synergistic effect of ischemic stroke and prothrombotic genotypes on the risk of VTE. The combined exposure to ischemic stroke and each of the individual SNPs (i.e. FVL, prothrombin G20210A, or variations in *FGG* or *F11)* resulted in an effect on VTE risk that exceeded the sum of the separate effects. When the 5-SNP risk score [10] was applied the number of prothrombotic risk alleles displayed a dose-response relationship with VTE risk, both in participants with and without stroke. The dose-response was particularly pronounced in stroke patients, and the risk of overall VTE and provoked VTE increased on average by 54% and 76%, respectively, per category increase in the genetic score. Furthermore, the combination of ischemic stroke and the high-risk category of the genetic

score (i.e. ≥5 risk alleles) yielded an effect on VTE risk that was greater than the sum of the separate effects. Of note, more than 80% of the VTE events occurring among study participants jointly exposed to ischemic stroke and the high-risk category of the genetic score appeared to be attributable to the interaction between the two risk factors. As risk estimates for provoked VTE were higher than for unprovoked events, our findings suggest that the increased risk of VTE in stroke patients was mainly driven by a combination of stroke-related provoking factors and genetic risk factors.

Recently, we reported that patients with ischemic stroke have a transient increased risk of VTE [7], which is consistent with previous registry-based population studies investigating the temporal relationship between ischemic stroke and VTE [1, 25]. This transient nature of VTE risk after stroke underscores the role of stroke-related factors as the main contributors to the development of thrombotic events. Indeed, measures of stroke severity have been shown to be strongly associated with risk of subsequent VTE [26]. Accordingly, our risk estimates were consistently higher for provoked than for unprovoked VTE events in study participants jointly exposed to stroke and prothrombotic risk genes. Therefore, it is likely that complications to acute stroke, such as prolonged immobilization, leg paralysis and secondary infections, which are all established risk factors of VTE [27-30], may contribute to the VTE risk. Previous studies have shown that individuals under prolonged immobilization [31, 32] or with upper respiratory tract infections [33], who are also carriers of prothrombotic risk genes, have increased risk of VTE that exceeds the sum of the separate effects of the risk factors. However, to the best of our knowledge, no other study have explored the joint effect of ischemic stroke and prothrombotic risk genes on the VTE risk.

Our findings of an increased risk of VTE after stroke in individuals with prothrombotic genotypes appear to be consistent with the thrombosis potential model. In this model, the thrombosis potential reflects the risk for VTE that is present during an individual's life, and each risk factor contributes to increase the thrombosis potential [34]. When sufficient risk factors have been accumulated, the thrombosis potential exceeds the 'thrombosis threshold' and a thrombotic event occurs [34]. Stroke is a strong risk factor of VTE [7]. Still, when the 5-SNP score was applied, the risk of VTE in stroke patients with one or no risk allele was not increased compared with participants without stroke. Our findings infer that the combination of stroke-related risk factors and prothrombotic risk genes are needed to sufficiently rise the thrombosis potential, leading to an incident VTE event. Moreover, the addition of risk alleles resulted in a dose-dependent increased risk of VTE. Stroke patients with ≥5 risk alleles had almost 12-fold higher risk of VTE than participants without stroke and ≤1 risk allele. All measures of interaction (i.e. RERI, AP and synergy index) between risk alleles and ischemic stroke on VTE risk were pointing towards a substantial synergistic effect. When these findings were analyzed in light of the thrombosis potential model, our findings suggest that ischemic stroke and the high-risk category of the 5-SNP score yielded a higher thrombosis potential together than separately [34].

Even though our results on the interaction between stroke and prothrombotic genotypes do not allow conclusions about biological mechanisms, as interaction is defined in numerical terms [34], one may still speculate on the pathophysiology behind these findings. A potential mechanism includes an association between prothrombotic genotypes and severity of stroke. If the prothrombotic genotypes are somehow related to severity of stroke, this may lead to a more pronounced or prolonged immobilization after acute stroke. However, whether the prothrombotic genotypes assessed in this study influence the

outcome of ischemic stroke is as yet unclear, as studies on this topic are scarce, and often limited by small sample sizes [16]. The risk of VTE in stroke patients could be further amplified by the hypercoagulable state associated with the prothrombotic genotypes, either through resistance to activated protein C due to FVL [35, 36], increased levels of prothrombin or factor XI in the presence of prothrombin G20210A or *F11* variation, respectively [37, 38], or changes in levels of fibrinogen γ', a product of alternative splicing of *FGG* that is inversely related to thrombotic risk [39]. The present study was not designed to investigate these proposed mechanisms. Still, our results may form the basis for further studies to confirm the interaction and to investigate the underlying mechanism(s).

International guidelines for VTE prevention after stroke suggest prophylactic-dose heparin (unfractionated or low-molecular-weight heparin) or intermittent pneumatic compression devices over no prophylaxis in patients with acute ischemic stroke and restricted mobility [40]. Prophylaxis should be initiated as early as possible and should be continued throughout the hospital stay or until the patient regains mobility. However, the guidelines are mainly based on one randomized study (the Clots in Legs or Stockings after Stroke [CLOTS] study), in which no information was provided regarding the prevalence of prothrombotic risk genes in the study population [41]. Despite the recommendation of anticoagulant use during the hospitalization, the high incidence of VTE observed after stroke indicates that VTE prophylaxis in stroke patients is inadequate. Indeed, data from clinical practice have shown that less than 50% of ischemic stroke patients at risk of VTE receive any form of thromboprophylaxis [42, 43]. This may be due to poor compliance following guidelines, or uncertainty on how to assess patients at increased risk of VTE. The Padua prediction score uses both ischemic stroke and known thrombophilic conditions to predict VTE in hospitalized patients [44]. However, the Padua score does not differentiate between

number of risk alleles, and external validation of the prediction score showed limited performance for predicting VTE among hospitalized medical patients [45]. Our finding of an increased risk of VTE after stroke with increasing number of prothrombotic risk alleles suggests that the number of prothrombotic risk alleles could be considered when assessing thrombosis risk in patients with ischemic stroke. Identification of patients in whom supra-additive effects on VTE risk are present due to the combination of stroke-related risk factors and prothrombotic risk alleles, would allow the implementation of more effective thromboprophylaxis, which may reduce the incidence of VTE after stroke. Therefore, new studies are warranted to explore to what extent assessment of prothrombotic genotypes in stroke patients would improve risk stratification of VTE and aid clinical decisions of therapeutic interventions.

Major strengths of our study include the prospective design with participants recruited from the general population, the large number of genotyped participants, the long-term follow-up, the wide age distribution and the validated events of both ischemic stroke and VTE. The high participation rate in the Tromsø Study and the broad age range formed a cohort that is representative of the general population and minimized selection bias in the sub-cohort. Some limitations merit attention. Even though our study was derived from a large cohort, the number of VTE events was low in some subgroups, particularly for the rare genetic variants, which resulted in limited statistical power. Our results on the measures that quantify interaction should therefore be interpreted with caution. Unfortunately, we did not have information about stroke severity or the number of patients with leg-paralysis or prolonged immobilization.

In conclusion, we found a synergistic effect of ischemic stroke and prothrombotic genotypes on the risk of subsequent VTE. In stroke patients, increasing number of risk alleles

showed a dose-dependent increased risk of VTE, particularly of provoked VTE events. Our findings suggest that genetic risk factors play an important role in the development of VTE after stroke, and may imply that the number of risk alleles could be considered when assessing the VTE risk in patients with ischemic stroke.

Addendum

L. B. Rinde and V.M. Morreli analyzed the data and drafted the manuscript. J.-B. Hansen and S. K. Brækkan designed the study, organized data collection, interpreted the results and revised the manuscript. F. R. Rosendaal,and B. Småbrekke interpreted the results and critically reviewed the manuscript. E. N. Smith and K. A. Frazer genotyped the case-cohort. I. Njølstad, E. B. Mathiesen, and M.-L. Løchen were responsible for data collection and revision of the manuscript. T. Wilsgaard provided statistical support and revision of the manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Tables

Table 1. Baseline characteristics of the study population with and without stroke (n = 2463). The Tromsø Study

	No stroke (n= 2200)	**Stroke (n=263)**
Age (years)	57 ± 14	67 ± 10
Sex (male)	44.4 (976)	46.0 (121)
BMI (kg/m^2)	26.0 ± 4.1	26.9 ± 4.4
Total cholesterol (mmol/L)	6.53 ± 1.31	6.87 ± 1.25
HDL (mmol/L)	1.54 ± 0.42	1.47 ± 0.40
Triglycerides (mmol/L)	1.63 ± 1.00	1.87 ± 1.06
Systolic blood pressure (mmHg)	142 ± 22	158 ± 27
Diastolic blood pressure (mmHg)	81 ± 13	88 ± 16
Hypertension*	52.0 (1144)	79.8 (210)
Hypercholesterolemia†	49.5 (1089)	58.9 (155)
Smoking ‡	34.0 (749)	31.9 (84)
Physical activity §	22.1 (486)	14.1 (37)
Education ‖	21.7 (478)	10.6 (28)
Self-reported diabetes mellitus	2.7 (60)	7.2 (19)
rs8176719 (*ABO*)		
≥1 risk allele	63.1 (1389)	67.7 (178)
rs6025 (*F5*)		
≥1 risk allele	8.8 (193)	8.4 (22)
rs1799963 (*F2*)		
≥1 risk allele	1.6 (34)	0.8 (2)
rs2066865 (*FGG*)		
≥1 risk allele	45.7 (1006)	43.7 (115)
rs2036914 (*F11*)		
≥1 risk allele	81.5 (1792)	78.7 (207)

Values are % (n) or mean ± SD
BMI indicates body mass index
Genes related to the single nucleotide polymorphisms are depicted between parentheses
*Mean systolic/diastolic blood pressure ≥140/≥90 mm Hg
†Total cholesterol ≥6.5 mmol/L
‡Self-reported daily smoking, yes/no.
§≥1hours of moderate or hard physical activity per week, yes/no.
‖>15 years of education.

Table 2. Characteristics of venous thromboembolism events (n = 660). The Tromsø Study

	No stroke (n = 600)	Stroke (n = 60)
Clinical characteristics % (n)		
Deep vein thrombosis	56.7 (340)	60.0 (36)
Pulmonary embolism	43.3 (260)	40.0 (24)
Provoked	48.3 (290)	75.0 (45)
Unprovoked	51.7 (310)	25.0 (15)
Clinical risk factors % (n)		
Estrogen*†	5.8 (35)	6.7 (4)
Pregnancy/puerperium*	0.8 (5)	-
Heredity‡	3.8 (23)	-
Provoking factors % (n)		
Surgery	16.8 (101)	11.7 (7)
Trauma	8.5 (51)	8.3 (5)
Cancer	27.2 (163)	16.7 (10)
Immobility§	16.3 (98)	61.7 (37)
Other||	6.0 (36)	3.3 (2)

*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 h 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 (e.g. intravascular catheter)

Table 3. Hazard Ratios of venous thromboembolism for individual single nucleotide polymorphisms and categories of the 5-SNP score according to ischemic stroke exposure. The Tromsø Study

	Risk Alleles	Events	HR (95 % CI)*
SNP (Gene)			
rs8176719 (*ABO*)			
No stroke	0	195	1 (Reference)
	≥1	405	1.32 (1.11-1.57)
Stroke	0	17	2.38 (1.44-3.92)
	≥1	43	2.49 (1.78-3.48)
rs6025 (*F5*)			
No stroke	0	511	1 (Reference)
	≥1	89	2.11 (1.68-2.64)
Stroke	0	51	2.02 (1.50-2.72)
	≥1	9	4.42 (2.28-8.59)
rs1799963 (*F2*)			
No stroke	0	586	1 (Reference)
	≥1	14	1.64 (0.96-2.78)
Stroke	0	59	2.04 (1.55-2.69)
	≥1	1	9.62 (1.32-69.04)
rs2066865 (*FGG*)			
No stroke	0	328	1 (Reference)
	≥1	272	1.02 (0.86-1.19)
Stroke	0	30	1.61 (1.10-2.36)
	≥1	30	2.90 (1.98-4.26)
rs2036914 (*F11*)			
No stroke	0	108	1 (Reference)
	≥1	492	1.06 (0.86-1.31)
Stroke	0	12	1.99 (1.09-3.63)
	≥1	48	2.20 (1.56-3.12)

Table 3. *(Continued)*

	Risk Alleles	Events	HR (95 % CI)*
5-SNP score			
No stroke	0-1	101	1 (Reference)
	2	161	1.07 (0.83-1.37)
	3-4	292	1.30 (1.04-1.63)
	≥ 5	46	1.97 (1.39-2.80)
	HR per increase of risk category		1.24 (1.05-1.46)
Stroke	0-1	6	0.96 (0.42-2.20)
	2	15	1.81 (1.03-3.17)
	3-4	35	3.54 (2.35-5.33)
	≥ 5	4	11.8 (4.17-33.3)
	HR per increase of risk category		1.54 (1.35-1.75)

CI, confidence interval; HR, hazard ratio; single nucleotide polymorphisms (SNP)
*Adjusted for age (as time scale) and sex.

Table 4. Measures of interaction on an additive scale between ischemic stroke and the individual single nucleotide polymorphisms or 5 or more risk alleles in the 5-SNP score. The Tromsø Study

	RERI (95% CI)	AP (95% CI)	Synergy index (95% CI)
SNP (Gene)			
rs8176719 (*ABO*)	-0.21 (-1.59 to 1.16)	-0.09 (-0.65 to 0.48)	0.87 (0.38 to 2.03)
rs6025 (*F5*)	1.29 (-1.70 to 4.28)	0.29 (-0.20 to 0.78)	1.61 (0.65 to 4.00)
rs1799963 (*F2*)	6.94 (-12.04 to 25.92)	0.72 (0.16 to 1.28)	5.13 (0.53 to 50.2)
rs2066865 (*FGG*)	1.28 (0.07 to 2.48)	0.44 (0.15 to 0.73)	3.03 (0.98 to 9.36)
rs2036914 (*F11*)	0.15 (-1.16 to 1.46)	0.07 (-0.52 to 0.65)	1.14 (0.34 to 3.85)
5-SNP score	9.84 (-2.13 to 21.82)	0.84 (0.67 to 1.00)	11.6 (3.18 to 42.1)

CI, confidence interval; single nucleotide polymorphisms (SNP); RERI, Relative excess risk due to interaction; AP, Proportion due to interaction

Table 5. Hazard Ratios for provoked and unprovoked venous thromboembolism for categories of the 5-SNP Score according to ischemic stroke exposure. The Tromsø Study

		Provoked VTE		Unprovoked VTE	
	Risk Alleles	**Events**	**HR (95 % CI)***	**Events**	**HR (95 % CI)***
5-SNP score					
No stroke	0-1	55	1 (Reference)	46	1 (Reference)
	2	86	1.06 (0.75-1.48)	75	1.08 (0.75-1.57)
	3-4	130	1.07 (0.78-1.46)	162	1.58 (1.14-2.19)
	≥ 5	19	1.48 (0.88-2.50)	27	2.57 (1.59-4.14)
	HR per increase of risk category		1.07 (0.93-1.23)		1.35 (1.18-1.55)
Stroke	0-1	5	1.51 (0.59-3.83)	1	0.33 (0.05-2.45)
	2	10	2.22 (1.10-4.46)	5	1.33 (0.52-3.44)
	3-4	26	5.10 (3.10-8.39)	9	1.79 (0.84-3.83)
	≥ 5	4	22.6 (7.7-66.2)	0	-
	HR per increase of risk category		1.76 (1.51-2.06)		1.17 (0.92-1.50)

CI, confidence interval; HR, hazard ratio; single nucleotide polymorphisms (SNP)
*Adjusted for age (as time scale) and sex.

Figures

Figure 1. Flowchart illustrating the composition of the case–cohort study. FVL, Factor V Leiden; n, number of participants; VTE, venous thromboembolism

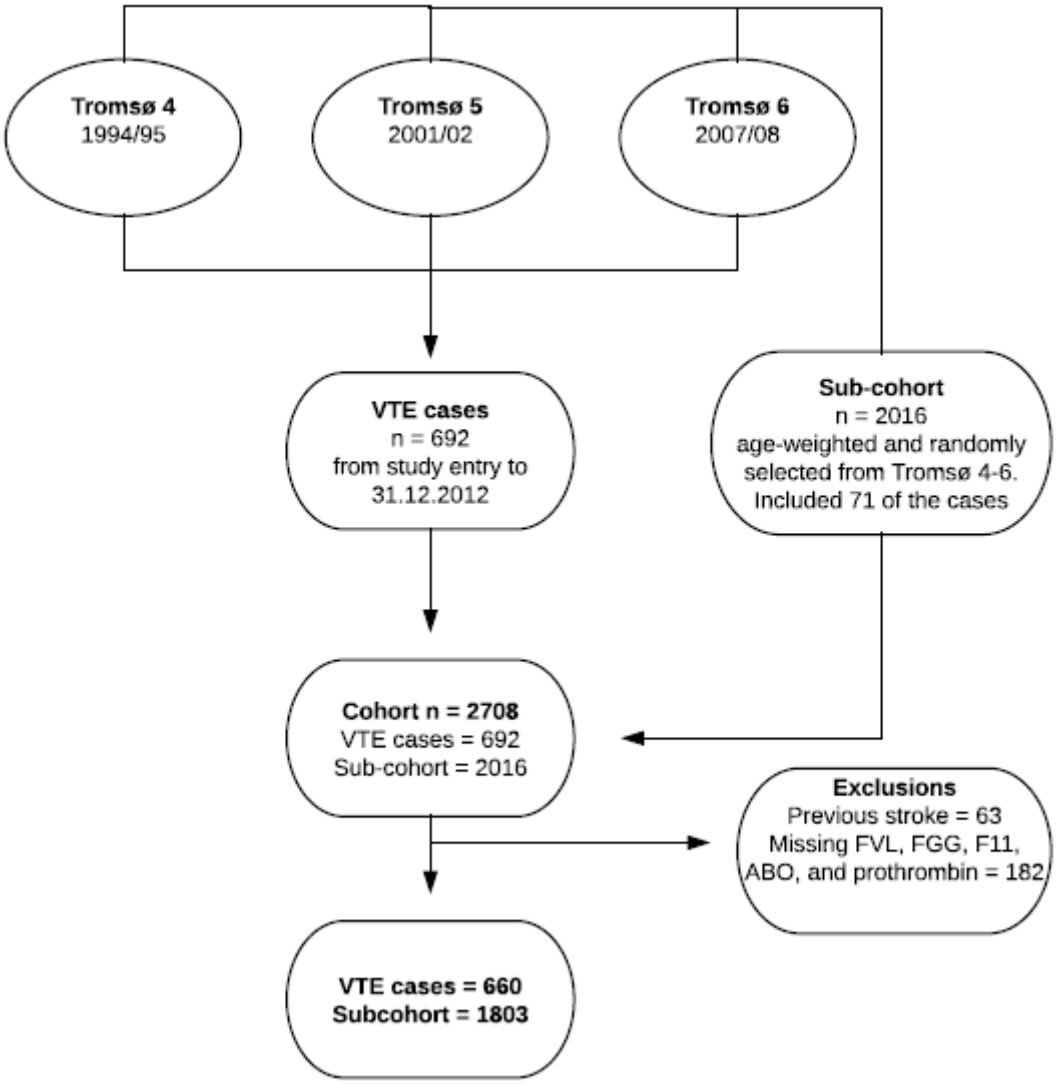
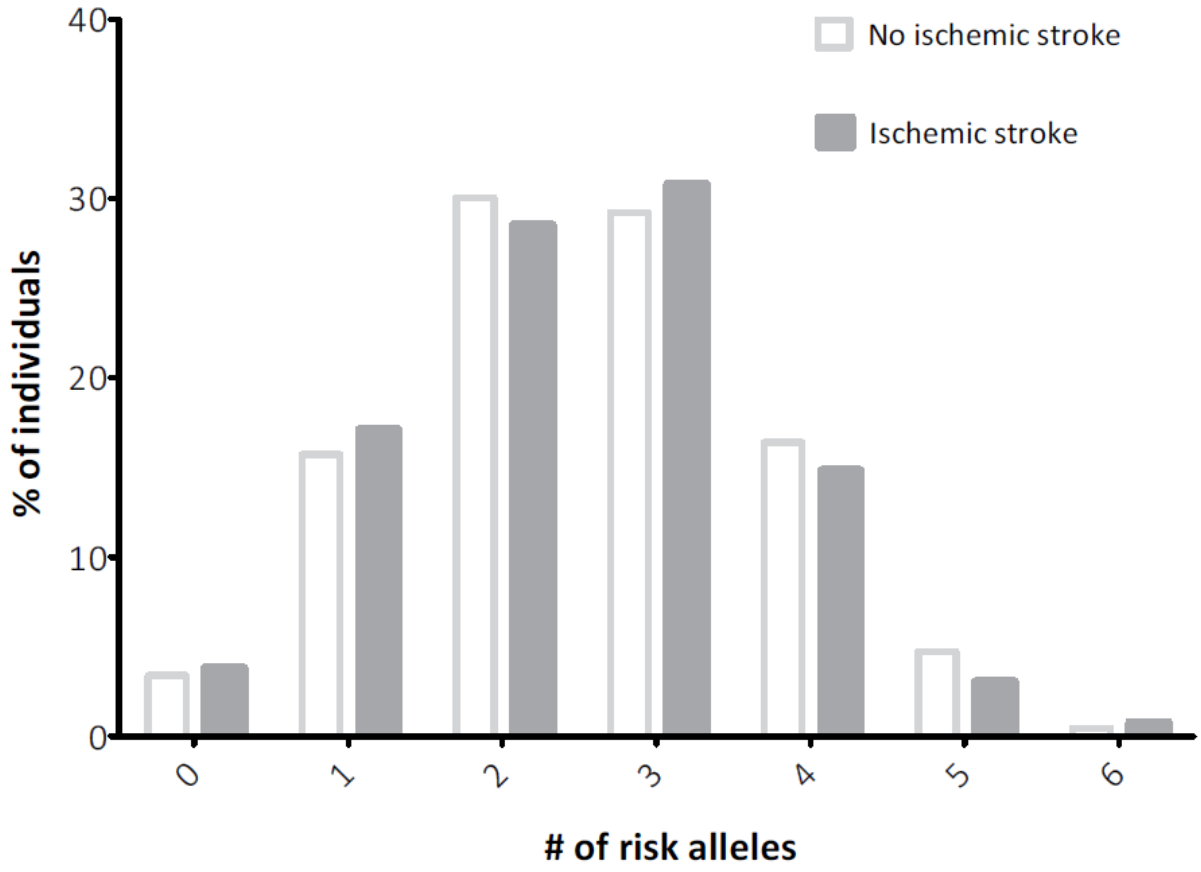


Figure 2. Distribution (%) of individuals across number of risk alleles in study participants with and without previous ischemic stroke



Supplementary material

Supplementary Table 1. Hazard Ratios for deep vein thrombosis and pulmonary embolism for individual single nucleotide polymorphisms and categories of the 5-SNP Score according to ischemic stroke exposure. The Tromsø Study

		Deep vein thrombosis		Pulmonary embolism	
	Risk Alleles	**Events**	**HR (95 % CI)***	**Events**	**HR (95 % CI)***
SNPs (Gene)					
rs8176719 (*ABO*)					
No stroke	0	103	1 (Reference)	92	1 (Reference)
	≥1	237	1.44 (1.14-1.82)	168	1.19 (0.92-1.53)
Stroke	0	14	3.95 (2.24-6.95)	3	0.82 (0.26-2.61)
	≥1	22	2.57 (1.61-4.12)	21	2.37 (1.46-3.85)
rs6025 (*F5*)					
No stroke	0	275	1 (Reference)	236	1 (Reference)
	≥1	65	2.82 (2.15-3.70)	24	1.26 (0.83-1.92)
Stroke	0	28	2.21 (1.48-3.30)	23	1.82 (1.17-2.82)
	≥1	8	7.84 (3.85-15.96)	1	0.98 (0.14-6.98)
rs1799963 (*F2*)					
No stroke	0	334	1 (Reference)	252	1 (Reference)
	≥1	6	1.20 (0.53-2.68)	8	2.26 (1.12-4.58)
Stroke	0	35	2.30 (1.60-3.31)	24	1.76 (1.14-2.70)
	≥1	1	16.51 (2.28-119.48)	0	-
rs2066865 (*FGG*)					
No stroke	0	184	1 (Reference)	144	1 (Reference)
	≥1	156	1.03 (0.83-1.28)	116	1.00 (0.78-1.27)
Stroke	0	19	1.97 (1.22-3.19)	11	1.23 (0.66-2.28)
	≥1	17	3.16 (1.90-5.25)	13	2.62 (1.47-4.68)
rs2036914 (*F11*)					
No stroke	0	60	1 (Reference)	48	1 (Reference)
	≥1	280	1.09 (0.82-1.44)	212	1.02 (0.75-1.40)
Stroke	0	8	2.59 (1.23-5.46)	4	1.36 (0.49-3.77)
	≥1	28	2.51 (1.58-3.97)	20	1.87 (1.10-3.19)

Supplementary Table 1. (Continued)

		Deep vein thrombosis		Pulmonary embolism	
	Risk Alleles	**Events**	**HR (95 % CI)***	**Events**	**HR (95 % CI)***
5-SNP score					
No stroke	0-1	55	1 (Reference)	46	1 (Reference)
	2	89	1.08 (0.77-1.52)	72	1.05 (0.72-1.52)
	3-4	169	1.37 (1.01-1.86)	123	1.22 (0.87-1.70)
	≥ 5	27	2.08 (1.31-3.29)	19	1.85 (1.08-3.17)
	HR per increase of risk category		1.24 (1.09-1.41)		1.16 (1.00-1.35)
Stroke	0-1	4	1.38 (0.49-63.87)	2	0.58 (0.14-2.40)
	2	10	2.47 (1.22-4.97)	5	1.16 (0.45-2.98)
	3-4	20	4.51 (2.62-7.77)	15	2.63 (1.42-4.88)
	≥ 5	2	13.30 (3.08-57.5)	2	9.97 (2.28-43.5)
	HR per increase of risk category		1.67 (1.41-1.98)		1.39 (1.14-1.69)

CI, confidence interval; HR, hazard ratio; single nucleotide polymorphisms (SNP)
*Adjusted for age (as time scale) and sex.

Supplementary Table 2. Measures of interaction on an additive scale between ischemic stroke and five or more risk alleles in the 5-SNP score in deep vein thrombosis and pulmonary embolism. The Tromsø Study

	RERI (95% CI)	AP (95% CI)	Synergy index (95% CI)
Deep vein thrombosis	10.85 (-8.28 to 30.0)	0.82 (0.55 to 1.08)	8.42 (1.54 to 46.7)
Pulmonary embolism	8.54 (-5.78 to 22.9)	0.86 (0.66 to 1.05)	20.88 (1.88 to 232)

CI, confidence interval; single nucleotide polymorphisms (SNP); RERI, Relative excess risk due to interaction; AP, Proportion due to interaction

Supplementary table 3. Hazard Ratios for provoked and unprovoked venous thromboembolism for individual single nucleotide polymorphisms according to ischemic stroke exposure. The Tromsø Study

		Provoked VTE		Unprovoked VTE	
	Risk Alleles	**Events**	**HR (95 % CI)***	**Events**	**HR (95 % CI)***
SNP (Gene)					
rs8176719 (*ABO*)					
No stroke	0	100	1 (Reference)	95	1 (Reference)
	≥1	190	1.21 (0.95-1.55)	215	1.44 (1.13-1.84)
Stroke	0	11	2.90 (1.55-5.44)	6	1.78 (0.78-4.09)
	≥1	34	3.82 (2.56-5.70)	9	1.07 (0.53-2.13)
rs6025 (*F5*)					
No stroke	0	255	1 (Reference)	256	1 (Reference)
	≥1	35	1.65 (1.16-2.35)	54	2.58 (1.92-3.46)
Stroke	0	39	3.08 (2.17-4.37)	12	0.95 (0.53-3.72)
	≥1	6	5.74 (2.54-12.99)	3	3.03 (0.97-9.50)
rs1799963 (*F2*)					
No stroke	0	285	1 (Reference)	301	1 (Reference)
	≥1	5	1.20 (0.50-2.91)	9	2.05 (1.06-3.99)
Stroke	0	45	3.17 (2.28-4.40)	14	0.95 (0.55-1.64)
	≥1	0	-	1	20.21 (2.78-146.85)
rs2066865 (*FGG*)					
No stroke	0	161	1 (Reference)	167	1 (Reference)
	≥1	129	0.98 (0.78-1.24)	143	1.05 (0.84-1.31)
Stroke	0	23	2.44 (1.56-3.82)	7	0.76 (0.36-1.64)
	≥1	22	4.39 (2.78-6.92)	8	1.49 (0.72-3.06)
rs2036914 (*F11*)					
No stroke	0	60	1 (Reference)	48	1 (Reference)
	≥1	230	0.89 (0.67-1.19)	262	1.27 (0.93-1.73)
Stroke	0	10	2.93 (1.49-5.75)	2	0.76 (0.18-3.15)
	≥1	35	2.86 (1.87-4.39)	13	1.35 (0.73-2.52)

CI, confidence interval; HR, hazard ratio; single nucleotide polymorphisms (SNP)
*Adjusted for age (as time scale) and sex.

Stiftelsen Kristian Gerhard Jebsen

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