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Risk Factors and Triggers of Venous Thromboembolism in Patients with Myocardial Infarction

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Acknowledgements

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Summary

During the past decades, extensive data from the general population have revealed that patients with acute myocardial infarction (MI) are at increased risk of venous thromboembolism (VTE, i.e., deep vein thrombosis [DVT] and pulmonary embolism [PE]). The risk is highest in the initial 0-6 months following an acute MI, and declines rapidly thereafter. The explanation for the observed association between MI and future risk of incident VTE is yet unknown. The overall aim of the present thesis was to identify triggers and risk factors of VTE in patients with MI that potentially can be used to identify MI patients with high risk of VTE.

In Paper I, we used a case-crossover designed study with the incident VTE cases recruited from the fourth survey of the Tromsø Study. A case-crossover design is well-suited for studying transient risk factors or triggers on the risk of acute events. The study populations for Paper II and Paper III were recruited from the fourth, fifth and sixth surveys of the Tromsø Study. In Paper II, the participants consisted of a subgroup with extended genetic information. Study subjects in Paper II and Paper III were followed from the first survey they attended to the date of an incident VTE, the date of death or migration, or until administrative censoring at the end of follow-up.

First, we found that an MI is a strong trigger factor for VTE, and that indirect risk factors related to the MI, in particular acute infections and immobilization, may to a large extent explain the observed association between MI and VTE. Second, we showed that five prothrombotic genotypes did not explain the increased risk of VTE in MI patients, implying that the prothrombotic genotypes may not play a crucial role in the development of VTE after MI. Third, we demonstrated that the combined effect of MI and obesity on overall VTE risk exceeded the sum of the separate effects. In non-obese subjects, MI was not associated with DVT and unprovoked VTE. Thus, the increased risk of these subtypes of outcomes in MI patients appeared to be dependent on the presence of obesity.

Our findings imply that an acute MI is a strong trigger factor for VTE, and that indirect factors related to hospitalization for MI (i.e., acute infections and immobilization) and concomitant obesity could be essential in the risk assessment of VTE after MI.

Sammendrag

I løpet av de siste tiårene har flere befolkningsstudier vist at pasienter med akutt hjerteinfarkt har økt risiko for å utvikle venøs tromboembolisme (VTE, fellesbetegnelse på dyp venetrombose [DVT] og lungeemboli [LE]). Hjerteinfarktpasienter har særlig økt risiko for å utvikle LE de første seks månedene etter infarktet. Mekanismene bak denne sammenhengen er fremdeles uklare. Formålet med denne avhandlingen har vært å studere risikofaktorer for VTE hos pasienter med hjerteinfarkt.

I artikkel I brukte vi et case-crossover design bestående av studiedeltakere fra den fjerde Tromsøundersøkelsen. Et case-crossover design er velegnet for å studere hvordan forbigående risikofaktorer påvirker akutt oppståtte sykdommer. Studiedeltakerne i artikkel II og III ble rekruttert fra den fjerde, femte og sjette Tromsøundersøkelsen. Studiedeltakerne i artikkel II bestod av en undergruppe som fikk utført genetiske analyser. I artikkel II og artikkel III ble studiedeltakerne fulgt fra første undersøkelse de deltok i til en VTE hendelse oppstod, til de døde eller flyttet, eller til studieslutt.

Vi fant at akutt hjerteinfarkt var en sterk trigger for VTE, samt at mye av risikoen kunne forklares av andre tilleggsrisikofaktorer slik som akutte infeksjoner og immobilisering. Videre, så fant vi at felles risikogener ikke kunne forklare den økte risikoen for VTE hos infarktpasienter, noe som kan bety at de genetiske risikofaktorer undersøkt i denne studien ikke spiller en sentral rolle for utvikling av VTE hos pasienter med infarkt. Til slutt fant vi at kombinasjonen av akutt hjerteinfarkt og fedme (dvs. BMI≥30) hadde en synergistisk («*mer enn summen av*») effekt på risikoen for VTE. For deltakerne som hadde en BMI<30, var ikke hjerteinfarkt en risikofaktor for hverken DVT eller uprovosert VTE. Dette betyr at fedme mest sannsynlig er en sentral risikofaktor for å utvikle DVT og uprovosert VTE hos pasienter med hjerteinfarkt.

Våre funn tyder på at akutt hjerteinfarkt er en sterk trigger for VTE, og at tilleggsrisikofaktorer som akutte infeksjoner og immobilisering, samt fedme, vil kunne være lovende prediktorer for VTE hos hjerteinfarktpasienter.

List of papers

The thesis is based on the following papers:

I. **Myocardial infarction as a Transient Risk Factor for Incident Venous Thromboembolism: Results from a Population-Based Case-Crossover Study**

    Joakim K. Sejrup, Trond Børvik, Gro Grimnes, Trond Isaksen, Kristian Hindberg, John-Bjarne Hansen, Vania M. Morelli, Sigrid K. Brækkan

II. **Myocardial Infarction, prothrombotic genotypes, and venous thrombosis risk: The Tromsø Study**

    Joakim K. Sejrup, Vania M. Morelli, Maja-Lisa Løchen, Inger Njølstad, Ellisiv B. Mathiesen, Tom Wilsgaard, John-Bjarne Hansen, Sigrid K. Brækkan

III. **Joint effect of myocardial infarction and obesity on the risk of venous thromboembolism: The Tromsø Study**

    Joakim K. Sejrup, Vania M. Morelli, Maja-Lisa Løchen, Inger Njølstad, Ellisiv B. Mathiesen, Tom Wilsgaard, John-Bjarne Hansen, Sigrid K. Brækkan

    Manuscript

Abbreviations

AP	Attributable proportion
APC	Activated protein C
AUC	Area under receiver-operating characteristic curve
BMI	Body mass index
CAD	Coronary artery disease
CI	Confidence interval
COC	Combined oral contraceptive pills
COPD	Chronic obstructive pulmonary disease
CTPA	Computer-tomography pulmonary angiography
CVD	Cardiovascular disease
CVC	Central venous catheterization
DVT	Deep vein thrombosis
ECG	Electrocardiogram
EU	European Union
EV	Extracellular vesicle
FGG	Fibrinogen gamma chain
FVL	Factor V Leiden
GWAS	Genome wide association study
HFrEF	Heart failure with reduced ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HR	Hazard ratio
HRT	Hormone replacement therapy
IBD	Inflammatory bowel disease
ICD	International Classification of Diseases
IR	Incidence rate
KHB method	Karlsson, Holm and Breen method
MAR	Missing at random
MEGA study	Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study
MNAR	Missing not at random

MI	Myocardial infarction
OC	Oral contraceptives
OR	Odds ratio
PCI	Percutaneous coronary intervention
PE	Pulmonary embolism
PTS	Post-thrombotic syndrome
RA	Rheumatoid arthritis
RAM	Risk assessment model
RERI	Relative excess risk due to interaction
RNA	Ribonucleic acid
RR	Relative risk
SNP	Single nucleotide polymorphism
TF	Tissue factor
UNN	University Hospital of North Norway
VTE	Venous thromboembolism
vWF	von Willebrand factor
WHO	World Health Organization

1. Introduction

The first well-documented description of a case compatible with deep vein thrombosis (DVT) can be found in "*La vie et les miracles de Saint Louis*" by Guillaume de Saint Pathus [1]. In 1271, a 20-year-old cobbler named Raoul suffered from unilateral pain and swelling of the right calf that extended up to the thigh. His surgeon advised him to wait and see but Raoul's symptoms worsened, and he developed a leg ulcer. Raoul was then advised to visit the tomb of King Saint Louis where he spent several days praying to the saint. He decided to collect the dust that he found below the tomb and applied it directly to the ulcer. Raoul was miraculously healed. After this first description of a possible DVT case the number of reported cases has increased steadily and important breakthroughs in epidemiology, diagnostics, prophylaxis, and treatment has been made.

Figure 1 Clinical signs of a DVT include the classical signs of inflammation: Swelling, pain, redness, and loss of function. Illustration from Heilman, via Wikimedia Commons [8]

Venous thrombosis is a disease of blood coagulation. Venous thromboembolism (VTE) is a common term used to describe blood clots in the large, deep veins of the body [2], mainly in the deep vessels of a leg (DVT) or in the pulmonary circulation (pulmonary embolism [PE]). Rarely, VTE occurs in the upper extremities, portal, mesenteric or large cerebral veins as well [3]. Studies indicate that up to 50% of PEs originate from a DVT, where the complete thrombus or parts of a thrombus have dislodged and traveled with the blood stream and wedged in the pulmonary vasculature as the vessels decrease in diameter [4]. Alternatively, an embolus might have formed in the right atrium of the heart, or the thrombus could arise de novo in the lungs due to local inflammation-driven coagulation or stasis in the cardiopulmonary circulation. Approximately 20% of PE events in the Tromsø Study was explained by atrial fibrillation [5].

Clinical symptoms of VTE are vague and unspecific. A lower limb DVT often presents with swelling, pain and redness of the affected limb (Figure 1) [6-8]. Depending on the size and location, a PE may present with shortness of breath,

pleuritic chest pain, fever, hemoptysis, apprehension or ultimately sudden death [9, 10]. Because of the unspecific symptoms and potentially fatal complications of DVT, referral for investigation from primary care is a common issue in the emergency departments. In fact, the diagnosis of DVT is confirmed in only 20-30% of referred patients [11]. The first step in the diagnostic process is to assess the clinical probability of VTE, which is done by applying the Wells score and Geneva score for DVT and PE, respectively [12]. A low Wells score (i.e., *unlikely* DVT) in combination with a negative D-dimer value (i.e., lower than cut-off) excludes the diagnosis of DVT [12]. Further, a high score (i.e., *likely* DVT) leads to a definite diagnostic radiological procedure, and the DVT diagnosis is in most cases made by ultrasound examination of the lower extremities. The diagnosis of PE is made by multidetector-row computer-tomography-scanning (CT) with contrast injection (CT- pulmonary angiography [CTPA]) to visualize emboli in the pulmonary arteries. The CTPA investigations in clinical practice today possess a high sensitivity, which has led to increased ability to detect less severe PEs with unknown clinical significance [13].

We can differentiate between acute and chronic complications of VTE. The most frequent and potentially fatal acute complication of DVT is pulmonary embolization, while a rare complication is lower limb ischemia due to venous congestion (i.e., phlegmasia cerulea dolens) [14, 15]. The most common chronic complication is venous insufficiency ultimately leading to the post-thrombotic syndrome; a condition with chronic pain and swelling of the affected extremity that ultimately may lead to venous ulcerations. PTS affects at least one-third of DVT patients within 10 years [16]. Acute complications of PE include pulmonary infarction [17] that leads to disturbances of blood oxygenation and acute heart failure due to increased right ventricular workload and ultimately sudden death [18] while chronic thromboembolic pulmonary hypertension (CTPH) characterized by shortness of breath and chest pain is a well-described rare long-term complication with an estimated incidence of 0.5-1.5% [19]. About half of PE patients develop "post PE syndrome", a condition characterized by chronic activity related dyspnea that influence quality of life [20]. Moreover, around 30% of VTE survivors experience a recurrent event within ten years [16]. The risk of recurrence is high for patients with persisting risk factors such as underlying inherited thrombophilia or malignancy, while the recurrence risk is low for patients with transient risk factors such as surgery. In addition, data from two Norwegian cohorts [21] and one

Danish cohort [22] have shown that VTE is associated with permanent work-related disability. Thus, indirect costs due to loss of work time may add to the economic burden [23] of VTE.

VTE is treated with anticoagulants [12, 24]. Direct oral anticoagulants (DOACs) are the preferred treatment option and have replaced vitamin K antagonist (VKA) monotherapy due to the lower bleeding risk, rapid onset of action, predictable dose-effect response and ease of administration that does not require regular monitoring. In the setting of severe and potential life-threatening VTE, intravenous unfractionated heparin (UFH), systemic thrombolysis, catheter-directed thrombolysis or thrombectomy are recommended treatment options. Additionally, in several high-risk situations, anticoagulants are given as prophylaxis, aiming to prevent VTE [25, 26].

1.1 Epidemiology- VTE

VTE is a major health concern in the European countries [27], where DVT and PE affects approximately 680 000 and 435 000 individuals per year, respectively [28]. The incidence rates (IRs) of VTE in the general population have increased slightly over time, mainly due to an increase in PE [29-32]. Better diagnostic tools may to some extent explain the increased PE incidence. The increase in VTE incidence may, however, have several explanations [16]. First, the incidence could reflect an enlargement of the population at risk as the population is getting older and have more comorbidities [31]. Second, exposure of the population to more risk factors [33] such as surgery, obesity and cancer could also be a potential explanation. Third, despite an increased focus on thromboprophylaxis [34], inadequate identification of high-risk individuals with subsequent under-utilization of prophylaxis may also contribute to the increased VTE incidence. Of note, IRs of VTE are lower in Asians, Pacific Islanders and Hispanics than in Caucasians [2], and studies have reported almost 25% higher rates in African-Americans [2].

VTE is associated with considerable mortality, and the rates differ between DVT and PE (1). Almost 25% of all PE cases essentially present as sudden death [18], and autopsy data show that 3-5% of all adult deaths may be a result of PE [35]. The one-week survival rate for overall VTE have been reported to be 70-90%, and for DVT and PE around 96% and 59%, respectively [16, 36, 37]. The 1-year all-cause mortality after VTE is approximately 10-30% [38, 39]. Indeed, epidemiological models estimate that there are approximately 540 000 VTE-related deaths each year in Europe [28].

Currently well-known risk factors for VTE accounts for 60-75% of all VTE cases in the general population [18, 30], implying that 25-40% of all VTE cases in the community are unprovoked. Classification of a VTE event as either provoked or unprovoked has important prognostic implications [40]. A provoking environmental factor may be either transient (e.g., surgery, puerperium) or persistent (e.g., metastatic cancer), depending on the length of time it takes for the risk factor to resolve. Patients with neither a transient nor persistent provoking risk factor for VTE are often classified as unprovoked VTE.

1.2 Pathophysiology- Thrombosis

1.2.1 Hemostasis in the wrong place

Hemostasis is a sophisticated, finely tuned process balancing pro- and anticoagulant forces. Primary hemostasis is the process of glycoreceptor-mediated binding of platelets to von Willebrand factor (vWF), with further aggregation, activation, and release of secondary aggregators like thromboxane A_2, ADP and serotonin. This results in the assembly of platelets that expose anionic phospholipids serving as a platform for coagulation factor enzymes [41]. The coagulation cascade is further triggered by the expression of tissue factor (TF) that binds circulating factor VII [42] (the extrinsic pathway of coagulation) (Figure 2). Factor VII, an inactive zymogen, is then converted into factor VIIa. The TF:VIIa complex activates factor IX and factor X into factor IXa and factor Xa, respectively. Thrombin is subsequently formed. Several potential activators of the intrinsic pathway of coagulation have been proposed, like extracellular ribonucleic acid (RNA) and polyphosphates. Factor XIIa activates factor XI, and factor XIa further activates factor IX to factor IXa. Factor

Figure 2 A brief overview of the coagulation cascade, including the intrinsic (blue), extrinsic (green) and common (orange) pathways. Product: Fibrin (clot). Adapted from Mackman N., J Clin Invest 2012

IXa forms a complex with factor VIIIa, which ultimately activates factor X to factor Xa in the common pathway. The end-product of coagulation is cross-linked fibrin (secondary hemostasis). Primary and secondary hemostatic mechanisms reinforce each other, as thrombin generation amplifies activation of platelets and coagulation factors [43]. Further, thrombosis was described as *hemostasis in the wrong place* by MacFarlane in 1977 [44] as various pathological processes trigger intravascular thrombosis associated with different diseases [42, 45].
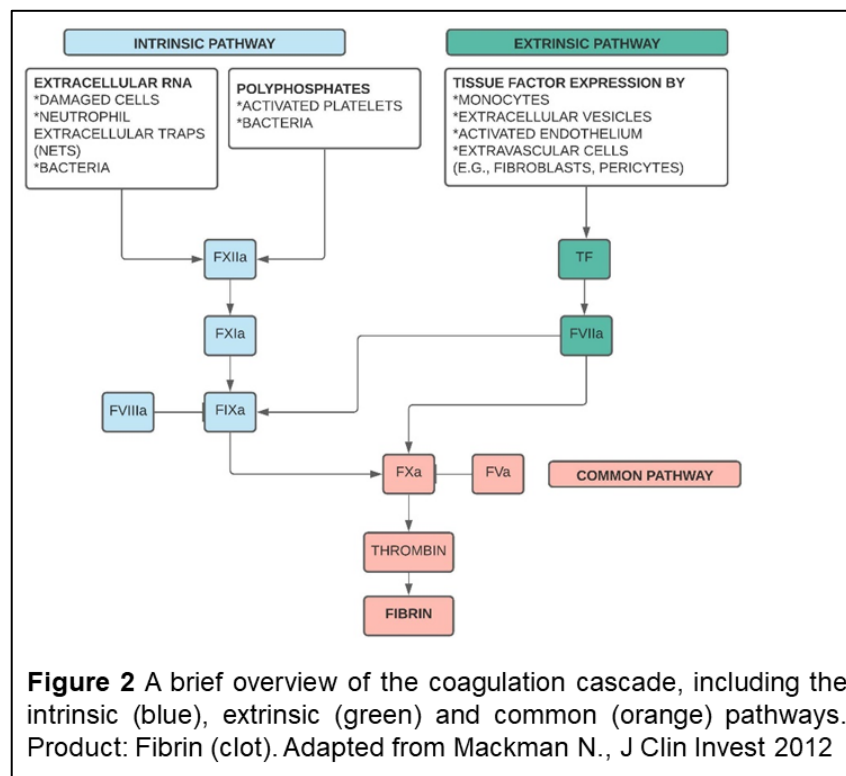
1.2.2 Venous thrombosis

Under normal circumstances the venous blood flow is pulsatile with valve leaflets opening and closing approximately 20 times each minute when standing, which ensures a vortical flow in the valve pockets that prevents stasis [45]. The turbulent blood flow and effect of gravity may lead to formation of secondary vortexes, which induces localized and intermittent hypoxia in the valve pocket [46]. Hypoxia might cause downregulation of natural anticoagulant proteins on the endothelial surface (i.e., protein C- receptor, tissue factor pathway inhibitor, thrombomodulin and heparin-like proteoglycans), promoting prothrombotic processes [46]. Additionally, the valve leaflets do not contain vasa vasorum (i.e., intrinsic blood supply to the vessel wall) and are thus reliant on venous blood oxygenation [45]. Furthermore, hypoxemia also promotes activation of leukocytes and platelets, which in turn release TF-containing extracellular vesicles (EVs) [47]. The activated leukocytes themselves might express TF on the cell surface, recruit platelets and release neutrophile extracellular traps [48, 49]. Neutrophile extracellular traps are part of the innate immune response and suggested to play a key role in inflammatory-mediated thrombosis.

1.3 Risk Factors for Incident VTE

A risk factor is any factor that affects the incidence of disease occurrence [50, 51]. Thus, the *probability* of disease occurrence is higher in the presence of these factors, which can be genetic, acquired or a combination. A trigger factor is the proximal cause of disease, closer to the onset of outcome than most of the other causes under consideration [52]. Therefore, a trigger is a relative term. The thrombosis potential model was first described in 1999 [53]. The idea of this model is that all individuals are at risk for VTE during life, which is reflected as the "thrombosis potential", and that all risk and trigger factors contribute to the potential (Figure 3). Combination of risk factors, either persistent or transient, contribute to the thrombosis potential ultimately leading to a VTE event when sufficient pro coagulant factors are present and outweighs anticoagulant mechanisms, and the thrombosis threshold is exceeded [53].
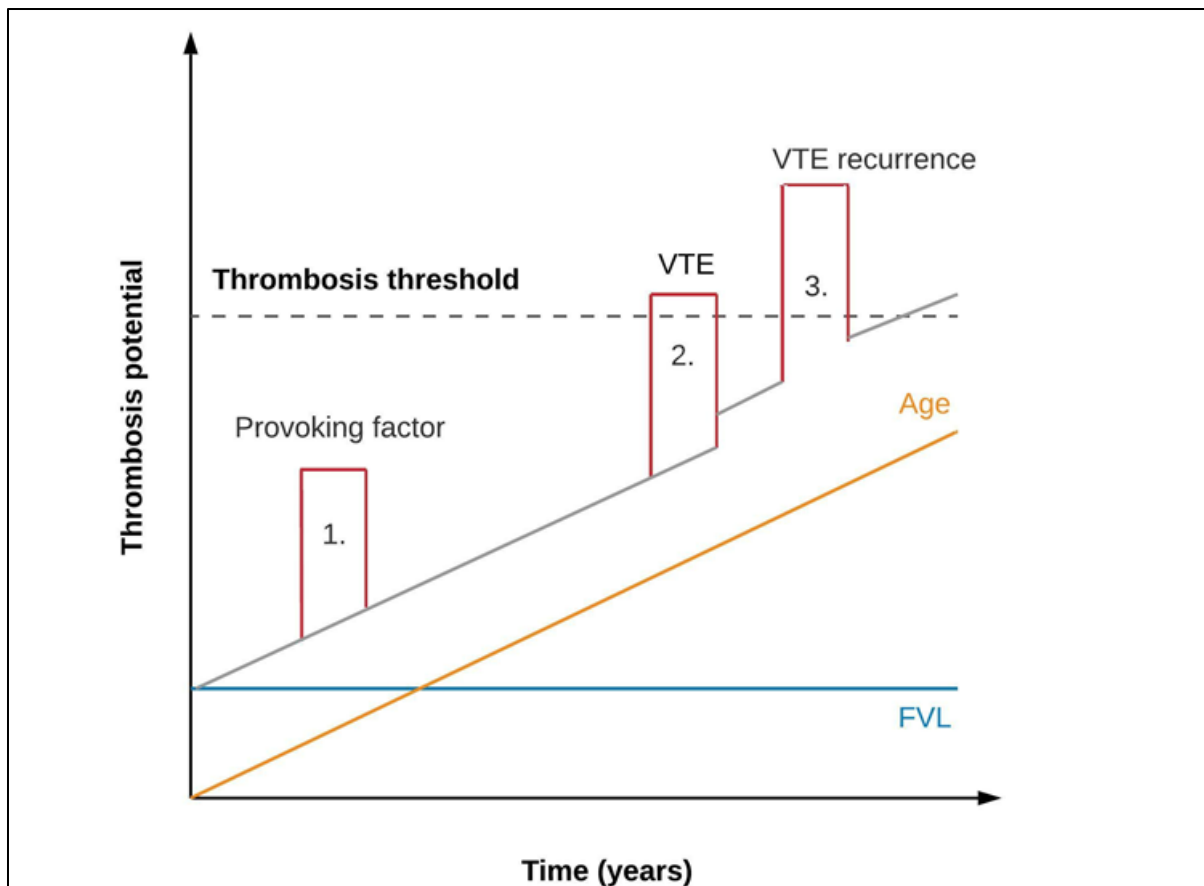
Figure 3 The thrombosis potential model adapted from Rosendaal [53]. The blue line represents the effect of a genetic exposure (here: Factor V Leiden [FVL]), which is stable over time). The orange line represents the effect of age. The grey line represents the joint effect of a genetic exposure and age, in combination with provoking risk factors (red) early (1.) and later in life (2. and 3.). The second provoking factor causes the patient to cross the thrombosis threshold and causes a VTE event. The third flare causes a recurrent VTE event

In 1862, Dr. Rudolph Karl Ludwig Virchow postulated a triad of pathophysiological mechanisms of thrombosis [54], that included (i) altered hemostatic balance (hypercoagulability), (ii) reduction in flow velocity or pulsatility (stasis) and (iii) loss of vascular wall integrity (endothelial damage or dysfunction). The different risk factors for VTE acts through one or more of the branches of Virchow's triad in promoting venous thrombus formation (Figure 4).

Figure 4 Virchow's triad flowchart
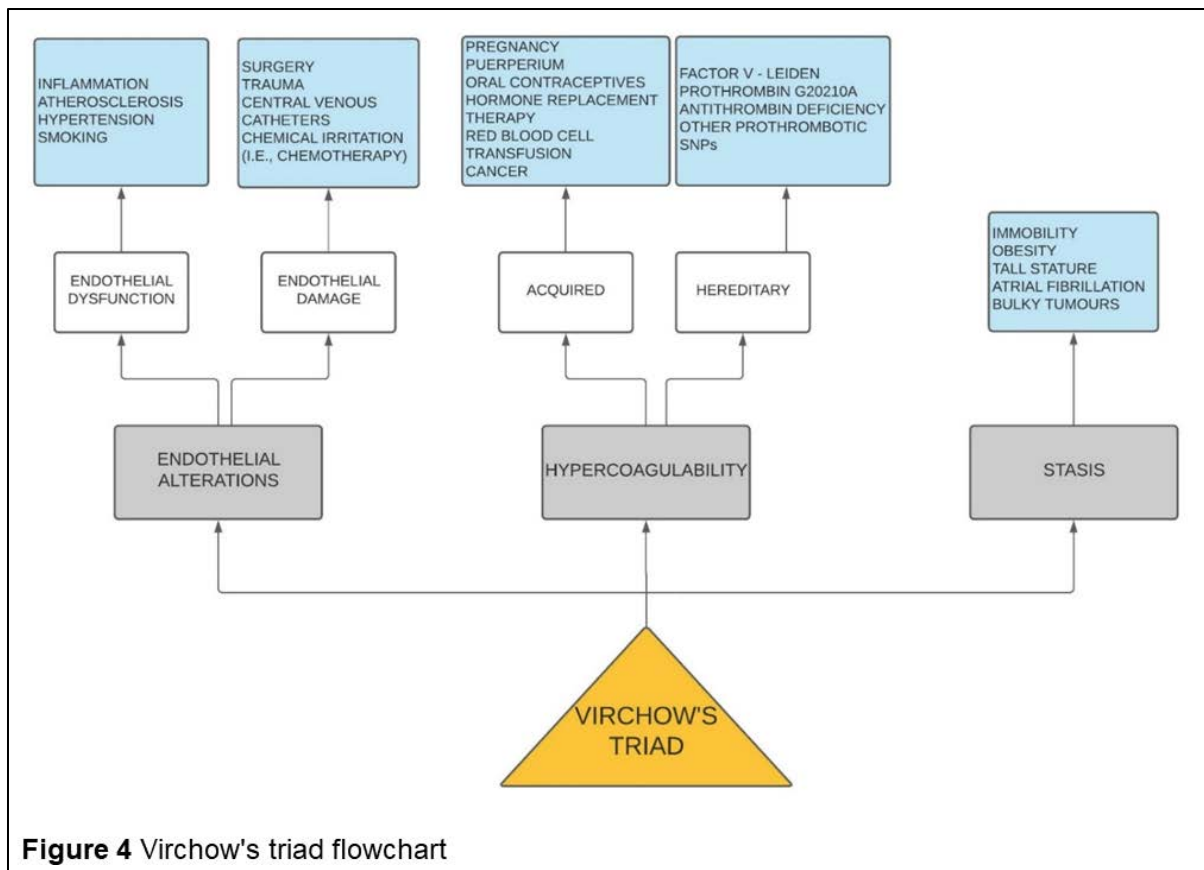
1.4 Heritability and SNPs

Family- and twin studies have demonstrated that VTE is a highly heritable disease [55, 56]. The estimated heritability of VTE is approximately 60% [55, 57] and 50% of patients with a first unprovoked VTE have an identifiable inherited thrombophilia [3]. Single nucleotide polymorphisms (SNPs) are base pair substitutions at specific DNA loci that appears in the population at different frequencies (reported as mean allele frequency [MAF]) [58], and several SNPs associated with increased thrombosis tendency (prothrombotic genotypes) have been identified [3, 59, 60]. Two mechanisms by which SNPs increase thrombosis tendency are gain-of-function or loss-of-function mutations. Gain-of-function mutations lead to an up-regulation of the *concentration* (e.g., non-O blood type and Prothrombin G20210A) or *activity* of a normal protein, or impaired down-regulation of a normal protein (e.g., Factor V Leiden [FVL]). Loss-of-function or inactivation mutations are less common and lead to gene products having less or no function (e.g., Antithrombin- and Protein C/Protein S deficiencies).

1.4.1 Genetic risk factors for VTE

1.4.1.1 Gain-of-function mutations

The *ABO* locus has three main allelic forms- A, B and O, which give rise to six possible genotypes and four possible phenotypes [61]. ABO blood group carbohydrate structures are expressed on various cells including platelets and vascular endothelium [62]. The most prevalent phenotype in most populations is blood group O, with a frequency of approximately 40% among Caucasians. Furthermore, the rs8176719 in *ABO* is the most common prothrombotic genotype and differentiates non-O from O blood types. Non-O type is present in 60-70% of the population [63] and is correlated with higher levels of factor VIII and vWF in plasma [61, 64]. The non-O blood type is known to increase the risk of VTE 1.5-2.0-fold [63, 65, 66].

Table 1. The relationship between blood group (phenotype), antigen(s) present on the erythrocytes, antibodies in serum and corresponding genotype(s) [61]

Blood Group	Antigen(s) present on erythrocytes	Antibodies present in serum	Genotype(s)
A	A antigen	Anti-B	AA or AO
B	B antigen	Anti-A	BB or BO
AB	A antigen and B antigen	None	AB
O	None	Anti-A and anti-B	OO

The Prothrombin G20210A variant is present in 2-4% of the population, mainly in Caucasians, and is the second most common prothrombotic genotype [3]. The variant is a single base mutation in the untranslated region of the gene promoter, which leads to an overproduction of Prothrombin by altering messenger-RNA (mRNA) expression. Heterozygous subjects have a 30% increase in the concentration of Prothrombin, while homozygous subjects have at least 70% increased concentration [67]. The G20210A variant in the Prothrombin gene is associated with a 2.5-fold increased risk of venous thrombosis [65].

FVL is found in up to 5% of the Caucasian population, while in Asian, African and indigenous Australian populations, the mutation is rare [68]. The variant is caused by a point mutation (missense) in the Factor V gene, which eliminates one of the three activated protein C (APC) cleavage sites, and causes the majority of APC resistance

[69]. Activated Factor V does not act as an enzyme but as a co-factor in the coagulation cascade. Protein C is a natural anticoagulant protein that cleaves and thereby inactivates procoagulant Factor Va and VIIIa resulting in downregulation of Thrombin production [70]. Studies have reported a 2-3 and 15-20-fold increased risk of venous thrombosis conferred by FVL for heterozygous and homozygous carriers, respectively [70].

The rs2066865 in the *FGG* locus appears in the general population with an allele frequency of 25% [65]. The variant is associated with decreased Fibrinogen gamma chain (FGG) -concentration which enhances thrombosis risk 1.5-fold [65]. The mechanism behind the prothrombotic properties of the FGG variant is not fully understood, however, the FGG has demonstrated to increase APC sensitivity [71]. Lowered levels of the FGG could therefore lead to APC resistance.

The rs2036914 in the *F11* locus is common, with a risk allele frequency of 52%. Results from the Leiden Thrombophilia (LETS) and Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) studies have shown that the SNP is independently associated with DVT, increasing VTE risk 1.4-fold, presumably through increased FXI levels [65, 72].

1.4.1.2 Loss-of-function mutations

Antithrombin, Protein C and Protein S are all natural anticoagulants produced in the liver. Antithrombin deficiency is a rare genetic variant present in 0.2% of the population [68]. Antithrombin, a serine protease inhibitor, inhibits the function of Thrombin and Factor Xa. Antithrombin deficient patients are heterozygous for the Antithrombin defect as homozygosity is not compatible with life [3]. Antithrombin deficiency may present as either type I deficiency (both Antithrombin activity and antigen are reduced to the same extent) or type II deficiency (higher Antithrombin antigen than activity levels), the latter is indicative of a functional defect in the Antithrombin molecule [3]. Antithrombin deficiency is associated with a 10-50-fold increased risk of VTE [73]. Protein C and Protein S deficiency is present in 0.03-0.2% of the population [65]. Protein S serves as a cofactor for Protein C, together inactivating Factor Va and VIIIa [68]. In heterozygous carriers, Protein C/Protein S-deficiency is associated with a 10-fold increased risk of VTE [74].

Table 2. Known prothrombotic genotypes associated with VTE risk [65]

Gene	Site	Phenotype	Frequency	VTE OR
Genes associated with VTE identified before GWAS				
F2	rs1799963	↑FII	0.02	2.5
F5	rs6025	APC resistance	0.05	3
FGG	rs2066865	↓Fibrinogen γ	0.25	1.47
ABO	rs8176719	↑VWF, ↑FVIII	0.3	1.5
HIVEP1	rs169713	VTE	0.21	1.2
PROCR	Multiple	↓Protein C	rare	~10
PROS1	Multiple	↓Protein S	rare	~10
SERPINC1	Multiple	↓Antithrombin	rare	~10
Novel SNPs associated with VTE identified by GWAS				
VWF	rs1063856	↑VWF	0.37	1.15
TC2N	rs1884841	↑VWF	0.44	1.27
STXBP5	rs1039084	↑VWF	0.46	1.11
GP6	rs1613662	↑platelet function	0.82	1.15
F11	rs2289252	↑F11	0.41	1.35
F11	rs2036914	↑F11	0.52	1.35
C4BPB/C4BPA	rs3813948	↑C4BP	0.08	1.18
KNG1	rs710446	↓aPTT	0.45	1.2
SERPINC1	rs710446	↓Antithrombin	0.1	1.29
TSPAN15	rs78707713	Unknown	0.88	1.28

1.4.2 GWAS and De Haan Score

During the past two decades, high-throughput micro-array-based genotyping and genome-wide association studies (GWAS) have identified genes that underlie common diseases and related quantitative/complex traits [75-77]. *Morange and Tregouet* have described some of the novel associations from the GWAS era in venous thrombosis [65] (Table 2), and the reported effect sizes regarding VTE risk associated with the SNPs are generally low (odds ratios [ORs] ranging between 1.11 to 1.35). A more recent GWAS study including a transcriptome-wide association study (TWAS) has discovered additional gene variants and loci associated with the risk of VTE [78] and demonstrate similar effect sizes on VTE risk as previous studies. Indeed, genetic variants with a strong impact on VTE risk are uncommon and are often private mutations (i.e., present in less than 0.1% of the population) [76]. Moreover, the strength of association and effect sizes obtained suggest that the most important genetic variants associated with VTE risk have been discovered [78, 79]. Even though the new SNPs are associated with a low risk increase of VTE, they may be of clinical

significance if they interact with other VTE risk factors (either genetic or acquired), yielding supra-additive effects on VTE risk.

Hugoline de Haan and colleagues investigated whether 31 venous thrombosis associated SNPs could predict VTE risk [80]. A model based on 31 SNPs and a more parsimonious model based on the 5 most strongly associated SNPs with VTE risk had similar predictive abilities, with areas under receiver-operating characteristic curve (AUC) or c-statistics of 0.70 and 0.69, respectively (Figure 5). When environmental risk factors were included (i.e., obesity, recent leg injury, surgery, immobilization, pregnancy/puerperium, oral contraceptive use, hormone replacement therapy and cancer), the c-statistic further increased. Thus, the discriminative accuracy of the model improved. Genetic prediction scores might be clinically useful and cost-effective in high-risk patients, and identification of risk factors and biomarkers can prove helpful in identifying subjects who will benefit from genetic profiling.
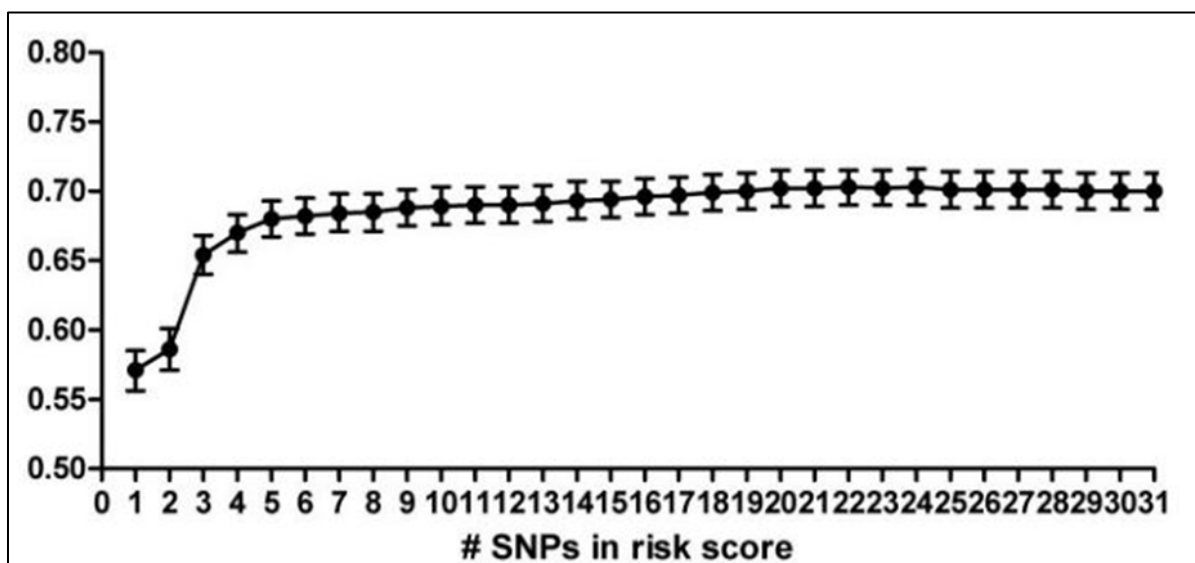

Figure 5 AUC of genetic risk scores based on increasing number of SNPs [80]. SNPs were added in order of the OR as found in the literature. The slope of the curve flattened out when the five SNPs with the highest OR for VTE had been added

1.5 Acquired risk factors

1.5.1 Age and gender

Increasing age is a well-established risk factor for VTE in both men and women [29]. The risk increases exponentially with age from 1 per 10 000 in young adults to 1 per 100 in elderly [2, 50]. During childbearing age, the IRs are somewhat higher in females. However, between the age of 45 to 70 years the rates are essentially higher in men (Figure 6) [2, 16, 30]. The estimated lifetime risk of VTE is marginally higher in women than in men when competing risk of death is taken into account [81]. PE

Figure 6 Age- and sex specific incidence rates of VTE. The Tromsø Study 1996-2012 [30]
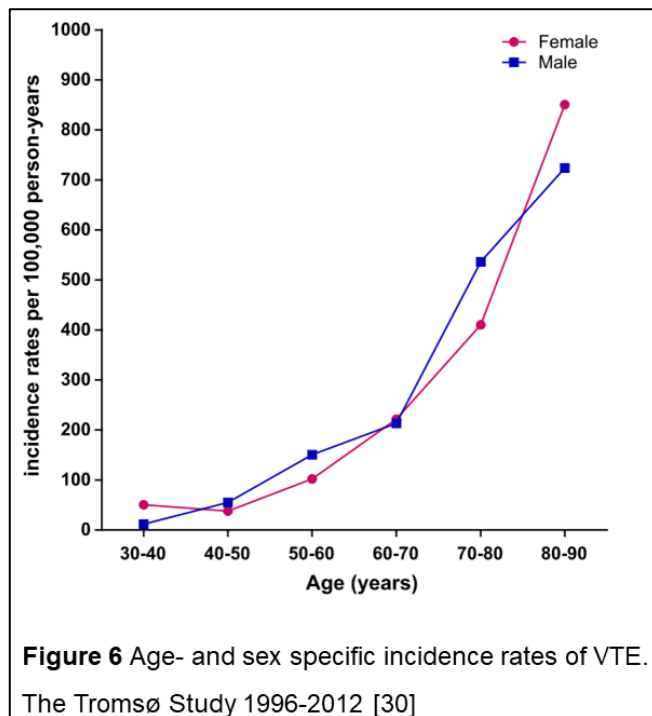
accounts for an increasing proportion of VTE with increasing age for both genders [82]. Therefore, a higher case fatality of VTE is seen in the older ages [39, 83]. The reason why age is such an important risk factor for venous thrombosis is not completely elucidated, however, accumulation of other illnesses affecting the thrombosis potential may be a plausible explanation. *Increasing age* probably is a container concept with a mix of unknown and known risk factors that either become stronger or more prevalent with age. Furthermore, the biological effects of aging on blood vessels, connective tissue and valves, may promote thrombosis as well [83], and a larger increase in procoagulant (e.g., FVII, FVIII, FX and Fibrinogen) than anticoagulant factors with age presumably play a role [84].

1.5.2 Obesity

The rising prevalence of obesity, especially in the Western countries, is a public health concern. Almost 40% of the world's population was either overweight (body mass index [BMI]≥25 kg/cm^2) or obese (BMI≥30 kg/cm^2) in 2016 according to WHO [85]. Obesity is associated with a 2-3-fold increased risk of VTE [86]. Moreover, a wide range of obesity measures (e.g., BMI, WC [waist circumference], HC [hip circumference], WHR [waist-hip-ratio], WHtR [waist-to-height-ratio]) have demonstrated to be associated

with VTE risk [87], though WC identified most subjects at risk and was the strongest predictor of VTE. Additionally, emerging evidence from Mendelian randomization studies have reported BMI as a causal risk factor for VTE [88, 89], and the association is stronger as the BMI increases [90]. The underlying pathophysiological mechanisms by which obesity increases the risk of venous thrombosis could be physical immobilization, mechanical impact exerted by adipose tissue on veins that impairs venous blood flow, or through the production of prothrombotic adipokines or estrogens [91-93]. C-reactive protein (CRP) was a mediator of VTE risk in a population-based study [94], implying that low-grade inflammation could play a pivotal role in the chain of causation in obesity related VTE.

1.5.3 Reproductive-related risk factors

Current use of estrogens in terms of **combined oral contraceptive pills** (COCs) and **hormone replacement therapy** (HRT) are associated with increased risk of VTE [95]. VTE is a well-known complication even of the contemporary low-dose COCs, and is associated with a 2-3-fold increased risk in users compared to non-users [96]. The risk of VTE in users of COCs decreases with the duration of use, and the risk is highest the first year [97]. Further, HRT is associated with a 2-4-fold increased risk of VTE in postmenopausal women [98-101], and as for COC users the elevated risk is restricted to the first year of treatment.

Pregnancy can be seen as a hypercoagulable condition, which presumably evolved to protect women from hemorrhage during miscarriage and childbirth [102, 103]. The increased risk of VTE associated with pregnancy is higher than that associated with COC use [104]. The elevated VTE risk is present as early as the first trimester because of lowered Protein S levels and increased concentration of Fibrinogen, factors VII, VIII, X and vWF, however, the risk is particularly high during the third trimester (6-fold increased) and persists for about 3 weeks post-partum (puerperium) (22-fold increased) [105]. With regard to prophylaxis and treatment of pregnancy related VTE, low-molecular-weight heparin (LMWH) has demonstrated to be the drug of choice because the molecule does not cross the placenta [102].

1.5.4 Surgery and trauma

One well-described risk factor for VTE is recent surgical intervention [82], and hospitalization for surgery accounts for 24% of VTE cases [18]. Patients hospitalized for surgery had a nearly 22-fold increased odds of VTE [16], however, the risk

estimates differ both according to surgery and anesthesia methods. Major orthopedic surgery is the strongest risk factor for VTE, where studies on total hip (HR of 15.84 [95% CI: 13.12-19.12]) [106] and knee replacement (OR 1.96 [95% CI: 1.35-1.61]) [107] demonstrate the highest risk estimates. Further, studies report a lower VTE risk associated with regional anesthesia (i.e., spinal or epidural) than general anesthesia. Duration of general anesthesia also plays a role for VTE risk as operations requiring anesthesia for more than 30 minutes are associated with highest risk [40]. Recent trauma requiring hospital admission (i.e. major fracture or severe soft tissue injury) is a risk factor for VTE with a reported 13-fold (OR 12.69, 95% CI: 4.06-39.66) increased odds [82]. Surgery and major trauma lead to a hypercoagulable state with immune- and coagulation activation through tissue and vessel wall damage, and are potent initiators of thrombosis [108].

1.5.5 Hospitalization for medical conditions

Up to 50% of VTE cases in the general population can be attributed to **hospitalization** [109, 110], and 22% of VTE cases in general populations are attributed to hospitalization for medical illness [18, 111]. Additionally, hospitalization is a strong trigger factor for VTE (OR 12.3 [95% CI: 6.4-23.6]) [109]. Between 70-80% of all fatal PE events occur in hospitalized medical patients [112].

Immobilization contributes substantially to the VTE risk among hospitalized patients [109]. Patients can be immobilized because of an acute or chronic medical condition, after surgical procedures, paresis after stroke or because of increasing age and frailty, which hampers mobility [2, 113]. Among hospitalized patients, immobilization is associated with a 1.5-3.0-fold increased risk of VTE [114]. When comparing immobilization for >72h and >7 days regarding VTE risk, a slightly stronger association is observed in patients confined to bed >7 days [115]. Immobility, especially in the supine position, increases thrombosis risk presumably via calf muscle and diaphragm dysfunction, which decreases venous flow in the deep veins of the legs and inferior vena cava, and causes venous stasis [114].

Medical complications after **stroke** are common and associated with prolongation of the hospital stay and increased mortality [116]. VTE is a stroke-related complication in 1-4% of strokes patients, where DVT is more common than PE [117]. However, PE accounts for up to 25% of early deaths after stroke [118]. DVT is a major concern particularly in patients with limb paralysis, where the degree of paralysis

correlates with DVT risk. Prospective studies that used 125-labeled fibrinogen imaging of the lower limbs post-stroke have detected DVT, in the absence of anticoagulation, in 50% of patients 2 weeks after the acute stroke event [118]. Another study on patients in a stroke rehabilitation unit, demonstrated bilateral DVT on venography in 33% of cases 9 weeks after stroke. Ischemic stroke increases VTE risk 10-fold during the 3-month period after the acute event, and the risk is particularly high for provoked VTE [119]. Stroke was a strong trigger factor for VTE in a case-crossover study [120], and the association between stroke and VTE risk appeared to be largely explained by immobilization and infection. Comorbidity, particularly cancer, further increases the risk of VTE within 3 months after stroke [121].

Acute inflammation in response to severe **infection** may result in a systemic activation of procoagulant mechanisms [122, 123], mainly coagulation activation by TF [123]. Hospital-diagnosed systemic respiratory tract infections, urinary tract infections, skin infections, intraabdominal infections and septicemia were associated with a 3.3-fold increased risk of VTE, while outside-of-hospital antibiotic treatment was associated with a 2.6-fold increased risk in a population-based case-control study from Northern Denmark [124]. In a case-crossover study of 707 VTE patients from the Tromsø cohort, acute infection was associated with a high VTE risk (OR 24.2 [95% CI: 17.2-34.0]) that was attenuated after adjustment for immobilization (OR 14.6 [95% CI: 10.1-21.2]) [125]. In the Health and Retirement Study, infection was the most common trigger for hospitalization for VTE [126].

The IR of VTE after a **cancer**-diagnosis was 12.4 per 1000 per year during the first 6 months in a cohort study [2]. Metastatic disease and treatment with chemotherapy and hormonal therapies further increase the risk [127]. Cancer originating from the pancreas, ovaries, lymphatic tissue, bone and brain were associated with the highest IRs [2]. In addition, cancer and institutionalization (hospitalization or nursing home confinement) jointly accounted for 65% of the incident VTE cases in the general population [18]. Cancer-related VTE is associated with a high case-fatality rate, with a 1-year case-fatality rate reported to range from 63% to 88% [39, 128]. *Khorana and colleagues* developed a prediction model for VTE in cancer patients in 2008 [129], and several risk assessment models (RAMs) have been developed during the recent years [130]. However, none of the models had sufficient discriminating properties to justify introduction to clinical practice. Why cancer patients

are at increased risk of VTE could be due to multiple interacting factors, including hospitalization, surgery, chemotherapy, or a direct mechanical effect by the tumor on venous drainage. It has also been shown that certain cancer types produce thrombogenic TF- and polyphosphate loaded EVs which activate both the extrinsic and intrinsic pathways of coagulation [83, 131, 132], and EVs may therefore potentially serve as a link between cancer and VTE.

Heart failure increases the risk of VTE (PE in particular), and the increased risk persists through long term follow-up [133-135]. Results from the Atherosclerosis Risk in Communities (ARIC) cohort demonstrated that heart failure is associated with a long term 3-fold increased risk of VTE. The risk of VTE is similar in patients with heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) [134]. This indicates that all components of Virchow's triad are part of the pathogenesis of heart failure-related VTE.

During the period of hospitalization, both **central venous catheterization** (CVC) and transfusion of blood products may be required for various purposes. Prior CVC was responsible for 9% of VTE cases in a population based study from Olmsted county [18]. Placement of a CVC was a strong risk factor for upper extremity VTE, with a more than 5-fold increased risk even after adjustment for comorbid conditions in which such catheters likely would be used   [82]. Furthermore, **red blood cell transfusion** was a trigger for hospitalization for VTE in a case-crossover study of patients ≥51 years from the United States [126].

Chronic inflammatory disorders are not considered traditional risk factors for VTE. However, emerging evidence have demonstrated that the conditions probably play a role in VTE, especially during periods of flare of disease activity. A prospective cohort study based on the Tromsø Study have reported a 1.6-fold increased VTE risk in subjects with **chronic obstructive pulmonary disease** (COPD), driven primarily by secondary events (i.e., immobilization or bronchial superinfection) in relation to hospitalization for acute exacerbations [136]. The combination of a COPD diagnosis with lowered SpO_2 and respiratory symptoms (e.g., dyspnea, cough and phlegm) had additive effects on VTE risk [137]. Results from a large case-control study from Denmark illustrated that **autoimmune connective tissue diseases**, in particular juvenile rheumatoid arthritis and systemic lupus erythematosus (SLE), were associated with VTE risk, whereas autoimmune skin diseases were not associated with

risk of VTE [138]. In addition, studies have reported **rheumatoid arthritis** (RA) as a risk factor for VTE in both hospitalized medical patients (relative risk [RR] 1.99 [95% CI: 1.98-2.00]) [139] and in the general population (RRs around 1.4) [140, 141], even after adjustment for several VTE risk factors that could potentially confound the relationship between RA and VTE. Systematic reviews and meta-analyses showed essentially similar risk estimates [142, 143]. Studies on **inflammatory bowel disease** (IBD) (i.e., morbus Crohn and Ulcerative colitis) have also demonstrated an association between IBD and VTE, with an approximately two-fold increased risk of VTE [144], and in periods of flares the risk increase is even more prominent (RR 15.8 [95% CI: 9.8-25.5]) [145].

1.6 Risk Factor Interactions

As described in Section 1.4 of this thesis, VTE has a well-known and strong genetic component [146, 147]. In combination with exposure to provoking risk factors or triggers of VTE during life, the thrombosis potential may exceed the thrombosis threshold and lead to clinical manifest venous thrombosis. Therefore, VTE is a complex, multifactorial disease encompassing environmental and genetic risk factors, as well as environment-environment, gene-environment, and gene-gene interactions [53, 59, 148]. Indeed, results from large population-based studies conducted during the last decades have demonstrated more than additive effects of several exposures on VTE risk. A report from the Tromsø Study showed synergistic effects between increasing BMI and tall stature on VTE risk [149] with an almost 5-fold increased risk in tall, obese men, and 3-fold increased risk in tall, obese women. Reports from the MEGA Study have demonstrated that obese women who used oral contraceptives (OCs) had a 24-fold higher VTE risk than women with a normal BMI who did not use OCs [150]. Results from the same MEGA Study have demonstrated joint effects of FVL and obesity, and Prothrombin G20210A and obesity on VTE risk with 8- and 7-fold increased risk [150] compared to normal weight non-carriers, respectively. Further, data from The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study have shown that the combined effect of OC use and FVL yielded an almost 16-fold increased risk of VTE [151]. Prothrombotic genotypes, either evaluated as individual SNPs or as part of a genetic risk score, augmented VTE risk in patients with ischemic stroke in a report from the Tromsø Study [152]. Moreover, the combination of cancer and variants in the *F5* gene yielded a synergistic increased risk of VTE in the Tromsø Study [153].

1.7 Myocardial Infarction (MI) – Epidemiology, Atherothrombosis and Cardiometabolic Risk Factors

The most common coronary artery disease (CAD) is ischemic heart disease, with a spectrum of clinical manifestations ranging from stable angina pectoris to fulminant ST-elevation MI [154]. An MI is defined by the European Society of Cardiology (ESC) as myocardial injury with necrosis in a clinical setting consistent with ischemia [155]. The prevalence of CAD in U.S. adults ≥20 years of age is 2.8% (4.0% for men and 1.8% for women) [156]. Globally, CAD is the leading cause of morbidity and mortality [157]. Cardiovascular diseases (CVDs) are responsible for about 51% of deaths in women and 42% of deaths in men, with CAD contributing to about half of these deaths [158].

Atherosclerosis is hardening of an artery specifically due to an atheromatous plaque, a process that starts early in life and progresses at different rates depending on environmental exposures and genetic factors [159, 160]. A thrombus may form on top of a ruptured atherosclerotic plaque (i.e., atherothrombosis) in a coronary artery, leading to clinical manifest MI as ruptured atherosclerotic plaques expose collagen, vWF and TF to circulating platelets, coagulation factors, immune cells, and other blood components [161, 162]. The arterial thrombi are platelet rich and often referred to as white thrombi, and typically form in locations where shear stress is high on damaged endothelium. On the contrary, venous thrombi are rich in fibrin and red blood cells (red thrombi) and are typically formed under low shear stress on intact endothelium.

The well-established cardiometabolic risk factors contributing to the development of MI are hypertension, diabetes mellitus, dyslipidemia, smoking and abdominal obesity [163, 164]. Additionally, the metabolic syndrome is a constellation of risk factors that separately and together is associated with CVD risk [165]. The syndrome consists of increased waist circumference, dyslipidemia, insulin resistance and hypertension, which in combination increases the CVD risk 2-fold [166]. Recent results from the Tromsø Study show that secondary prevention after MI is suboptimal as a low proportion achieve the treatment targets for the cardiometabolic risk factors [167, 168].

1.8 The Link Between MI and VTE

1.8.1 MI as a risk factor for VTE

Despite differences in epidemiology [28, 169], pathophysiology [47] and treatment approaches [170], a link between arterial and venous thrombosis have been demonstrated. First, 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 two weeks after the MI event [171]. Second, a relation between MI and VTE was supported in a cross-sectional study where an association between CAD and PE was found in patients aged 60 years or older [172]. Third, in two large, Danish registry-based case-control studies, *Sorensen and colleagues* found that the risk of VTE, and PE in particular, was substantially increased in the first months immediately after an MI after taking age, sex, and information regarding obesity, medication use and comorbidities into consideration [173, 174]. The increased risk associated with an MI diagnosis seemed to extend beyond 3 months past the initial hospitalization for MI, but the association was much weaker over the long term. Accordingly, results from the large prospective Tromsø Study, which included 29 506 participants with confounder information and validated VTE outcomes, showed that patients with MI are at increased short-term risk of VTE, with a nearly 5-fold increased risk during the 6 month period after the MI [175]. In subgroup analysis, MI was associated with a nearly 8.5-fold increased risk of PE during the first 6 months after the acute MI event. In accordance with the findings from the two Danish case-control studies, PE risk remained almost 4-fold higher from 6 months to 1 year after the incident MI compared to the non-exposed group, and the association disappeared after 1 year of follow-up.

1.8.2 An MI could have a direct or an indirect effect on VTE risk

The explanation for the observed association between MI and future risk of incident VTE is yet unknown. The short-term increased VTE risk after MI, and provoked VTE in particular, suggests that mechanisms related to the MI itself (a direct causal effect) or indirect mechanisms due to hospitalization for MI may play a role [176]. Thus, induction of a procoagulant state due to tissue damage or hospitalization accompanied by concomitant presence of transient risk factors for VTE (e.g., immobilization, infections, major surgery, CVC), may potentially play a role in MI-related VTE [177, 178].

The Chest guidelines recommend anticoagulant thromboprophylaxis for high-risk hospitalized medical patients unless the risk of bleeding complications is too high [25]. However, less than 40% of hospitalized medical patients receive appropriate anticoagulant prophylaxis [179]. Current knowledge on risk factors and triggers of VTE in MI patients, particularly in the first month after the MI, is scarce. Therefore, there is an unmet need for discovery of risk factors that can be used to identify MI patients at high risk of VTE. This can potentially improve the benefit-to-harm ratio, as anticoagulant treatment is a two-edged sword due to the increased bleeding risk, which may be high during the acute phase of an MI [180, 181], and should only be given to patients at high VTE risk.

1.8.3 Shared Risk Factors between MI and VTE

Atherosclerosis was not a risk factor for future VTE in previous population-based cohort studies [182, 183] including the Tromsø Study [184], even after taking regression dilution into account [185]. Additionally, except for obesity, traditional cardiometabolic risk factors such as hypertension, diabetes mellitus and dyslipidemia, are not shared between arterial and venous thrombosis, even after correction for regression dilution bias [127, 186-190]. Apparently, cigarette smoking is not an independent risk factor for VTE as the increased risk of provoked VTE associated with smoking presumably is related to the development of comorbid conditions associated with cigarette smoking (e.g., cancer, MI, stroke) [191].

Several meta-analyses published during the recent years have reported effects of well-known venous thrombosis associated genotypes on MI risk, with studies on non-O blood type, Prothrombin G20210A and FVL reporting ORs ranging from 1.1-1.9 [66, 177, 192-195]. In addition, family history of MI is a risk factor for both MI and VTE [196, 197], which is independent of intermediate development of MI or aggregation of cardiometabolic risk factors [198]. Indeed, prothrombotic genotypes may augment VTE risk under conditions of high thrombosis risk related to the MI (e.g., hospitalization with subsequent immobilization, tissue damage with a prothrombotic response, and heart failure with stasis in the cardiopulmonary circulation). Whether the relationship between MI and VTE can be explained by common prothrombotic genotypes has not been well addressed.

Additionally, a large proportion (52%) of the world's adult population is overweight or obese [85]. Obesity doubles the risk of VTE in population-based studies, and mendelian randomization studies have shown that obesity is a causal risk factor for VTE [88, 89]. Obesity is a shared risk factor for MI and VTE, although the pathophysiological mechanisms for the associations appear to differ for the two diseases [94, 199]. Obesity is common in patients with MI, but whether obesity is associated with a more than additive effect on risk of VTE in combination with MI has not been investigated.

2. Aims of the Thesis

The overall aim of the present thesis was to identify triggers and risk factors of VTE in patients with MI.

The specific aims of the thesis were:

❖ To study the role of MI as a trigger factor for incident VTE by adjusting for other concomitant triggers, and evaluate to what extent the relationship between MI and VTE is mediated through well-known risk factors for VTE in a case-crossover study derived from the Tromsø Study (Paper I)

❖ To investigate if the association between MI and VTE could be explained by the presence of prothrombotic genotypes and to assess the combined effect of MI and prothrombotic genotypes on the risk of VTE in a case-cohort recruited from the general Tromsø population (Paper II)

❖ To study the joint effect of MI and obesity on the risk of VTE, and to evaluate whether obesity yields a more than additive effect on VTE risk in MI patients in a population-based cohort study based on the Tromsø Study (Paper III)

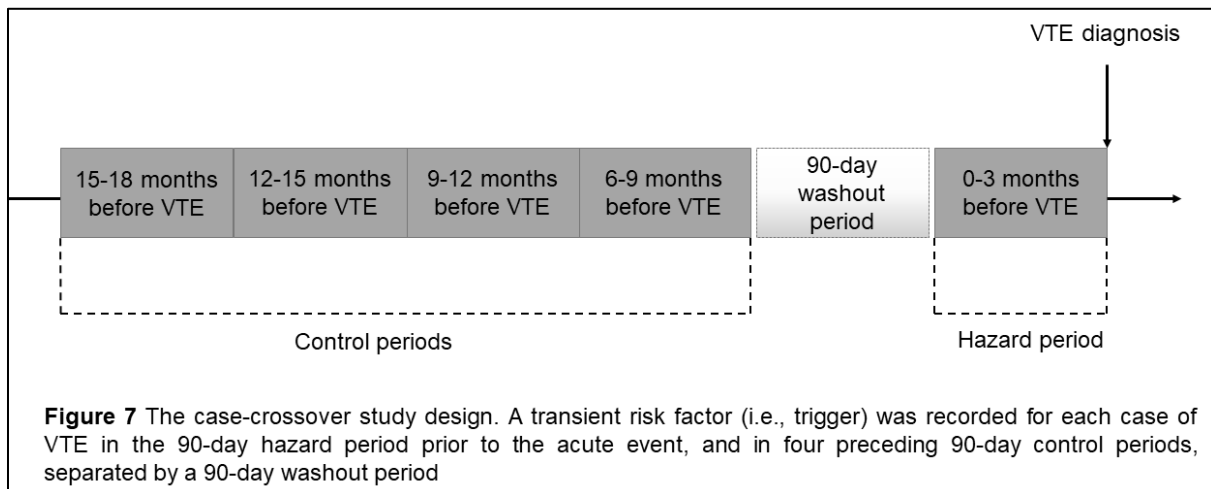# 3. Study Population and Methodological Description

3.1 The Tromsø Study and Study Designs

The Tromsø Study is a unique single-center population-based prospective cohort study initiated in 1974 where the main objective has been to elucidate causes of the high cardiovascular mortality in Northern Norway [200]. The study is conducted by the Department of Community Medicine at the University of Tromsø. From 1974 to 2016, seven surveys (referred to as Tromsø 1-7) have been conducted and the 8th survey is planned to be carried out in 2024-2025. The aim has been to include large, representative samples of the Tromsø population, with invitation of whole birth cohorts and random samples. VTE registration started on January 1, 1994, and ended on December 31, 2012. All potential cases of incident and recurrent VTE during this time-period was recorded.

The papers included in the present thesis are all based on prospective follow-up data on subjects who participated in these surveys. Paper I include participants from Tromsø IV only, while Paper II and III additionally include subjects from Tromsø V and VI. Tromsø IV was conducted in 1994-95 and all inhabitants aged >24 years were invited. A total of 27 158 subjects participated, which corresponded to 77% of the eligible population. All participants aged 55-74 years and 5-10% random samples in the other 5-year birth cohorts were invited to a more thorough second screening visit, and 6 889 subjects participated (78% of those invited). Further, Tromsø V was conducted in 2001-02 and included 8 130 subjects aged 30-89 years of age (78% of the eligible population). Lastly, Tromsø VI was conducted in 2007-08 and included 12 984 subjects aged 30-87 years (66% of the eligible population). In all papers, participants with a previous VTE before baseline were excluded.

The study population in Paper I consisted of all incident VTE cases (n=707) registered among the Tromsø IV participants and were included in a case-crossover study design. The case-crossover study was designed with a 90-day risk or hazard period, four 90-day control periods (180 – 540 days before the VTE) and a 90-day washout period in between. Conditional logistic regression was applied to calculate ORs for VTE according to acute MI, and after adjustment for other triggers. The method developed by Karlson, Holm and Breen (KHB method) was applied to investigate

whether and to what extent known risk factors or triggers mediate the relationship between MI and VTE.

Figure 7 The case-crossover study design. A transient risk factor (i.e., trigger) was recorded for each case of VTE in the 90-day hazard period prior to the acute event, and in four preceding 90-day control periods, separated by a 90-day washout period

For Paper II, we conducted the analysis using a case-cohort design based on the entire Tromsø IV, V and VI cohorts. Subjects that attended at least one of these surveys were followed from the date of enrolment until the date of an incident VTE, death, migration, or administrative censoring on December 31st, 2012, whichever occurred first. In total, 689 VTE cases were included during follow-up and an age-weighted sub-cohort of 1 761 subjects were randomly selected from the entire cohorts. In Paper III, a cohort design was applied, and participants were followed from the date of inclusion in Tromsø IV, Tromsø V or Tromsø VI until December 31st, 2014. For both Paper II and Paper III, MI was entered as a time-varying covariate and Cox regression models used to obtain hazard ratios (HRs) of VTE with corresponding 95% confidence intervals (CIs) according to combinations of MI and prothrombic genotypes, and MI and obesity, respectively.

3.2 Exposure Assessment in the Case-Crossover Study

Trained personnel reviewed the medical records of each incident VTE case for relevant risk factors, diagnostic procedures, medical and surgical treatment, laboratory tests and diagnoses during hospitalization, day care and outpatient clinic visits in any of the risk or control periods. If a risk factor was present in the 90-day period prior to the VTE event it was included as a trigger in that period. If a trigger occurred over several days, it was considered to have occurred if any of the days of the exposure fell within the 90-day period. Acute MI was defined as the presence of an MI diagnosis in the medical

record that was based on a combination of clinical symptoms and signs together with electrocardiographic findings and measurements of cardiac enzymes in blood samples. Moreover, immobilization was defined as the presence of one or more of the following features: Bed rest for three days or more, Eastern Cooperative Oncology Group (ECOG) score of four, or other immobilizing factors specified in the medical records (e.g., confinement to wheelchair, cast immobilization, etc.). The definition of infection included both community-acquired infection that required hospital admission and hospital-acquired infections and was recorded if an acute infection was described by a physician in the patient's medical record. Major surgery, trauma, red blood cell transfusion, and CVC were recorded if noted in the medical record.

3.3 Cohort Baseline Measurements

At baseline inclusion, information was obtained by validated self-administered questionnaires, physical examination and blood samples, and citrated plasma and DNA was stored at -70ºC in biobanks. Information from questionnaires provided information regarding smoking habits, physical activity, education level, diabetes, family history of MI, medication use and use of OCs or HRT. Further, body weight and body height were measured with subjects wearing short-sleeved garments and no shoes. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured by trained personnel with an automatic device (Dinamap Vital Signs Monitor, 1846, Critikon Inc., Tampa, FL, USA). Three recordings were performed on the upper right arm with two-minutes intervals, and the mean of the last two readings was used.

We genotyped the SNPs rs8176719 in *ABO* (ABO blood group), rs6025 in *F5* (factor V Leiden [FVL]), rs1799963 in *F2* (Prothrombin G20210A) and rs2036914 in *F11* using the Sequenom platform, and rs2066865 in *FGG* using the TaqMan platform. For Sequenom, 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. For TaqMan, an initial input of 100 ng of DNA was used, and samples were genotyped using the Applied Biosystems 7900HT according to the recommended protocol. Subjects were categorized as carriers of the risk gene when ≥1 risk allele was present. For rs2036914, the minor allele was associated with a lower risk of VTE, and therefore, we considered the major

allele as the risk allele. Based on the paper by *de Haan et al* [80], we composed a 5-SNP score by summarizing the number of risk alleles from the five sequenced SNPs. These were categorized into 0-1, 2, 3 and ≥4 risk alleles.

3.4 Cohort Classification of MI

The national Norwegian identification number consisting of 11-digits allowed linkage to national and local diagnosis registries. All incident MI cases were identified by linkage to the hospital discharge diagnosis registry at the University Hospital of North Norway (UNN) and the National Causes of Death Registry at Statistics Norway. Events of possible incident MI were identified by a broad search for the International Classification of Diseases (ICD) 9th revision codes 410-414, 427, 428, 430-438, and 798-799 in the period 1994-1998, and for the ICD 10th edition codes I20-I25, I46 to I48, I50, I60-I69, and R96, R98, and R99, thereafter. The Causes of Death registry covered study subjects registered as living in Norway at the time of their death, regardless of whether the death took place in Norway or abroad.

The hospital medical records were thoroughly reviewed for case validation according to the World Health organization (WHO) MONICA/MORGAM criteria, which include clinical symptoms and signs,

Definite MI	1. Typical, atypical, or inadequately described symptoms plus a definite new infarction in ECG recordings 2. Typical symptoms plus significantly higher myocardial enzyme and/or troponin levels 3. Atypical or inadequately described symptoms plus significantly higher myocardial enzyme and/or troponin levels plus a probable new infarction in ECG recordings 4. Postmortem evidence of a recent MI or thrombosis
Probable MI	1. Typical, atypical, or inadequately described symptoms plus a probable new infarction on ECG recordings plus moderately increased myocardial enzyme and/or troponin levels 2. Typical symptoms plus moderately increased myocardial enzyme and/or troponin levels 3. Atypical or inadequately described symptoms plus significantly higher myocardial enzyme and/or troponin levels 4. Atypical or inadequately described symptoms plus moderately higher myocardial enzyme and/or troponin levels plus probable new infarction shown in ECG 5. Sudden death with no evidence of noncoronary cause of death
Possible MI	An event that can be dated and for which secondary data of typical history in combination with ECG findings and/or echocardiography and/or autopsy are consistent with MI but for which no primary data source is available

Figure 8 Classification algorithm for myocardial infarction. The Tromsø Study

findings in electrocardiograms (ECG), values of cardiac enzymes and findings from autopsy records (when applicable) [201]. Incident events classified as definite, probable, or possible MI based on the WHO algorithm were included by the endpoint committee and applied in the analysis in Paper II and Paper III (Figure 8). Linkage to the National Causes of Death Registry at Statistics Norway provided information on all-cause mortality. Information from death certificates was used to collect relevant information of the event from additional sources, including autopsy reports and records from nursing homes, ambulance services and general practitioners.

3.5 Outcome ascertainment – Venous Thromboembolism

UNN is the only hospital in the municipality of Tromsø, and all hospital-based medical care and VTE related health care in the region is provided exclusively by this hospital. The hospital discharge diagnosis registry, the autopsy registry, and the radiological procedure registry at the UNN were used to identify VTE subjects during follow-up. Relevant ICD-codes of revision 9 (ICD-9) for the period 1994-1998 were 325, 415.1, 452, 453, 671.3, 671.4 and 671.9, and for the period 1999 to 2012 relevant ICD-10 codes were I26, I80, I82, I67.6, O22.3, O22.5, O87.1 and O87.3 [196]. Trained personnel, who were blinded to the individuals' baseline variables, reviewed the medical records for case validation.

All four following criteria had to be recorded for inclusion as a VTE case:

i. Presence of symptoms and signs indicative of either a DVT, PE or both.
ii. Objective diagnostic confirmation either radiologically (i.e., compression ultrasound, ventilation-perfusion scan or CTPA) or by autopsy.
iii. A diagnosis of DVT or PE made by a physician in the patient's medical records.
iv. Initiation of treatment for VTE (i.e., anticoagulants, thrombolysis or thrombectomy), or treatment was planned for, but not started due to contraindication(s).

A VTE case was only included from the autopsy registry if the autopsy report stated that VTE was the cause of death (part one of the death certificate), or a significant factor associated with the cause of death (part two of the death certificate).

4. Main Results

4.1 Paper I

MYOCARDIAL INFARCTION AS A TRIGGER FACTOR FOR INCIDENT VENOUS THROMBOEMBOLISM

This study was undertaken to investigate the role of MI as a transient risk factor for first lifetime VTE using a case-crossover design. Conditional logistic regression was applied to calculate ORs with 95% CIs for VTE according to MI and after adjustment for other transient risk factors. Immobilization and infection can be consequences of an MI and could potentially mediate the relationship between MI and VTE. Accordingly, the KHB-method was used to investigate whether and to what extent immobilization and infection mediate the relationship between MI and VTE. Among the 707 VTE cases, there were 408 DVTs (57.7%) and 299 PEs (42.3%) with or without concomitant DVT. Moreover, 13 (1.8%) had a diagnosis of MI in a hazard period and 6 (0.2%) in a control period. Immobilization occurred in 222 (31.4%) of the hazard periods and in 57 (2.0%) of the control periods. Acute infection occurred in 267 (37.8%) of the hazard periods and in 107 (3.8%) of the control periods. The OR for VTE associated with MI was 11.9 (95% CI: 3.9 – 36.7) in the crude model and decreased to 2.7 (95% CI 0.6 – 11.2) after adjustment for immobilization and infection. When added one-by-one, the OR for VTE associated with MI adjusted for immobilization was 8.1 (95% CI: 2.2 – 30.2), whereas adjustment for infection alone yielded an OR of 3.9 (95% CI: 1.0 – 14.7). When the other VTE risk factors (i.e., major surgery, trauma, red blood cell transfusion, and CVC) were added along with immobilization and infection, the OR was only marginally attenuated (OR 2.6 95% CI: 0.6 – 11.9). The ORs for DVT and PE in subgroup analyses were essentially similar as for overall VTE. In the KHB model, MI was entered as the main exposure, hazard or control period defined as outcome, infection and immobilization as mediators, and major surgery, trauma, red blood cell transfusion, and CVC as covariates. The analysis showed that approximately 60% of the association between MI and VTE was mediated through immobilization and infection, of which 72% was attributed to infection and 28% to immobilization. In conclusion, MI is a transient risk factor for VTE, and indirect risk factors related to the MI, in particular infection and immobilization, may to a large extent explain the observed association between MI and VTE.

4.2 Paper II

THE COMBINED EFFECT OF MYOCARDIAL INFARCTION AND PROTHROMBOTIC GENOTYPES ON THE RISK OF VENOUS THROMBOSIS

In this study, we sought to investigate the role of five prothrombotic genotypes as potential common risk factors for MI and VTE. Within a case-cohort derived from the Tromsø Study, 641 subjects were included as incident VTE cases, and a sub-cohort (n=1 761) was created by randomly sampling participants from the source cohort weighted for the age distribution of the cases in 5-year age groups. Cox regression models with age as time scale adjusted for sex were used to estimate HRs with 95% CIs for VTE by MI status, adjusted for each of the prothrombotic SNPs (rs8176719 [ABO], rs6025 [F5], rs1799963 [F2], rs2066865 [FGG] and rs2036914 [F11]) individually and in a multivariable model. Further, we estimated HRs for combinations of MI and the individual SNPs (0 versus ≥1 risk allele) and combinations of MI and categories of the 5-SNP score. In our study, 274 participants developed an incident MI during a median of 15.7 years of follow-up. Those who developed MI where on average older and had higher values of total cholesterol, triglycerides, and BMI than those who did not develop MI. Moreover, 47 of the 641 VTE events occurred in subjects with MI and the proportion of PE was higher among MI patients compared to study subjects that did not experience an MI (55% and 42%, respectively). Incident MI was associated with a 1.4-fold increased risk of VTE (HR 1.44 95% CI: 1.07 – 1.96) compared to subjects without MI. Adjustments for each of the SNPs did not alter the risk estimates, and the HR for MI in the model that included all 5 SNPs as covariates was 1.52 (95% CI: 1.12 – 2.07).   For all SNPs except for the variant in *F11*, the joint exposure of MI and risk alleles was not associated with excess risk of VTE compared to MI free subjects with zero risk alleles. In study participants without MI, the risk of VTE increased linearly across increasing categories of risk alleles of the 5-SNP score. In patients with MI, there was no increase in risk across categories of the same score. Results from subgroup analysis regarding risk of DVT and PE as well as provoked and unprovoked VTE, showed essentially similar results as those for overall VTE. In conclusion, our findings suggest that the increased risk of VTE in patients with MI cannot be explained by these five prothrombotic genotypes. The combined effect of the five prothrombotic SNPs and MI did not result in excess risk of VTE.

4.3 Paper III

JOINT EFFECT OF MYOCARDIAL INFARCTION AND OBESITY ON VENOUS THROMBOSIS RISK

The purpose of this study was to investigate the joint effects of incident MI and obesity on the risk of VTE in a prospective population-based cohort study. Using three surveys of the Tromsø Study, 29 410 participants were recruited and followed through 2014. At baseline inclusion in each survey, information was obtained by questionnaires, physical examination, and blood samples. Cox regression models with age as time scale and MI as a time-dependent variable were used to calculate HRs of VTE (adjusted for sex) by combinations of MI exposure and obesity status with exposure to neither risk factors as reference category. The presence of interaction on an additive scale was evaluated with the relative excess risk due to interaction (RERI) and attributable proportion (AP). During a median of 19.6 years of follow-up, 2090 study participants developed a first-time MI and 784 participants developed a first-time VTE. Among those with MI, 55 developed a subsequent VTE, yielding an overall IR of 5.3 pr 1000 person-years (95% CI: 4.1 – 6.9) after MI. In the combined exposure group (i.e., MI + / Obesity +), the IR was 11.3 per 1000 person-years, and the HR indicated a 3-fold increased risk (HR 3.16 [95% CI: 1.99 – 4.99]) for overall VTE compared with the reference group after adjustment for age and sex. Subgroup analyses indicated that in non-obese subjects, MI was associated with PE (HR 1.54, 95% CI: 0.98 – 2.43), but not with DVT (HR 0.84 (0.50 – 1.43). In non-obese subjects, MI was associated with increased risk of provoked (HR 1.48, 95% CI: 0.98-2.24), but not unprovoked VTE (HR 0.76, 95% CI: 0.41-1.39). Estimation of AP due to interaction revealed that 46% of the VTE events in participants with both MI and obesity were attributable to interaction between the two exposures. In subgroup analysis, 34% of PEs and 56% of DVTs in participants with both MI and obesity, respectively, were attributable to interaction between the two exposures. Similar numbers as for PE and DVT were observed for provoked VTE and unprovoked VTE. In conclusion, the presence of obesity yielded an excess risk of VTE in subjects with MI, suggesting an interaction between MI and obesity on the risk of VTE. Subgroup analyses indicated that the effect of interaction was more pronounced for the risk of DVT and unprovoked VTE, and the increased risk of these subtypes of outcomes in MI patients appeared to be dependent on the presence of obesity.

5. General Discussion

One of the central objectives of epidemiologic research is to disentangle causal associations between exposure and disease [202]. If we determine causal factors affecting disease risk, then we can reduce disease occurrence [51]. Several checklists with characteristics of a causal relationship have been proposed and sir Austin Bradford Hill described the famous Hill criteria [51, 203] (i.e., strength of the observed association, consistency across studies, specificity, temporality, biological gradient [dose-response], biological plausibility, experimental evidence, coherence and analogy). The only characteristic that is truly a causal criterion is temporality, which implies that the cause comes before the effect. Indeed, this is part of the definition of a cause. Furthermore, to test theories, we draw samples from a population to avoid studying the entire population. The representativeness of the sample is the main concern for generalizing to the background population (i.e., generalizability) and determines whether the findings can tell us what to expect in people or settings that were not studied.

5.1 Methodological Considerations

5.1.1 Study Designs

Paper III in the present thesis follows the design of a prospective cohort study. In a cohort study, baseline information is gathered at the start of follow-up, and the defined population is followed until the outcome of interest, or another censoring event occurs (i.e., death, migration, or end of study period). Exposure status is ascertained prior to the outcome occurrence. Hence, the prospective design in combination with close follow-up and calculation of person-time at risk allows for estimation of IRs as a measure of absolute risk, which can be used to derive relative risk estimates, expressed as the HR [204]. Our cohort was based on the Tromsø Study population, which is a large cohort with high participation or response rates (ranging from 66-77%) of the eligible population. The external validity, or generalizability, of the study is increased due to the high response rates and the study subjects recruited from a general Caucasian population. One of the main limitations of a cohort study is that it requires a large study population and long-term follow-up for an appropriate number of outcomes to occur to detect differences between the comparison groups. Consequently, the cohort design is not suited to study diseases with low IRs. VTE occurs in 1-2 persons per 1000 annually and the absolute number of persons who

develop both MI and VTE is low, and thus, statistical power is an issue in both Paper III and Paper II.

The main feature of a case-cohort design (Figure 9) is that controls or the *sub-cohort* is sampled from the entire source population at the start of follow-up rather than from the non-cases at the end (cumulative sampling). Accordingly, every person from the source population has the same chance of being included in the study as a sub-cohort member, regardless of whether the subject becomes a case. Indeed, the sampling process is based on a number of people in the source population, not an average of person-time that mirrors the distribution of person-time of exposed subjects in the source population (density based sampling) [51]. In addition, because the sub-cohort is sampled without regard of any outcome it may serve as a comparison group for several different diseases [205]. Because a random sample from the source population would not be representative of the age-distribution among cases, the sub-cohort in paper II was weighted for the age-distribution among cases. The case-cohort design was chosen to limit the costs and time required for genotyping as covariate data is only required for cases and sub-cohort members. Risk estimates are, however, similar in the case-cohort as in the full cohort with adequate sampling [206]. Like cohort studies, a strength of the case-cohort design is that temporality is known, which rules out reverse causation. However, reverse causation is in general not a problem when studying genetic exposures because genes are present from study start and is not altered by environmental factors.

Figure 9 Pictorial representation of the case-cohort design. Included in the study are sub-cohort subjects randomly sampled from the original cohort, together with all incident cases. Because the sub-cohort is a random sample from the whole original cohort, it includes some cases
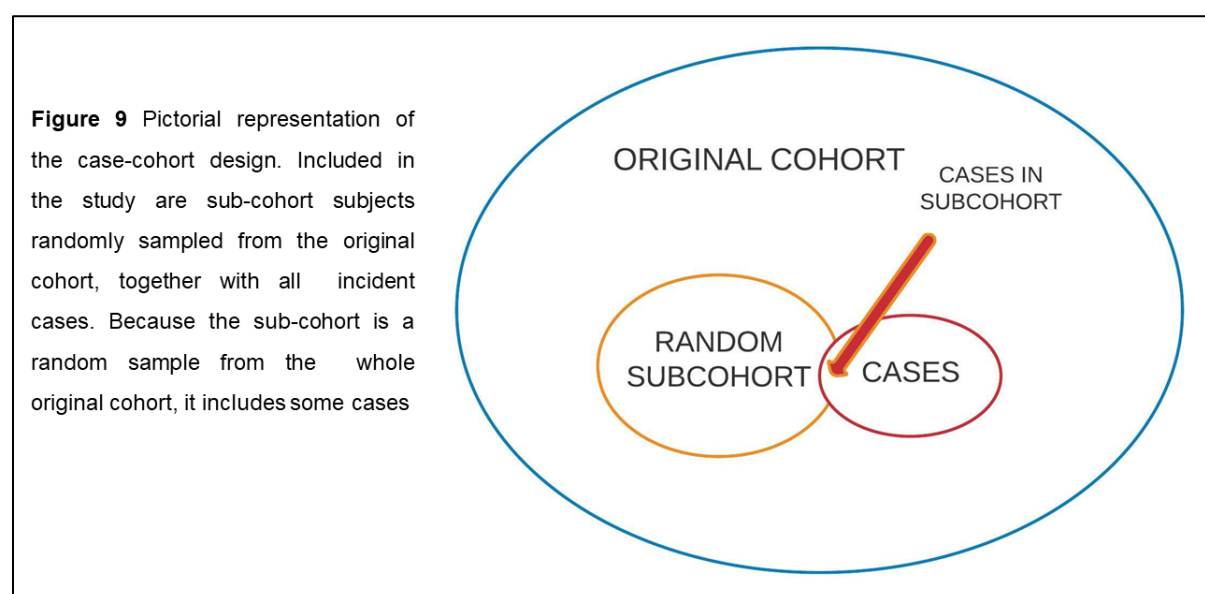
Only a certain type of study hypothesis can be evaluated by the case-crossover design as the disease under study must have an abrupt onset and the exposure should trigger a short-term effect on outcome [51]. The case-crossover design was first proposed by Maclure [207] and can be considered a variant of a case-control study where all subjects included are cases. However, rather than being a different set of sampled subjects, the controls are represented by exposure information drawn from the cases themselves. The self-matching is a key strength of the case-crossover design, ensuring that fixed factors (i.e., genetics, comorbidities, health behavior) that do not vary within an individual are controlled for through the design.

As described in Section 3.1 of this thesis, the case-crossover study was designed with a 90-day risk or hazard period, four 90-day control periods (180 – 540 days before the VTE) and a 90-day washout period in between. Each of the VTE cases were classified as exposed or unexposed depending on whether there was any exposure during the defined hazard or control period. A commonly applied definition of a trigger factor for VTE is a risk factor that is present for up to 3 months before the VTE event [40], which is the rational for defining a 3-month hazard period. However, exposures in the past could be the cause of recent disease if the time from the defined control periods to the risk period is too short (i.e. the effect lasts longer) [207]. Carry-over effects have the potential to dilute the effect of a trigger on the outcome under study. With the aim of preventing potential carry-over-effects between control and risk periods, a wash-out period of 3 months was implemented.

Survivorship bias represent a potential limitation to the case-crossover design because those who die before receiving a diagnosis are left out. However, it is not very likely that subjects who die before being diagnosed with VTE would differ considerably regarding trigger status than the subjects who survive until diagnosis and are included in the study. Another limitation is the potential for confounding by factors that change over time. Comorbidities related to the risk of both MI and VTE (e.g., progressive cancer), may have developed during the 1.5-year follow-up. Thus, it is crucial to fit control periods as close to the hazard period as possible. Moreover, because we gathered information on potential triggers for VTE from medical records, we must assume that all the relevant triggers have been described by the physician to obtain valid risk estimates.

5.1.2 Random error and systematic error

Random error is variability in the data that affects the *precision* of our risk estimates (e.g., due to limitations of instruments or variations in procedures) and can be reduced by increasing study size [208]. Bias is another term for systematic error and affects the *validity* of the study. In principle, a study can be biased due to (i) the way in which subjects have been enrolled (i.e., selection bias), (ii) the way the variables in the study have been measured (i.e., information bias) or (iii) due to incomplete control of confounding factors [51]. Because the correct values for the estimates from an epidemiological study are always unknown, we cannot determine the actual amount of error present. However, we can work thoroughly with the design, conduction, and analysis to reduce the amount of error.

Selection bias is a systematic error in a study that manifest when the association between exposure and disease differs for those who participate and those who do not participate [209]. Subjects with serious illnesses, disabilities, or lower socioeconomic status (SES) tend to refrain from attaining health examinations, thereby producing the healthy volunteer effect in epidemiological research (i.e., self-selection or prognostic selection bias) [210-212]. This may result in a healthier study population than the general population, a low number of outcome events and potentially an underestimation of the true association between exposure and outcome. The participation rate in the Tromsø Study was high, with overall attendance rate in Tromsø IV, V and VI exceeding 70%. All inhabitants aged 25 years or older living in Tromsø were invited in Tromsø IV, and this ensures a broad spectrum of age groups. The high participation rate and broad age range are major strengths of the Tromsø Study that presumably makes our results generalizable to other Caucasian populations. Nevertheless, there is a relatively low participation rate for subjects under 40 years and over 80 years of age, and men have a lower attendance rate than women [200]. Indeed, this may influence the generalizability in these age groups. We have, however, mainly reported relative risk estimates rather than absolute risks, and therefore this variant of selection bias should not affect our results. Additionally, selection bias is particularly a problem when selection of study subjects is related to the outcome. In our study, the outcome status was not known at study start. Thus, selection was not related to the outcome and selection bias should not represent a limitation in Paper II and Paper III.

An issue that affects studies with long-term follow-up is loss of study participants (i.e., loss to follow-up) for reasons related to the exposure and/or disease. When large proportions of study subjects are lost to follow-up, the validity of the study may be threatened. In Paper II and III, all subjects were censored at the time of migration or death. In addition to the proportionality assumption [213], non-informative or random censoring is another important assumption when performing Cox regression. This implies that all study subjects should have the same probability of being censored during follow-up. There is no reason to believe that subjects that moved from Tromsø had a different risk of VTE than those who stayed. Therefore, simple censoring at the time of migration is considered adequate. Competing risk is a related concept that may influence risk measures [214]. Competing risk of death has shown to increase the risk estimates in cancer-associated VTE [215] because person-time at risk among cancer patients is reduced due to non-random censoring by death. Competing risk by death did not influence our risk estimates considerably when applying the Fine and Grey competing risk regression model in Paper II and Paper III. Additionally, our focus is not on calculating absolute risk. We are comparing exposed and unexposed individuals alive and at risk of developing VTE and calculating relative risk measures to study the aetiology of MI-related VTE, without the aim of outcome prediction [216].

When studying exposures that change over time (e.g., MI status) in a prospective cohort study, we should correct for the timing of the exposure during follow-up. This correction ensures accurate statistical modelling of observation periods. In Paper II and Paper III, the MI variable was included as a time-dependent covariate and updated during follow-up (i.e., Mantel-Byar or time-dependent exposure assessment method). This imply that all study subjects contribute with unexposed person-time from baseline inclusion to the MI event, and exposed person-time from the MI event until any censoring event. Study subjects with an MI before inclusion were excluded. An alternative method would have been to use baseline information regarding MI status (i.e., time-fixed approach) at study inclusion. This is, however, not an appropriate method because an MI event during follow-up may have occurred and caused the incident VTE event.

Information bias comes about when the information collected about or from study participants is erroneous and information about exposure and/or disease status therefore becomes misclassified [51]. Non-differential misclassification of the exposure is present when the misclassification is independent of the outcome and similar across the comparison groups. Differential exposure misclassification is present when misclassification is dependent of the outcome and differs between the comparison groups. Misclassification of disease outcome is also possible and is non-differential when it is not dependent on the exposure and differential when it is dependent on the exposure. A non-differential misclassification leads to more predictable biases, most often resulting in underestimation of the true association (towards the null value), whereas a differential misclassification may bias the association in either direction. Differential misclassification of exposure may be present in Paper I as immobilization is a well-known risk factor for VTE. Hence, clinicians might have been more prone to specify the exposure in the medical record (i.e., surveillance bias) in the hazard period during hospitalization for VTE than during hospitalization for other conditions in a control period. In prospective cohort studies, the exposure is measured prior to the occurrence of disease. Therefore, potential exposure misclassification is most likely non-differential, which leads to an underestimation of the true effect size. The VTE cases in Papers I-III were identified by trained personnel searching the medical records. Potential outcome misclassification cannot be completely ruled out as the registration was dependent on adequate and complete information from the medical records described by individual physicians. However, the potential misclassification is most likely non-differential because the end-point committee was blinded to baseline characteristics of the potential VTE cases. Similarly, the MI diagnosis and other triggers (infection and immobilization) in Paper I was based on review of medical records and may therefore be prone to misclassification. In contrast to Paper II and Paper III, the validation of MI cases was not done by an end-point committee in Paper I. However, a diagnosis of MI is based on objective criteria from ECG, cardiac enzymes in blood samples in combination with clinical symptoms and findings and is therefore unlikely to be missing or falsely described in the medical records.

5.1.3 Confounding

Confounding (from Latin *confundere* "to pour together or mix") is a major issue in observational studies as it limits the ability to provide insights into
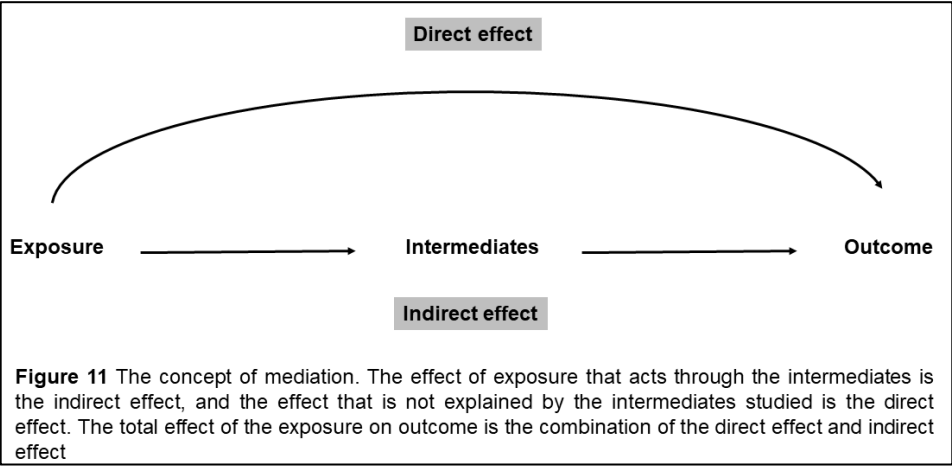

Figure 10 The concept of confounding
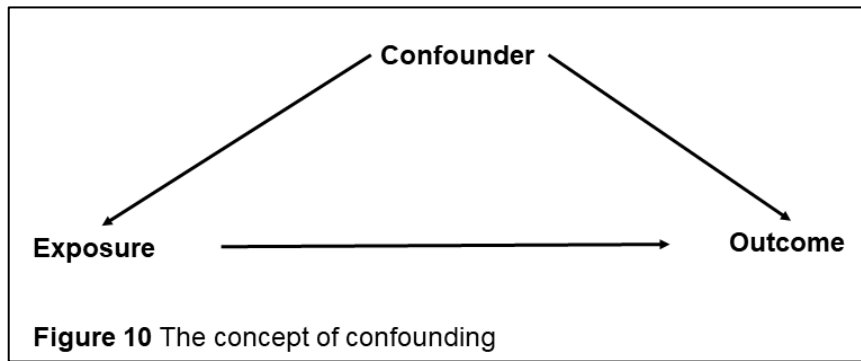
causality [50, 217]. Rothman describes confounding as a mixing of effects [51]. Indeed, a confounded association is real (not causal) and may be relevant in predictive studies [202]. A confounding variable is a variable that completely or partially explains the observed association under study. A confounder has the properties of being associated with both the exposure and disease (cannot be an effect of the disease) and is not on the causal pathway between an exposure and disease (i.e., mediator) (Figure 10). In Paper I-III, multivariable regression modelling (i.e., logistic regression and proportional hazard regression) was used to take into account confounding variables in the analysis [218, 219]. This entails a statistical approach where you obtain an estimate of the association between the independent and dependent variables that is conditioned for the effect of the other variables you include in the model. The estimates provided for the independent variables are mutually unconfounded. Therefore, the principal advantage of multivariable regression modelling is that several confounding variables can be controlled for simultaneously. In practice, if we see >10% change in the exposure coefficient when the covariate is added to the model, it is considered a confounder (if it also meets the conditions for being a confounder) [51]. In Paper I, the regression model yielded a crude OR of 11.9 when MI was included as main exposure. When infection was added as a covariate the OR decreased to a value of 3.9, which represents a 65% reduction of the risk estimate. Since infection also meets the criteria for being a confounder, we may conclude that infection essentially is a confounder of the relationship between MI and VTE. Moreover, since age is a strong confounder of the association between MI and VTE, we used age as timescale in our Cox models because this is a more efficient method to control for age than using time-on-study as timescale and adjusting for age at baseline [220]. This is because the hazard of disease is expected to change more as a function of age than as a function of time-on-study. This method is considered suitable in prospective studies with large

enough study populations that many people at each age are represented. Other commonly applied techniques in observational studies to reduce the potential for confounding are stratification, restriction, and matching. However, residual confounding is the potential distortion that remains after controlling for confounding in the statistical analysis or the design [221]. One reason for residual confounding could be that some confounders are unknown and therefore not measured. Another reason for residual confounding could be that data on certain factors was not collected, and therefore not available as covariates in the statistical model.

5.1.4 Mediation

Mediation (from Latin *medius* "the middle") analysis is applied to investigate the underlying mechanisms behind an

Figure 11 The concept of mediation. The effect of exposure that acts through the intermediates is the indirect effect, and the effect that is not explained by the intermediates studied is the direct effect. The total effect of the exposure on outcome is the combination of the direct effect and indirect effect

observed relationship between exposure and outcome [222-224]. The mediation model assumes that the exposure causes an intermediate variable, denoted *mediator* (i.e., intermediate), which in turn causes the outcome (Figure 11). This implies that a mediator is defined quite similar to a confounder, with the exception that the mediator is a presumed causal consequence of the exposure. Mathematically, there is no difference between a confounder and a mediator, and it is not always clear whether a variable is a confounder or a mediator. If the association between an exposure and outcome diminishes after adjusting for a variable, we cannot always conclude that the unadjusted association was due to confounding. In fact, adjusting for a variable in the causal pathway may have similar effects on the risk estimates as adjusting for a confounder. Unless the aim is to explore alternative mechanisms for an association, we should not include variables in the causal pathway in our regression model because these variables essentially are the reason why the exposure and outcome is associated. Adjusting for a mediator in the regression model yields the direct effect of

an exposure on outcome, by removing the indirect effect caused by the mediator. Indeed, the traditional way to assess the mechanism by which exposure leads to the outcome is to compare regression coefficients with and without the mediator included in the model [225].

The KHB method is a formal test of mediation applied in Paper I that allows for partitioning of the total effect of an exposure into indirect (acting through the mediator[s]) and direct effects (acting independent of the mediator[s]) while at the same time adjusting for potential exposure-outcome and mediator-outcome confounders [225, 226]. In Paper I, MI was included as main exposure, infection and immobilization as mediators, and major surgery, trauma, red blood cell transfusion, and CVC as confounders, and we observed that approximately 60% of the association between MI and VTE was mediated through concomitant infection and immobilization. The demonstration of an indirect effect strengthens the evidence that the total effect of MI on VTE risk is, at least partially, causal [224]. Because mediators are on the causal pathway, they are part of the mechanism by which the exposure causes the outcome [51]. Hence, knowing that an association is mediated through a set of variables provides insight into potential causality.

The presence of residual confounding cannot be completely ruled out as other unknown or unmeasured triggers could influence the relationship between MI, mediator and VTE. For unbiased estimation of the direct and indirect effects, we must assume that there is no unmeasured confounding of the exposure-outcome and mediator-outcome relationship. However, we have carefully selected and collected what we assume are the relevant variables that influence the relationship between MI, mediator and VTE. Furthermore, the study was not designed to determine the temporal sequence between exposure, intermediate and outcome. For the KHB method to be valid, we must assume that MI came first in the chain of causation. Since infection is not a strong risk factor for the development of MI [227], we consider this assumption to be valid as the MI patients most likely developed a nosocomial infection during hospitalization for the acute MI event [228]. However, we cannot rule out that an infection triggered the MI. Thus, our findings need to be confirmed in a study with a clear temporal relationship between these variables [223].

5.1.5 Regression dilution bias

When exposure information is collected at baseline and the time to an event is long, this may result in regression dilution bias because the exposure profile of study participants may change during follow-up. Regression dilution bias generally leads to an underestimation of the true association between the exposure and outcome [229]. Thus, regression dilution bias may lead to type II errors (i.e., failing to reject the null hypothesis when there is in fact a difference between the exposure groups) [230]. In Paper III, our risk estimates based on BMI measurement at baseline could be underestimated because body weight tend to increase with age [231] and has likely increased during follow-up in most subjects. However, variations in BMI in the Tromsø Study were small, and the risk estimates for VTE were essentially similar in time-fixed and time-varying analyses with repeated measurements of BMI that were updated during long-term follow-up [189].

5.1.6 Interaction

In several settings, the effect of one exposure may depend on the presence or absence of another exposure. This is the concept of interaction [232], and we should distinguish between statistical interaction (i.e., effect measure modification) and biological interaction. Effect measure modification means that the effect of one variable changes over values of another variable (i.e., an effect modifier) dependent on the underlying statistical model [51]. Logistic- and Cox regression models are exponential, which imply they are inherently multiplicative. This means that logistic- and Cox regression models evaluate departure from a multiplicative scale rather than departure from additivity in the presence of a cross-product term in the model [233]. Biological interaction measures joint or combined effects and refers to a mechanistic interaction that either exist or does not exist. The biological effect is not dependent on a statistical model (i.e., always refer to departure from additivity).

In Paper II and Paper III, we investigated combined effects. The exposures were combined and entered in the statistical models, using exposed to neither as the reference category, and adjusted for age (as timescale) and sex [234]. More than additive effects of the exposures were assessed with the RERI and the AP. These measures of interaction with their corresponding 95% CIs were calculated according to Andersson et al. [233]. In short, RERI is calculated as $HR_{AB} - HR_A - HR_B + 1$, where

HR_A is the hazard ratio for the first risk factor (i.e., MI) in the absence of the second risk factor (i.e., prothrombotic SNP or obesity), HR_B is the hazard ratio for the second risk factor in the absence of the first risk factor, and HR_{AB} is the hazard ratio when both risk factors are present. The RERI value should be interpreted as the part of the total effect that is due to interaction. AP corresponds to RERI/HR_{AB}, and should be interpreted as the proportion of cases in the combined group that is due to interaction between the two exposures. RERI and AP values of 0 indicates no interaction, while RERI and AP > 0 indicate positive interaction, i.e., that the effect of the combined exposure is greater than the sum of the individual effects [233, 235]. No biological interaction was observed in Paper II. In Paper III, biological interaction between MI and obesity on risk of VTE was demonstrated.

5.1.7 Missing

Missing data is unavoidable in observational studies and may potentially undermine the validity of study results. The consequences of missing values depend on the reason why they are missing. Missing values may be missing completely at random (MCAR) (rarely exists), missing at random (MAR) or missing not at random (MNAR) [236]. Missing values on important confounder variables is often a potential problem, as the variables are not available for adjustment in the regression model. A common way to address missing values in the statistical analysis is to include only cases with complete variable information (i.e., list-wise deletion). Exclusion of participants may both reduce the statistical power and introduce selection bias. Thus, the list-wise deletion approach requires that a small number of cases have missing variables, and/or the variable is not MNAR. In Paper II and Paper III, missing values regarding exposure (i.e., genetic variables and BMI) were handled with a list-wise deletion approach. The missing on prothrombotic SNPs and BMI is regarded MAR as the missingness is not related to the value of the specific variable. For SNPs, the missing is probably related to the quality and handling of blood samples, and a complete case analysis should not bias our results. Additionally, because of the small number of missing values (n=171 [i.e., <6% on all variables] and n=43 [i.e., <1% on all variables] for SNPs and BMI, respectively]) we may assume that the statistical power was not largely reduced.

5.2 Discussion of Main Results

5.2.1 MI as a trigger for VTE

In paper I, we reported that an MI is a strong trigger factor for incident VTE, and that the association was mainly driven by
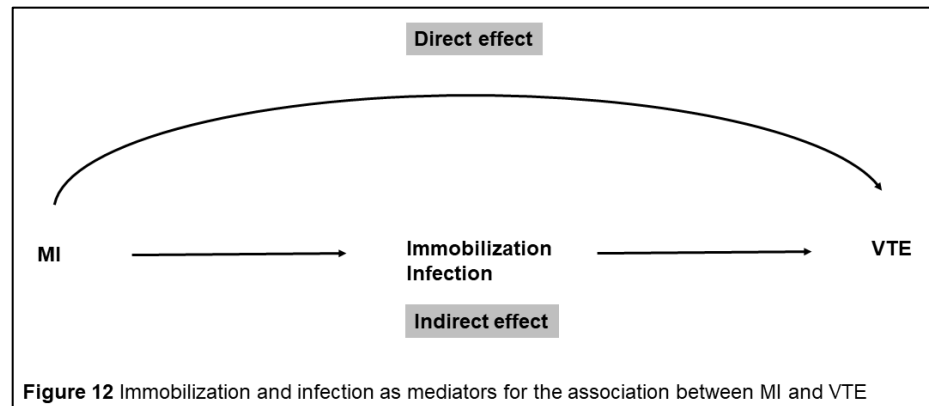

Figure 12 Immobilization and infection as mediators for the association between MI and VTE

concomitant infection and immobilization (Figure 12). The total mediating effect in our KHB model was approximately 60%. Our findings put further emphasis to the notion that an MI is a high-risk situation regarding VTE risk. Although acute MI has been demonstrated to be a risk factor for VTE in previous studies, the role of MI as a trigger factor for VTE is not well investigated. Previous studies on the association between MI and VTE were case-control studies and cohort studies, designed to answer the question "*why did the patient develop the disease?*". However, it is equally important to answer the question clinicians often ask their patients "*were you doing anything unusual just before the episode?*". In order to answer this question a case-crossover design is particularly suitable [52]. The results from Paper I are in line with a case-control study from Olmsted County reporting that the strength of the association between MI and VTE was attenuated after adjustment for all other risk factors for VTE [237]. Unfortunately, as described in Section 4.1 of this thesis, the temporal sequence between exposure, intermediate and outcome was not determined in our study. Nevertheless, the adjustment and mediation analysis showed that concomitant infection and immobilization were important factors contributing to VTE risk in MI patients.

Serious infections occur in up to 3-4% of MI patients treated with percutaneous coronary intervention (PCI) and coronary arterial bypass graft surgery, and are associated with adverse 90-day outcome and prolonged hospital stay [238, 239]. Despite the apparent low infection rate in patients with MI, infection may still play an important role in VTE risk stratification of MI patients. Moreover, immobilization is a recognized risk factor for VTE, and immobilization and infection often coexist [125,

240]. Indeed, joint exposure to immobilization and infection has been shown to yield a synergistic effect on VTE risk [125]. Current guidelines recommend anticoagulant thromboprophylaxis for medically ill patients at increased risk of VTE throughout the period of immobilization or hospital stay [25]. However, a publication from the Norwegian Coronary Stent Trial have demonstrated a considerable bleeding risk in patients with MI receiving dual antiplatelet therapy after PCI [180], and independent risk factors for major bleeding were chronic kidney disease, low body weight (<60 kg), diabetes mellitus, and advanced age (>80 years). Therefore, a RAM that discriminates MI patients at high and low risk of VTE is needed to identify those with a favorable benefit-to-harm ratio for pharmacological thromboprophylaxis. The VTE risk should also be balanced against the potential bleeding risk inflicted by the combination of dual antiplatelet therapy and anticoagulant medication (i.e., triple antithrombotic therapy) [241]. A trigger factor can be especially predictive of a given outcome because it is most proximal to outcome occurrence [242]. Hence, the results from the present study suggest that both acute infection and immobilization could be essential in the risk assessment of VTE in MI patients.

We could not differentiate between subtypes of MI in Paper I. In clinical practice, we often distinguish between type I and type II MIs based on underlying pathophysiology. A type I MI is the classical atherothrombotic event in the coronary circulation (described in Section 1.4 of this thesis), and a type II MI occurs due to low partial pressure of O_2 in blood (i.e., hypoxemia) which leads to O_2 deprivation in the myocardium and ischemia. The population of patients with type I and type II MIs differ considerably with regard to risk factor profile, comorbidity and mortality [243], which could potentially confound the association between MI and VTE. However, comorbidities are controlled for through the study design in the case-crossover study.

The association between MI and VTE was not completely explained by the presence of indirect risk factors related to the MI, as a direct effect of MI on VTE risk was observed after adjustment. Elevated levels of coagulation factors VIII, IX, XI, plasminogen activator inhibitor-1 and vWF are risk factors for both VTE [244] and MI [245, 246]. Increased levels of the coagulation factors have been measured immediately after an acute MI, and a persistent coagulation activation have been demonstrated even long after the acute event [247, 248]. Additionally, there is a complex crosstalk between the coagulation system and inflammatory pathways [49].

Indeed, inflammation has been demonstrated to be a risk factor for both VTE and MI [249-251], and markers of inflammation increase after an acute MI event [252, 253]. Therefore, activation of coagulation and inflammation could mediate the transient VTE risk observed in MI patients. In addition, release of procoagulant EVs secondary to hypoxemia and myocardial damage have also been demonstrated [254] and may serve as a mechanism [255] by which MI increases VTE risk.
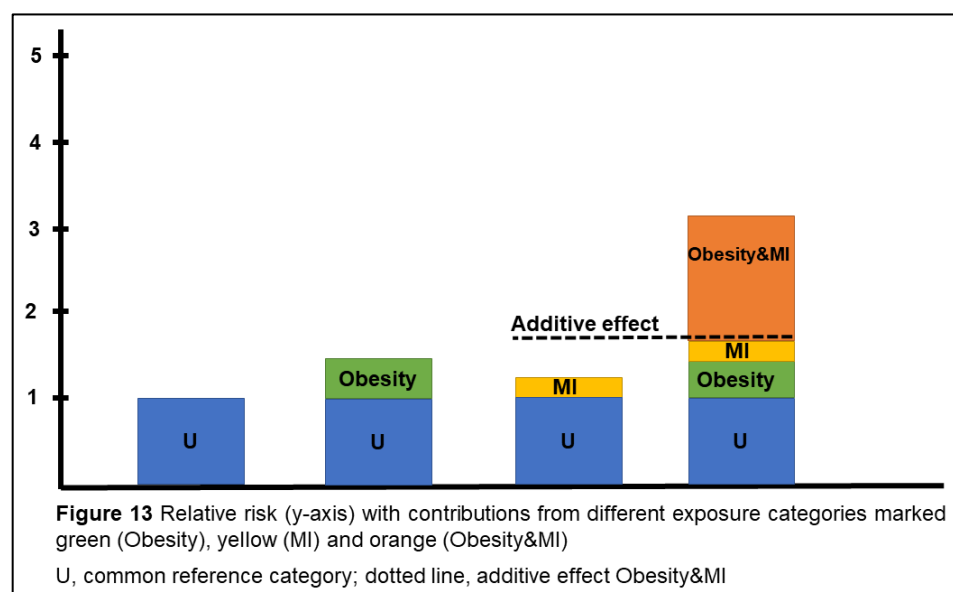
Other conditions that are frequent complications of an acute MI which could potentially result in an increased thrombotic potential, are heart failure with subsequent stasis in the cardiopulmonary circulation [256, 257] or atrial fibrillation with turbulent blood flow [258]. Heart failure post MI may add to the increased thrombosis tendency after the acute MI event [174, 259, 260], and current knowledge implies that both HFpEF and HFrEF increase VTE risk short- and long term [135]. Therefore, the observed short-term increased VTE risk after an MI could in part be explained by heart failure. All components of Virchow's triad are presumably part of the pathogenesis of heart failure related VTE. First, impaired myocardial contractility with subsequent reduced ejection fraction (HFrEF) promotes stasis in the cardiopulmonary circulation [261]. Second, regional up-regulation of TF on capillary endothelial cells leads to a hypercoagulable state (for both HFrEF and HFpEF) in the pulmonary vascular bed [135]. Third, stretch and injury to the vessel wall promotes endothelial dysfunction (for both HFrEF and HFpEF). Unfortunately, we did not have information on acute heart failure after MI in our study, and could therefore not take this into account in the analysis.

5.2.2 The impact of prothrombotic SNPs and obesity on the relationship between MI and VTE

As described in Section 5.2.1 of this thesis, MI patients receive dual antiplatelet therapy as secondary prevention after the acute event (12 months as a general recommendation [262]). Therefore, medical thromboprophylaxis with anticoagulants is particularly challenging due to the increased bleeding risk. By identifying high-risk groups or settings in which interaction occurs, preventive actions for VTE may be more targeted and effective.

In paper II of this thesis, we reported that incident MI and the prothrombotic SNPs were associated with VTE risk when analyzed separately. However, adjustment for the five prothrombotic SNPs (either individually or as a score) did not influence the relationship between MI and VTE. In individuals without MI, the risk of VTE increased linearly with increasing number of risk alleles in the 5-SNP score. In contrast, there was no association between increasing number of risk alleles and risk of VTE in MI patients. Our findings of no excess combined effect in Paper II could imply that an MI and the prothrombotic SNPs operate through different mechanisms and probably would be causes of different VTE cases, rather than acting together as cause of the same VTE case. Further, a potential explanation of no excess combined effect could be that mechanisms related to pathways other than those that lead to a hypercoagulable state in the presence of prothrombotic genotypes are crucial in MI-related VTE. However, in a study of patients with a broad spectrum of cardiometabolic disease (i.e., history of atherosclerosis, MI, and diabetes), a polygenic risk score based on 297 SNPs was an independent predictor of VTE after accounting for available clinical risk factors [263]. The findings from this study suggest that polygenic risk scores may potentially identify more patients at particularly high VTE risk.

In paper III, the combined exposure of MI and obesity was associated with a 3-fold increased risk of VTE compared to participants exposed to neither risk

Figure 13 Relative risk (y-axis) with contributions from different exposure categories marked green (Obesity), yellow (MI) and orange (Obesity&MI)

U, common reference category; dotted line, additive effect Obesity&MI

factors, which corresponded to an effect on VTE risk that exceeded the sum of the separate effects (Figure 13). Moreover, 46% of the VTE events that occurred among subjects jointly exposed to MI and obesity appeared to be attributable to interaction between the two risk factors. In analyses of overall VTE, the effect of MI on VTE risk in non-obese was small (15% increased). Subgroup analyses revealed that in non-

obese, MI was associated with provoked VTE, but not unprovoked VTE. Thus, it appears that in the absence of obesity, other provoking risk factors are required to increase the risk of VTE in MI patients. These findings fit well with the thrombosis threshold model where two or more risk factors are needed to exceed the thrombosis threshold [53]. Accordingly, we showed in Paper I that infection and immobilization mediated the VTE risk within the 3-month period after an MI by approximately 60%.

In Paper III, we found that the combined effect of MI and obesity had a stronger impact on the risk of DVT and unprovoked VTE than PE and provoked VTE. Obesity was apparently a prerequisite for the increased risk of DVT and unprovoked VTE in MI patients. The underlying mechanism behind such an interaction is unknown. However, as both obesity and MI are inflammatory conditions [253, 264], one might speculate that the observed excess VTE risk could be related to thromboinflammation. In addition, obesity is associated with both hypercoagulation and hypofibrinolysis [199], which could add to the hypercoagulable state induced by an MI, and resulting in an excess risk of VTE. Moreover, higher levels of procoagulant EVs have been reported in obese than normal weight subjects [265], and release of EVs following myocardial damage after an MI have been described [254]. Substantially increased levels of EVs may serve as initiators of the coagulation cascade [47, 255] in the acute phase of an MI, resulting in excess risk of VTE. As described in Section 5.2.1 of this thesis, MI is associated with heart failure [257], which is a risk factor for VTE, and potentially, heart failure induced stasis may be further enhanced in obese patients, leading to increased risk of DVT.

The age- and sex adjusted incidence of MI decreased by 3% each year from 1994 – 2008 in the Tromsø Study [266]. Changes in modifiable cardiometabolic risk factors such as dyslipidemia, hypertension, smoking, and physical inactivity accounted for 66% of the decline. Despite a decline in the incidence of MI during the past decades, acute MI is still a major contributor to the global disease burden [157]. Further, obesity is a well-known risk factor for MI, and the prevalence of obesity among MI patients is high [164]. Indeed, the prevalence of obesity in the population is increasing [85]. Because only 20% achieve the goal of a BMI<25 kg/m^2 after an incident MI [167], obesity will likely continue to be an important contributor to the increased VTE risk among MI patients in the coming years [157].

In Paper II and Paper III, we did not have information on frequent medication after MI like statins [267] and antithrombotic drugs [180]. However, the use of such medication is probably evenly distributed over the risk allele categories and therefore do not serve as confounders in Paper II. In Paper III, statin use would presumably be more frequent in overweight and obese subjects. However, if the effect of these medications would be sufficient to counterbalance the effect of obesity, they might have contributed to underestimate the risk of VTE post MI.

5.2.3 Clinical implications

In summary, our findings from Paper I suggest that the increased VTE risk after MI may to a large extent be explained by concomitant conditions related to the MI, in particular acute infections and immobilization. The results from Paper II suggest that the increased risk of VTE in MI patients is not explained by the five prothrombic SNPs. Therefore, the five prothrombotic SNPs presumably do not play an important role in the development of VTE after MI. The results from Paper III demonstrate that the combination of MI and obesity leads to an excess risk of VTE, suggesting that there is biological interaction between MI and obesity on the risk of VTE. An implication from the present thesis and initiative to improve patient care, is to acknowledge MI as a high-risk situation for VTE, and to avoid complications of MI related to increased VTE risk. Furthermore, half of the world's adult population is either overweight or obese, the prevalence of obesity is increasing, and obesity is a preventable shared risk factor for both MI and VTE [33, 164, 268]. Thus, interventions to reduce obesity will be important to reduce the impact of MI-related VTE at an individual and population level.

Around one quarter of incident VTE cases in the general population are attributed to hospitalization for acute medical illness [18] and approximately 6% of PE cases in the general population can be attributed to MI [175]. Current guidelines recommend medical thromboprophylaxis during the period of immobilization or hospital stay in acutely ill hospitalized medical patients at increased risk of thrombosis, but recommends against extending prophylaxis beyond the initial period of hospitalization [34, 269]. However, the increased VTE risk extends beyond hospital discharge, as a large proportion (approximately 70%) of hospital-related VTE cases occur after hospitalization [270-272] and the elevated VTE risk in MI patients extends up to 6 months after the event [175]. However, extending thromboprophylaxis 4-6 weeks after

hospital discharge in unselected medical patients reduced symptomatic VTE events at the expense of increased major and fatal bleedings [273]. Because MI patients receive dual antiplatelet therapy as secondary prevention after the acute event, medical thromboprophylaxis with anticoagulants is particularly challenging due to the increased bleeding risk inflicted by triple antithrombotic therapy. The findings from the present thesis suggest that acute infections, immobilization and obesity could be essential in the risk assessment of VTE in MI patients.

To improve patient care it is pivotal to develop a prediction model to accurately discriminate between MI patients at high and low VTE risk, and preferably also high and low bleeding risk, as accurate risk stratification could identify the MI patients with a positive risk-benefit ratio for thromboprophylaxis during hospitalization and in the first weeks after hospitalization [274]. A RAM can aid health care practitioners to assess an individual patient's risk of VTE based on a set of characteristics. The development of a RAM that identifies MI patients at particularly high thrombosis risk should be founded on clinical risk factors for VTE, potential biomarkers [115, 255, 275], and bleeding risk [180]. The potential discriminative capabilities of acute infection, immobilization, and obesity on VTE risk should be further investigated in a large prospective cohort of MI patients, and their predictive performance alone and combined in a RAM should be assessed. A successful prediction model that improves patient stratification and aids clinical decisions on medical thromboprophylaxis, will in turn contribute to reduce the incidence of VTE in the population.

6. Conclusions

❖ We found that an MI is a strong trigger factor for VTE, and that indirect risk factors related to the MI, in particular infection and immobilization, may to a large extent explain the observed association between MI and VTE. Even though the risk of VTE in patients with MI was largely explained by other risk factors, MI was still associated with an increased risk of VTE after adjustment for all the other well-established VTE triggers

❖ The five prothrombotic genotypes, either evaluated separately or as part of a genetic risk score, could not explain the association between MI and VTE as they did not increase VTE risk in MI patients

❖ We demonstrated that the combination of MI and obesity resulted in an excess risk of VTE, suggesting that there is biological interaction between MI and obesity on the risk of VTE. In non-obese subjects, MI was associated with PE and provoked VTE, but not associated with DVT and unprovoked VTE. However, the effect of the interaction was more pronounced for the risk of DVT and unprovoked VTE. Thus, the increased risk of these subtypes of outcomes in MI patients appeared to be dependent on the presence of obesity

7. References

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Paper I

Paper II

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in thrombosis & haemostasis

Myocardial infarction, prothrombotic genotypes, and venous thrombosis risk: The Tromsø Study

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Abstract

Background: The risk of venous thromboembolism (VTE) is increased after a myocardial infarction (MI). Some prothrombotic genotypes associated with VTE have also been associated with risk of MI. Whether prothrombotic single-nucleotide polymorphisms (SNPs) further increase the risk of VTE in MI patients is scarcely investigated.

Aim: To study the combined effect of MI and prothrombotic SNPs on the risk of VTE.

Methods: Cases with incident VTE (n = 641) and a randomly sampled subcohort weighted for age (n = 1761) were identified from the 4 to 6 surveys of the Tromsø Study (1994-2012). DNA was genotyped for rs8176719 (*ABO*), rs6025 (*F5*), rs1799963 (*F2*), rs2066865 (*FGG*), and rs2036914 (*F11*). Hazard ratios (HRs) for VTE with 95% confidence intervals (CIs) were estimated by categories of risk alleles and MI status.

Results: Patients with MI had a 1.4-fold increased risk of VTE, and adjustments for the 5 SNPs, either alone or in combination, did not affect this relationship (adjusted HR, 1.52; 95% CI, 1.12-2.07). In subjects without MI, an increased risk of VTE was observed for each of the individual SNPs (≥1 vs. 0 risk alleles), and the risk increased linearly with increasing number of risk alleles in the 5-SNP score. The combination of MI and prothrombotic genotypes, either as individual SNPs or in the 5-SNP score, did not result in an excess risk of VTE.

Conclusion: The relationship between MI and VTE was not explained by these 5 prothrombotic genotypes. Prothrombotic genotypes did not yield an excess risk of VTE in patients with MI.

KEYWORDS
epidemiology, genetics, myocardial infarction, pulmonary embolism, risk factors, thromboembolism, venous

Essentials

- The risk of venous thromboembolism (VTE) is increased after myocardial infarction (MI).
- Whether this association is explained by common prothrombotic genotypes is scarcely investigated.
- We investigated the combined impact of MI and prothrombotic genotypes on risk of VTE.
- In patients with MI, prothrombotic genotypes did not yield excess risk of VTE.

1 | INTRODUCTION

Several studies have indicated that patients with myocardial infarction (MI) have increased risk of subsequent venous thromboembolism (VTE).[1-3] The risk is highest during the first months after an MI[2,3] and appears to be particularly pronounced for pulmonary embolism (PE).

VTE is a multicausal disease encompassing both genetic and environmental risk factors, and a number of prothrombotic genotypes have been linked with VTE risk.[4] In a prediction study, de Haan and coworkers[5] investigated whether a set of 31 known VTE-associated single-nucleotide polymorphisms (SNPs) could be used to identify subjects with high risk of VTE. SNPs with the highest odds ratios in the literature were added one by one to build a risk score, ultimately using the most parsimonious model. A 5-SNP score, which consisted of rs8176719 (*ABO*), rs6025 (*F5*, factor V Leiden [FVL]), rs1799963 (*F2*, prothrombin G20210A), rs2066865 (*FGG*), and rs2036914 (*F11*), predicted VTE with an area under the receiver operating characteristic curve of 0.69.

The mechanism for the relationship between MI and VTE is not well understood. However, growing evidence indicates that atherosclerotic risk factors[6,7] and subclinical atherosclerosis[8-11] are not associated with VTE, and therefore cannot serve as common risk factors for the 2 conditions. It has been reported that certain SNPs associated with VTE risk also predisposes to acute MI.[5,12-15] Additionally, family history of MI has been demonstrated to be a risk factor for VTE in several studies,[16-18] and this risk is not explained by intermediate development of MI.[19] Whether the relationship between MI and VTE can be explained by common prothrombotic genotypes has not been well addressed. Therefore, the aim of this study was to investigate the combined effect of MI and the SNPs included in the 5-SNP risk score on VTE risk in a population-based case cohort.

2 | MATERIALS AND METHODS

2.1 | Study population

As described elsewhere,[20] the Tromsø Study is a unique Norwegian follow-up study with consecutive health surveys of the inhabitants of Tromsø. For the fourth survey (1994-1995), all inhabitants of the municipality of Tromsø aged >24 years of age were invited to participate. The overall attendance rate was high (77%) and 27 158 individuals participated. Repeated surveys were conducted in 2001-2002 and 2007-2008, with attendance rates of 78% and 66%, respectively. In total, 30 586 unique participants aged 25 to 97 years partook in ≥1 of the surveys, and of these, 30 361 consented to contribute to medical research.

From the date of inclusion in 1 of the 3 surveys until the follow-up ended (December 31, 2012), all incident VTE events were identified by performing an extensive search in the registries (diagnosis registry, autopsy registry, and radiology registry) at the University Hospital of North Norway (UNN). The UNN is the only hospital in the region, and all hospital care and relevant diagnostic radiological procedures is provided exclusively by this hospital. Each VTE was adjudicated and recorded after extensive review of medical records, as previously described.[21] The adjudication criteria for VTE were presence of signs and symptoms of deep vein thrombosis (DVT) or PE combined with objective confirmation by radiological procedures, which resulted in treatment initiation (unless contraindications were specified). A VTE was classified as either a DVT or PE, and if DVT and PE occurred concurrently, the VTE was classified as a PE.

During follow-up, 737 incident VTE events occurred. Subjects for whom blood samples were not available or of insufficient quality for DNA analysis were excluded (n = 45). The remaining 692 subjects were included as cases, and a subcohort (n = 2016) was created by randomly sampling participants from the source cohort weighted for the age distribution of the cases in 5-year age groups. As a consequence of the case-cohort design, 71 cases were also sampled to, and included in, the subcohort. Participants with MI before the enrollment date of the study were excluded (n = 135). Further, participants with at least 1 missing value for the risk allele variants were also excluded from the study (n = 171). Our final case cohort therefore comprised a total of 2402 subjects, consisting of 641 VTE cases and 1761 subjects in the subcohort (Figure 1). The regional committee for medical and health research ethics in Northern Norway approved the study. All participants provided informed written consent to participate.

2.2 | Baseline measurements

Baseline information was collected by questionnaires, physical examination, and blood samples. Nonfasting blood samples were collected from an antecubital vein, and citrated plasma and DNA were stored at −70°C in biobanks.

FIGURE 1 Flowchart illustrating the composition of the case-cohort study. FVL, factor V Leiden; VTE, venous thromboembolism
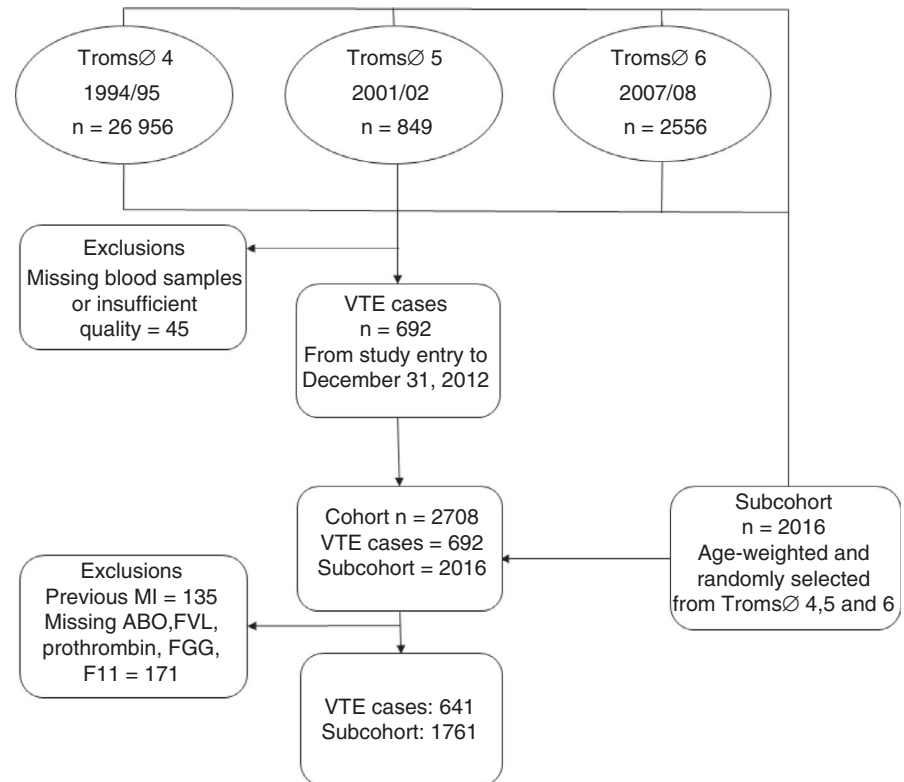
Hypertension was defined as mean systolic blood pressure ≥140 mm Hg or mean diastolic blood pressure ≥90 mm Hg or current use of antihypertensives. Hypercholesterolemia was defined as total cholesterol ≥6.5 mmol/L. From the self-administered questionnaires, baseline data on diabetes mellitus, cardiovascular disease (myocardial infarction, angina, or stroke), smoking (never/former/current), physical activity habits, and education level was obtained. Education level was categorized into basic schooling (7-10 years), high school/vocational school, and college/university. Further information about the baseline variables in the Tromsø Study can be found elsewhere.[20]

2.3 | Assessment of myocardial infarction

Identification of MI patients was performed searching hospital medical records and out-of-hospital medical records, autopsy records, and death certificates. The national 11-digit identification number facilitated linkage to local and national diagnosis registries. Possible cases of MI were identified by searching the hospital discharge registry at the UNN using relevant International Classification of Diseases codes, as previously described.[3] The medical records were reviewed, and all possible events were validated by an independent end-point committee. MONICA/MORGAM criteria were used to adjudicate MI cases, and these included clinical signs and symptoms of ischemic cardiac disease, findings on electrocardiograms, and elevated cardiac biomarkers. The autopsy record was used when applicable.

2.4 | Prothrombotic genotypes

We genotyped the SNPs rs8176719 in *ABO* (ABO blood group), rs6025 in *F5* (FVL), rs1799963 in *F2* (prothrombin G20210A), and rs2036914 in *F11* using the Sequenom platform, and rs2066865 in *FGG* using the TaqMan platform, as previously described.[22] For Sequenom, samples were genotyped using the Sequenom iPlex Gold Assay according to the recommended protocol, using an initial input of 10 to 20 ng DNA, and were analyzed using the MassARRAY Analyzer 4 (Agena Bioscience, San Diego, CA, USA). For TaqMan, an initial input of 100 ng of DNA was used, and samples were genotyped using the 7900HT (Applied Biosystems, Foster City, CA, USA) according to the recommended protocol.

Subjects were categorized as carriers of the prothrombotic risk gene when ≥1 risk allele was present. For rs2036914, the minor allele was associated with lower risk of VTE, and therefore we considered the major allele as the risk allele. Based on the paper by de Haan et al,[5] we composed a 5-SNP score by summarizing the number of risk alleles from the 5 sequenced SNPs. These were further categorized into 0 to 1, 2, 3, and ≥4 risk alleles.

2.5 | Statistical analysis

Statistical analysis was carried out using the STATA software version 15.0 (StataCorp, College Station, TX, USA). Cox proportional hazards regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for VTE by MI status, adjusted for each of

the prothrombotic genotypes individually and in a multivariable model. Further, we estimated HRs for combinations of MI and the individual SNPs. Participants without MI and with no risk alleles was used as the reference group. Moreover, we estimated HRs according to combinations of MI and categories of the 5-SNP score, using those without MI and 0 to 1 risk alleles as the reference group. Age was used as time scale in the Cox model, and MI was included as a time-dependent covariate in the Cox model. Thus, those who developed MI during follow-up contributed with both unexposed and exposed person-time (ie, unexposed person-time from baseline to the date of MI, and thereafter with exposed person-time from MI to the end of follow-up). All analyses were adjusted sex. The proportional hazards assumption was tested using Schoenfeld residuals and found not violated.

3 | RESULTS

Baseline characteristics of the study participants are shown in Table 1. In our cohort, 274 subjects experienced an incident MI

TABLE 1 Baseline characteristics of the study population with and without MI (n = 2402): The Tromsø study

	No MI (n = 2128)	MI (n = 274)
Age, y	57 ± 14	67 ± 10
Sex, male	41.9 (892)	54.0 (148)
Body mass index, kg/m^2	25.9 ± 4.2	26.7 ± 4.4
Total cholesterol, mmol/L	6.49 ± 1.31	7.17 ± 1.23
High-density lipoprotein, mmol/L	1.55 ± 0.42	1.47 ± 0.43
Triglycerides, mmol/L	1.60 ± 0.97	1.94 ± 1.08
Systolic blood pressure, mm Hg	142 ± 23	154 ± 23
Diastolic blood pressure, mm Hg	82 ± 13	87 ± 14
Hypertension[a]	52.0 (1107)	74.8 (205)
Hypercholesterolemia[b]	48.0 (1021)	71.5 (196)
Smoking[c]	33.7 (716)	38.1 (104)
Physical activity[d]	23.0 (484)	14.8 (40)
Education[e]	23.6 (503)	8.76 (24)
Self-reported diabetes mellitus	2.79 (59)	6.23 (17)
rs8176719 (ABO), ≥1 risk allele[f]	63.3 (1347)	65.3 (179)
rs6025 (F5), ≥1 risk allele[f]	9.1 (194)	5.1 (14)
rs1799963 (F2), ≥1 risk allele[f]	1.6 (35)	0.4 (1)
rs2066865 (FGG), ≥1 risk allele[f]	45.4 (966)	43.8 (120)
rs2036914 (F11), ≥1 risk allele[f]	81.6 (1736)	80.3 (220)

Note: Values are % (n) or mean ± standard deviation.
[a]Mean systolic/diastolic blood pressure ≥140/≥90 mm Hg or current use of antihypertensives.
[b]Total cholesterol ≥6.5.
[c]Self-reported daily smoking, yes/no.
[d]≥1 hour of moderate or hard physical activity per week, yes/no.
[e]>10 years of education.
[f]Percentage of participants with ≥1 risk allele(s).

during the median of 15.7 years of follow-up. On average, those who developed MI were older and had higher values of cardiometabolic risk factors, including total cholesterol, triglycerides, and body mass index than those who did not experience an MI. The proportion of men, smokers, and subjects with hypertension, hypercholesterolemia, and diabetes mellitus was higher in those with MI than in those without MI. The proportion of participants with ≥1 risk allele(s) for the SNPs investigated were essentially similar between the groups (Table 1).

Table 2 shows features of the VTE events in participants with and without MI in our study. In total, 47 of the 641 VTE events occurred in subjects with MI. The proportion of PEs was higher among subjects suffering from MI than among subjects who did not experience an MI (55% and 42%, respectively). The proportion of provoked events was higher in the MI group than in the non-MI group (62% and 53%, respectively).

Subjects with MI had an overall 1.4-fold higher risk of developing VTE (HR, 1.44; 95% CI, 1.07-1.96) compared to subjects without MI. Further adjustments for each of the prothrombotic SNPs did not alter the risk estimates (data not shown), and the HR for MI in the model that included all 5 SNPs was 1.52 (1.12-2.07).

The risk estimates for VTE by categories of the individual SNPs and MI status are shown in Table 3. In subjects with non-O

TABLE 2 Baseline characteristics of VTE events (n = 641): The Tromsø Study

	No MI (n = 594)	MI (n = 47)
Clinical characteristics, % (n)		
DVT	58.1 (345)	44.7 (21)
PE	41.9 (249)	55.3 (26)
Provoked	52.7 (313)	61.7 (29)
Unprovoked	47.3 (281)	38.3 (18)
Clinical risk factors, % (n)		
Estrogen[a,b]	12.2 (36)	3.85 (1)
Pregnancy/ puerperium[a]	1.9 (5)	–
Heredity[c]	3.71 (22)	–
Provoking factors, % (n)		
Surgery	14.8 (88)	21.3 (10)
Trauma	7.9 (47)	6.4 (3)
Cancer	23.4 (139)	27.7 (13)
Immobility[d]	21.0 (125)	21.3 (10)
Other[e]	5.1 (30)	4.3 (2)

[a]Only women included in the analysis.
[b]Current or previous use of hormone replacement therapy or oral contraceptives.
[c]Venous thromboembolism in a first-degree relative before 60 y of age.
[d]Bed rest >3 d; journeys of >4 h by car, boat, train, or air within the past 14 d; or other types of immobilization.
[e]Other provoking factor described by a physician in the medical record (eg, intravascular catheter).

TABLE 3 HRs with 95% CIs for VTE by combined categories of MI and prothrombotic genotypes: The Tromsø Study

	Risk alleles	Events	HR (95% CI)[a]
rs8176719 (*ABO*)			
No MI	0	181	Reference
	≥1	413	1.44 (1.21-1.72)
MI	0	23	2.38 (1.54-3.69)
	≥1	24	1.48 (0.97-2.29)
rs6025 (*F5*)			
No MI	0	499	Reference
	≥1	95	2.20 (1.76-2.73)
MI	0	45	1.54 (1.13-2.11)
	≥1	2	2.46 (0.61-9.91)
rs1799963 (*F2*)			
No MI	0	579	Reference
	≥1	15	1.64 (0.98-2.73)
MI	0	47	1.46 (1.10-2.00)
	≥1	0	–
rs2066865 (*FGG*)			
No MI	0	320	Reference
	≥1	274	1.06 (0.90-1.25)
MI	0	30	1.59 (1.08-2.32)
	≥1	17	1.34 (0.82-2.19)
rs2036914 (*F11*)			
No MI	0	106	Reference
	≥1	488	1.05 (0.85-1.30)
MI	0	8	1.21 (0.59-2.48)
	≥1	39	1.58 (1.10-2.30)
De Haan score			
No MI	0-1	87	Reference
	2	157	1.18 (1.00-1.54)
	3	185	1.47 (1.14-1.90)
	≥4	165	1.78 (1.37-2.31)
MI	0-1	14	2.19 (1.24-3.87)
	2	13	1.60 (0.89-2.87)
	3	10	1.68 (0.87-3.24)
	≥4	10	2.70 (1.40-5.21)

Abbreviations: CI, confidence interval; HR, hazard ratio; MI, myocardial infarction; VTE, venous thromboembolism.

[a]Adjusted for age (as time scale) and sex.

FIGURE 2 In individuals without MI, the risk of VTE increased linearly with increasing number of risk alleles in the 5-SNP score. In contrast, there was no association between increasing number of risk alleles and risk of VTE in MI patients. MI, myocardial infarction; SNP, single-nucleotide polymorphism; VTE, venous thromboembolism

blood type and no MI, the risk of VTE was 1.4-fold increased (HR, 1.44; 95% CI, 1.21-1.72) compared to subjects with blood type O without MI (reference category). In subjects with blood type O and MI, the risk was 2.4-fold higher (HR, 2.38; 95% CI, 1.54-3.69) than the reference category. However, the combination of non-O blood type and MI yielded a 1.5-fold increase (HR, 1.48; 95% CI, 0.97-2.29) in VTE risk compared to the reference category. In subjects without MI, FVL was associ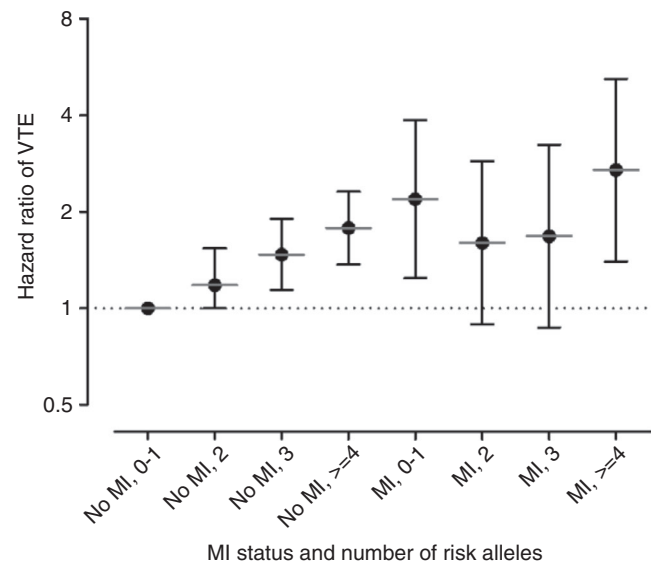ated with a 2-fold increased risk (HR, 2.20; 95% CI, 1.76-2.73) of VTE. Subjects with MI without FVL had a 1.5-fold increased risk (HR, 1.54; 95% CI, 1.13-2.11) of VTE, and MI patients with ≥1 risk alleles of FVL had a 2.5-fold higher (HR, 2.46; 95% CI, 0.61-9.91) risk of VTE than subjects without FVL and no MI. There was no association between *FGG* SNP and VTE risk in participants either with or without MI. In subjects without MI, the prothrombin mutation was associated with a 1.6-fold increased risk of VTE (HR, 1.64; 95% CI, 0.98-2.73). Due to lack of cases with MI and the prothrombin mutation, no combined effect could be calculated. In subjects without MI, there was no association between *F11* SNP and risk of VTE (HR, 1.05; 95% CI, 0.85-1.30). Subjects with MI and no risk alleles at *F11* had a HR for VTE of 1.21 (95% CI, 0.59-2.48), whereas MI patients with ≥1 allele had a 1.6-fold increased risk of VTE (HR, 1.58; 95% CI, 1.10-2.30).

In subjects without MI, the risk of VTE increased linearly across increasing categories of risk alleles of the 5-SNP score. In those with 2, 3 and ≥4 risk alleles, the risk was 1.2-fold (HR, 1.18; 95% CI, 1.00-1.54), 1.5-fold (HR, 1.47; 95% CI, 1.14-1.90) and 1.8-fold (HR, 1.78; 95% CI, 1.37-2.31) increased compared to subjects with 0 to 1 risk alleles. In patients with MI, there was no increased risk across categories of the 5-SNP score (Table 3 and Figure 2).

In subgroup analysis, we found that MI patients with ≥1 risk allele at *F11* had 1.8-fold higher risk of PE (HR, 1.79; 95% CI, 1.05-3.05). Otherwise, subgroup analysis on the association between the other SNPs studied and MI in relation to the risk of DVT and PE (Table S1), as well as provoked and unprovoked VTE, (Table S2) showed essentially similar results as those for overall VTE.

4 | DISCUSSION

We investigated the role of 5 prothrombotic genotypes as potential common risk factors for MI and VTE. Subjects with MI had increased risk of VTE, but adjustment for the 5 prothrombotic SNPs, either individually or as a score, did not influence this relationship. In individuals without MI, the risk of VTE increased with increasing number of risk alleles in the 5-SNP score. In contrast, there was no association between increasing number of risk alleles and risk of VTE in MI patients. Our findings suggest that the increased risk of VTE in patients with MI cannot be explained by these 5 prothrombotic genotypes.

Several studies,[1,2] including a former report from the Tromsø Study,[3] have shown that MI patients are at increased risk of VTE, particularly the initial months after the MI. Several lines of evidence support that this relationship is not explained by common atherosclerotic risk factors or subclinical atherosclerosis. A large meta-analysis of 9 population-based cohorts, including more than 240 000 individuals, showed no association between traditional atherosclerotic risk factors and VTE.[7] In the Tromsø Study, the risk of VTE after MI remained after adjustment for atherosclerotic risk factors.[3] Moreover, previous cohorts,[11] including the Tromsø Study,[8,9] consistently showed that subclinical atherosclerosis was not associated with VTE.

Non-O blood group, FVL, prothrombin G20210A, and SNPs in *FGG* and *F11* are recognized risk factors for VTE,[23–25] and some of these SNPs have also been associated with coronary artery disease.[13,14,26,27] For instance, non-O blood group is associated with a modestly increased risk of coronary heart disease,[28,29] and 2 meta-analyses[14,30] concluded that the FVL and prothrombin G20210A variants have weak or moderate associations with MI risk. Moreover, in a case-control study comprising only men, Doggen et al[31] reported a higher risk of MI in individuals with factor XI levels in the highest versus lowest quintile. In our study, rs2036914 in *F11* was the only SNP that showed an association with VTE in MI patients. However, the risk allele in the *F11* SNP was the most frequent allele (ie, major allele), and adjustment for this allele did not influence the association between MI and VTE, indicating that the aforementioned SNP could not explain the increased risk of VTE after MI. Indeed, all our adjustment models indicated that the presence of these 5 prothrombotic genotypes, either evaluated as individual SNPs or combined in a genetic risk score, could not explain the higher VTE risk observed in patients with MI compared to the general population.

The increased risk of VTE after MI could be explained by mechanisms other than shared risk factors such as obesity and advancing age.[32] Indeed, the short-term nature of the increased VTE risk reported in several studies points toward mechanisms related to the MI itself or subsequent complications after the MI.[1–3] Potential mechanisms may be venous stasis because of heart failure or disturbances in the electromechanical function of the heart (eg, atrial fibrillation),[33] or release of procoagulant extracellular vesicles following hypoxia and myocardial damage.[34] Furthermore, the short-term risk of provoked VTE after MI[3] infers that complications related to the MI may be important contributors. Concomitant presence of transient risk factors such as infection, immobilization, or cardiac surgery following an acute MI could give rise to a short period with particularly high thrombosis risk.[35–37] Our finding of no combined effect of prothrombotic SNPs and MI on VTE risk, indicates that the pathogenesis of VTE in subjects with MI probably involves mechanisms related to pathways other than those that lead to a hypercoagulable state in the presence of prothrombotic genotypes.

Developing a risk assessment model to distinguish MI patients with high and low risk of VTE is pivotal, and future studies should aim at identifying predictors of VTE following MI. The findings from this study may indicate that the SNPs included in the 5-SNP score are not critical in the risk assessment of VTE in MI patients.

Our study has several strengths, such as the prospective design, recruitment of participants from a general population, well-validated events of both VTE and MI, and the long follow-up period. The high participation rate and the broad age range formed a source cohort that presumably is representative of a general Caucasian population. The study was limited by a low number of VTE events in certain subgroups, particularly for the SNPs with a low prevalence (eg, the prothrombin mutation). Hence, the risk estimates must be interpreted with caution. Further studies with more statistical power are warranted to explore this association is subgroups of MI patients. Both statins and antithrombotic medications are frequently used after MI, and these therapies also reduces the risk of VTE. Unfortunately, we lacked information on the use of medications after MI. However, the use of such medications would presumably be evenly distributed among the prothrombotic genotypes and categories of the 5-SNP score, and thus, not serve as confounders. However, if the effect of these therapies were sufficient to counterbalance the risk of VTE due to prothrombotic genotypes in MI patients, they may have contributed to dilute or underestimate the effect of prothrombotic genotypes. Finally, as in all observational studies, the potential presence of residual confounding cannot be ruled out.

The combination of MI and 5 prothrombotic genotypes, either as individual SNPs or as a 5-SNP score, did not result in an excess risk of VTE. Our findings imply that the increased risk of VTE after an acute MI is not explained by these 5 prothrombotic genotypes.

RELATIONSHIP DISCLOSURE

The authors report nothing to disclose.

AUTHOR CONTRIBUTIONS

JKS analyzed the data and drafted the manuscript. VMM was involved in interpretation of the results and critical revision of the

manuscript. SKB and JBH designed the study and were involved in data collection, interpretation of results, and critical revision of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Paper III

Joint effect of myocardial infarction and obesity on the risk of venous thromboembolism: The Tromsø Study

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Essentials

- Myocardial infarction (MI) is associated with an increased risk of venous thromboembolism (VTE)

- Obesity is a shared risk factor for MI and VTE

- We investigated the joint effect of MI and obesity on VTE risk

- Obesity resulted in an excess risk of VTE in subjects with MI

Abstract

Background: Myocardial infarction (MI) is associated with an increased risk of venous thromboembolism (VTE). Obesity is a recognized risk factor for both MI and VTE. Whether obesity further increases the risk of VTE in MI patients is scarcely investigated.

Aim: To study the joint effect of MI and obesity on the risk of VTE.

Methods: Study participants (n=29 410) were recruited from three surveys of the Tromsø Study (conducted in 1994-1995, 2001, and 2007-2008) and followed up through 2014. All incident MI and VTE cases during follow-up were recorded. Cox regression models with MI as a time-dependent variable were used to estimate hazard ratios (HRs) of VTE (adjusted for age and sex) by combinations of MI exposure and obesity status. Joint effects were assessed by calculating relative excess risk and attributable proportion (AP) due to interaction.

Results: During a median of 19.6 years of follow-up, 2090 study participants experienced an MI and 784 experienced a VTE. Among those with MI, 55 developed a subsequent VTE, yielding an overall incidence rate (IR) of VTE of 5.3 per 1000 person-years (95% CI: 4.1-6.9). In the combined exposure group (MI+/Obesity+), the IR was 11.3 per 1000 person-years, and the adjusted HR indicated a 3-fold increased risk of VTE (HR 3.16, 95% CI: 1.99-4.99) compared with the reference group (MI-/Obesity-). The corresponding AP was 0.46 (95% CI: 0.17-0.74).

Conclusions: The combination of MI and obesity yielded a supra-additive effect on VTE risk of which 46% of the VTE events were attributed to the interaction.

Keywords: epidemiology, obesity, risk factor, myocardial infarction, venous thromboembolism.

Introduction

Several large cohort studies have reported a link between myocardial infarction (MI) and venous thromboembolism (VTE) (*i.e.,* deep vein thrombosis [DVT] and pulmonary embolism [PE]) (1-4). MI is associated with a short-term increased risk of VTE that appears to be particularly pronounced for PE, with an approximately 8-fold increased risk of PE during the first 6 months after the acute MI event (1, 5). The mechanism for this relationship is not well established. However, neither atherosclerosis nor traditional cardiometabolic risk factors such as hypertension, dyslipidemia, or diabetes mellitus are associated with risk of VTE (6, 7), and are therefore not likely shared risk factors for the two conditions (8, 9). The American College of Chest Physicians guidelines recommend thromboprophylaxis with anticoagulants for high-risk hospitalized medical patients (10), but currently, no risk assessment model exists for identifying MI-patients at particularly high risk of VTE. Therefore, there is a need to identify risk factors that are associated with VTE in MI patients.

Obesity is recognized as a risk factor for both MI and VTE (11) and a large proportion (13%) of the world's adult population is obese according to the World Health Organization (WHO) definition(12). The risk of VTE increases with increasing body mass index (BMI), and obesity (BMI$\geq$30 kg/m^2) is associated with a two-fold increased risk of VTE in the general population (13, 14). In addition, obesity is shown to interact with other risk factors, such as oral contraceptives (15), body height (16) and some prothrombotic genotypes (15, 17), yielding a more than additive effect on VTE risk. Even though obesity is a common feature in patients with MI, the role of obesity on VTE risk in MI patients has not been specifically explored, and it has not been investigated whether obesity yields a more than additive effect on risk of VTE in combination with MI. The aim of the present study was therefore to explore the joint effects of MI and obesity on VTE risk in a large population-based cohort.

Materials and methods

Study population

The Tromsø Study is a Norwegian single-center prospective follow-up study with consecutive health surveys of the inhabitants of the Tromsø municipality, and has been described thoroughly elsewhere (18). The fourth survey (T4) was initiated in 1994-1995, where all inhabitants ≥25 years were invited to the study. The participation was high (77%), and 27 158 individuals participated. Further surveys were conducted in 2001 (T5) and 2007-2008 (T6) with participation of 78% and 66%, respectively. In sum, 30 288 participants aged 25 to 97 years took part in ≥1 of the surveys. Participants not officially registered as inhabitants of the municipality of Tromsø at the date of study enrollment (n=21) and participants with a VTE or an MI before the enrollment date (n=82 and n=732, respectively) and missing data on BMI (n=43) were excluded. Hence, 29 410 study subjects were enrolled and followed from study inclusion to end of follow-up (December 31, 2014) or to the date of VTE, migration or death, whichever came first (Figure 1). The Regional committee for medical and health research ethics in Northern Norway approved the study, and all participants provided informed written consent to participation.

Baseline measurements

At baseline inclusion in each survey, information was obtained by questionnaires, physical examination, and blood samples. Blood samples were collected from an antecubital vein in a non-fasting state. From the self-administered questionnaires, data on diabetes mellitus and smoking status (never/former/current) was collected. Blood pressure was measured as previously described (1), and hypertension was defined as mean systolic blood pressure ≥140 mmHg or mean diastolic blood pressure ≥90 mmHg or current use of antihypertensive

medication. Hypercholesterolemia was defined as serum total cholesterol ≥6.5 mmol/L or current use of anticholesteremic medication. Further information regarding baseline variables in the Tromsø Study can be found elsewhere (18).

Assessment of obesity

Body height and weight were measured with subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). The World Health Organization (WHO) defines a BMI ≥30 kg/m^2 as obesity, and this definition was applied in the present study. *No obesity* was defined as a BMI of <30 kg/m^2.

Assessment of MI

The national Norwegian identification number consisting of 11 digits allowed linkage to national and local diagnosis registries. All incident MI events were identified by linkage to the hospital discharge diagnosis registry at the University Hospital of Northern Norway (UNN) and the Norwegian Cause of Death registry at Norwegian Institute of Public Health. The UNN is the only hospital in the region, and all in-hospital and outpatient care of MI and VTE is provided solely by this hospital. Events of possible incident MI were identified by a broad search for the International Classification of Diseases (ICD) 9th revision codes 410-414, 427, 428, 430-438, and 798-799 in the period 1994-1998, and for the ICD 10th edition codes I20-I25, I46-I48, I50, I60-I69, and R96, R98, R99 thereafter.

The hospital medical records were thoroughly reviewed for case validation according to the WHO MONICA/MORGAM criteria, which include clinical symptoms and findings of MI,

ischemic changes in electrocardiograms (ECG), values of cardiac enzymes and findings from autopsy records, when applicable (19).

Outcome ascertainment – venous thromboembolism

All incident VTE cases were identified from the date of inclusion in one of the three surveys (T4-6) until end of follow-up (December 31, 2014) by an extensive search in registries (radiology registry, discharge diagnosis registry and autopsy registry) at the UNN. Each VTE event was adjudicated after review of medical records, as described previously (20). The criteria for adjudication were presence of symptoms and findings of DVT or PE combined with objective confirmation by a radiological procedure, which resulted in treatment initiation unless contraindications were specified. The VTE events from the autopsy registry were included when the death certificate indicated VTE as the cause of death or a significant condition associated with death. Further, a VTE was classified as either a DVT or PE, and if DVT and PE occurred at the same time, the VTE was classified as a PE. The VTE cases were classified as provoked or unprovoked according to the presence of provoking factors at the time of diagnosis. Provoking risk factors were immobilization (*i.e.,* bed rest for >3 days, wheelchair use, or long-distance travel exceeding 4h within the 14 days prior to the event), recent surgery or trauma within the previous 8 weeks, active cancer, or any other potentially VTE provoking factor described by a physician in the medical record (*e.g.,* central venous catheterization).

Statistical analysis

Statistical analysis was performed using the STATA software version 16.0 (StataCorp, College Station, TX, USA). Cox proportional hazards regression models were applied to estimate hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) for VTE by

combinations of MI exposure and obesity status in a multivariable model. Subjects without MI and with *no obesity* were implemented as reference group. Further, age was used as time scale in the regression model (21), and MI was included as a time-dependent covariate. Accordingly, those who developed MI during follow-up contributed with unexposed person-time from baseline to the date of MI, and exposed person-time from MI to the end of follow-up. Subgroup analyses were conducted according to the localization of the thrombotic event (PE or DVT), and the presence of provoking factors at the time of diagnosis. All analyses were adjusted for age (as time scale) and sex, and the proportional hazards assumption was tested using Schoenfeld residuals and found not violated.

To investigate whether the effect of MI and obesity on VTE risk is larger than the sum of the separate risk factors combined, the presence of interaction on an additive scale was evaluated with the relative excess risk due to interaction (RERI) and attributable proportion (AP). These measures of interaction with their corresponding 95% CIs were calculated according to Andersson (22) et al., using an excel sheet (epinet.se/res/xls/epinetcalculation.xls). In short, RERI is calculated as $HR_{AB} - HR_A - HR_B + 1$, where HR_A is the hazard ratio for the first risk factor (*i.e.,* MI) in the absence of the second risk factor (*i.e.,* obesity), HR_B is the hazard ratio for the second risk factor in the absence of the first risk factor, and HR_{AB} is the hazard ratio when both risk factors are present. AP corresponds to $RERI/HR_{AB}$, and should be interpreted as the proportion of cases in the combined exposure group that is due to interaction between the two exposures. RERI and AP >0 indicates positive interaction, i.e., that the effect of the combined exposure is greater than the sum of the individual effects (22, 23).

Results

In total, 26 073 participants were recruited from T4, and 850 and 2487 new participants were recruited from T5 and T6, respectively (Figure 1). During a median of 19.6 years of follow-up, 2090 (7.1%) study participants experienced a first-time MI, and 784 (2.7%) participants had a first-time VTE. Among those with MI, 55 developed a subsequent VTE, yielding an overall incidence rate (IR) of VTE of 5.3 per 1000 person-years (95% CI: 4.1-6.9) after MI. The baseline characteristics of participants with and without incident MI during follow-up are presented in Table 1. Those who experienced an MI were on average older and had higher BMI, and included a higher proportion of men, subjects with hypertension, hypercholesterolemia, and smokers than those without MI (Table 1).

Clinical characteristics of the 784 VTE events are shown in Table 2. There were 451 DVTs (57.5%) and 333 PEs (42.5%). Additionally, 423 events (54.0%) were classified as provoked VTE, and 361 events (46.0%) were classified as unprovoked VTE. Active cancer, immobilization, and surgery within 8 weeks prior to the VTE event were the most frequent provoking factors (Table 2).

IRs and HRs of VTE and subtypes of VTE according to MI exposure and obesity status are shown in Table 3. Among study subjects without MI and with no obesity the IR of VTE was 1.5 per 1000 person-years. In obese participants with no MI, the IR was 3.1 per 1000 person-years and the corresponding HR indicated a 57% increased risk after adjustment for age and sex (HR 1.57, 95% CI: 1.30-1.89). In subjects with MI and no obesity the IR was 4.1 per 1000 person-years and the adjusted HR was 1.15 (95% CI: 0.81-1.61). In the combined exposure group, the IR was 11.3 per 1000 person-years, and the HR indicated a 3-fold increased risk (HR 3.16, 95% CI: 1.99-4.99) compared with the reference group after adjustment for age and sex. Subgroup analyses indicated that in non-obese subjects, MI was associated with PE (HR 1.54, 95% CI: 0.98-2.43), but not with DVT (HR 0.84, 95% CI: 0.50-1.43). However, in

the joint exposure group the HR was 3.49 (95% CI: 1.78-6.81) for PE and 2.91 (95% CI: 1.55-5.49) for DVT, respectively, when compared with the reference category.

In analyses stratified by the presence of provoking factors, MI without obesity was associated with a 1.5-fold increased risk (HR 1.48, 95% CI: 0.98-2.24) while the combination of MI and obesity was associated with a 2.8-fold higher risk (HR 2.78, 95% CI: 1.43-5.41) of provoked VTE compared with the reference category (Table 4). In the absence of obesity, MI was not associated with increased risk of unprovoked VTE (HR 0.76, 95% CI: 0.41-1.39), whereas the combination of MI and obesity was associated with a 3.6-fold increased risk of unprovoked VTE compared with the reference category (HR 3.59, 95% CI: 1.90-6.79).

As shown in Table 5, measures quantifying interaction on an additive scale (*i.e.,* RERI and AP) suggested a supra-additive effect of the combination of MI and obesity on the risk of VTE (Figure 2). The AP measure revealed that 46% of the VTE events in participants with both MI and obesity were attributable to interaction between the two exposures. In subgroup analysis, 34% of PEs and 56% of DVTs in participants with both MI and obesity, respectively, were attributable to interaction between the two exposures. Similar numbers as for PE and DVT were observed for provoked VTE and unprovoked VTE.

Discussion

In the present cohort of participants recruited from the general population, we found that the joint exposure of MI and obesity yielded a supra-additive effect on the risk of VTE. Individuals exposed to both MI and obesity had a 3-fold higher risk of VTE compared with individuals exposed to neither risk factors, and the combined effect of the two exposures exceeded the sum of the separate effects. Accordingly, 46% of the VTE events occurring among study participants jointly exposed to MI and obesity were estimated to be attributable to interaction between the two risk factors. Subgroup analyses indicated that the effect of the interaction was more

pronounced for the risk of DVT and unprovoked VTE, and obesity was apparently a prerequisite for increased risk of these outcomes in MI patients.

Obesity is recognized as a causal risk factor for VTE (24, 25), associated with a 2-fold increased risk (compared to normal weight) in population-based studies. Obesity is associated with both provoked and unprovoked VTE (13, 26) and has been shown to interact with environmental (*e.g.,* oral contraceptive use) and genetic factors (*e.g.,* Factor V Leiden) to yield synergistic effects on VTE risk (13, 15, 16). Obesity is also a risk factor for MI (27), and as expected, the prevalence of obesity was higher among those who developed MI than those who did not in our study. In analyses of overall VTE, the effect of MI on VTE risk in non-obese was small (15% increased), while the combined effect of MI and obesity exceeded the sum of the expected individual effects, yielding a >3-fold increased risk. Subgroup analyses revealed that in non-obese subjects, MI was associated with provoked, but not unprovoked VTE. Thus, it appears that in the absence of obesity, presence of other provoking factors is required to increase the risk of VTE in MI patients. These findings fit well with those of a previous case-crossover study (28), where we showed that infection and immobilization, which are known provoking factors for VTE, mediated the VTE risk within the 3-month period after an MI by approximately 60% (28).

Our findings further suggest that the increased risk of unprovoked VTE and DVT is dependent on the presence of obesity in MI patients, as the thrombosis risk among non-obese participants with MI was not increased in these subgroups. Our results indicate that 63% and 56% of the events in the joint exposure group could be attributed to an interaction between the two exposures. The potential mechanism behind such interaction is unknown. However, as both obesity and MI are associated with an inflammatory state (29, 30), one might speculate that the observed excess VTE risk could be related to thromboinflammation (31). Moreover, obesity is associated with both hypercoagulability and hypofibrinolysis (13), which could add to the

hypercoagulable state induced by an MI (32), and resulting in an excess risk of VTE. MI is associated with subsequent heart failure (33), which is a risk factor for VTE (34), and potentially, heart failure induced stasis may be further enhanced in obese patients, leading to increased risk of DVT. We are not aware of studies investigating the combined effect of heart failure and obesity on VTE risk. Unfortunately, we did not have information on heart failure in our study, and thus, we could not assess the potential role of heart failure as a mediator for the observed excess risk.

Development of a risk prediction model to recognize MI patients with a particularly high risk of VTE is pivotal, and future studies should aim at identifying predictors of VTE following MI. The findings from the present study of a supra-additive effect of MI and obesity on VTE risk suggest that obesity could be an important factor for risk assessment of VTE in MI patients. Designated prediction studies in large cohorts of MI patients are warranted to explore the predictive capability of obesity and assess to what extent obesity would facilitate risk stratification of VTE and aid clinical decisions regarding thromboprophylaxis.

Strengths of our study include the prospective design with participants recruited from a general Caucasian population, the well-validated events of both MI and VTE, and the long follow-up period. The high participation rate in the Tromsø Study and the broad age range minimized the risk of self-selection bias. A limitation of our study is that despite the large cohort size, the number of events were small in some subgroups and our findings need to be interpreted with caution. Additionally, because interaction is defined in numerical terms, we cannot draw any conclusions about the underlying mechanisms for the excess combined effect of MI and obesity on risk of venous thrombosis (35). We cannot exclude the potential presence of unrecognized residual confounding, which may influence the impact of MI and obesity on VTE risk.

In conclusion, the combination of MI and obesity resulted in an excess risk of VTE, suggesting an interaction between MI and obesity on VTE risk. Future studies are warranted to explore the predictive capability of obesity in MI patients, and to what extent obesity would improve the risk stratification of VTE.

Author contributions

JKS analyzed the data, interpreted results, and drafted the manuscript. BGT and VMM were involved in analyses, interpretation of the results and critical revision of the manuscript. MLL, IN, TW and EBM were responsible for data collection, interpretation of results, and revision of the manuscript. SKB and JBH designed the study, and were involved in data collection, interpretation of results, and critical revision of the manuscript.

Conflict of interest

None.

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Table 1. Baseline characteristics of study subjects without and with incident myocardial infarction (MI) during follow-up (n=29 410): The Tromsø Study

	No MI (n= 27 320)	MI (n= 2 090)
Age (years), mean ± SD	45 ± 14	61 ± 13
Sex (male), % (n)	45.8 (12 503)	61.3 (1 282)
BMI (kg m^{-2}), mean ± SD	25.2 ± 3.9	26.5 ± 4.0
Obesity (BMI ≥ 30 kg m^{-2}), % (n)	11.0 (2 998)	16.1 (336)
Hypertension *, % (n)	31.3 (8 562)	68.2 (1 425)
Hypercholesterolemia †, % (n)	29.8 (8 151)	61.4 (1 283)
Smoking ‡, % (n)	35.5 (9 682)	41.9 (875)
Self-reported diabetes mellitus, % (n)	1.4 (393)	6.0 (126)

BMI, body mass index; SD, standard deviation

* Mean systolic/diastolic blood pressure of ≥140 mmHg/≥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.

Table 2. Characteristics of venous thromboembolism events (n= 784): The Tromsø Study

	% (n)
Clinical characteristics	
Deep vein thrombosis	57.5 (451)
Pulmonary embolism	42.5 (333)
Provoked	54.0 (423)
Unprovoked	46.0 (361)
Provoking factors	
Surgery	15.9 (124)
Trauma	9.1 (71)
Cancer	24.1 (189)
Immobility *	21.1 (165)
Others †	4.5 (35)

* Bed rest for > 3 days, journeys > 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 (e.g., intravascular catheter).

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) and obesity exposure: The Tromsø Study.

		Person-years	VTE	IR (95% CI)*	HR (95% CI)†
Overall VTE					
MI -	Obesity -	392 287	589	1.5 (1.4 – 1.6)	Reference
MI -	Obesity +	45 290	140	3.1 (2.6 – 3.6)	1.57 (1.30 – 1.89)
MI +	Obesity -	8 707	36	4.1 (3.0 – 5.7)	1.15 (0.81 – 1.61)
MI +	Obesity +	1 688	19	11.3 (7.2 – 17.7)	3.16 (1.99 – 4.99)
PE					
MI -	Obesity -	392 287	239	0.6 (0.5 – 0.7)	Reference
MI -	Obesity +	45 290	64	1.4 (1.1 – 1.8)	1.75 (1.33 – 2.32)
MI +	Obesity -	8 707	21	2.4 (1.6 – 3.7)	1.54 (0.98 – 2.43)
MI +	Obesity +	1 688	9	5.3 (2.8 – 10.2)	3.49 (1.78 – 6.81)
DVT					
MI -	Obesity -	392 287	350	0.9 (0.8 – 1.0)	Reference
MI -	Obesity +	45 290	76	1.7 (1.3 – 2.1)	1.44 (1.12 – 1.85)
MI +	Obesity -	8 707	15	1.7 (1.0 – 2.9)	0.84 (0.50 – 1.43)
MI +	Obesity +	1 688	10	5.9 (3.2 – 11.0)	2.91 (1.55 – 5.49)

CI, confidence interval

* Per 1000 person-years.

† Age as time scale, adjusted for sex.

MI +/- indicates incident MI/no incident MI during follow-up, respectively. Obesity +/- indicates BMI $\geq$/< 30 kg/m^2 at baseline.

Table 4 Incidence rates (IRs) and hazard ratios (HRs) of provoked and unprovoked venous thromboembolism (VTE) according to myocardial infarction (MI) and obesity exposure: The Tromsø Study.

		Person-years	VTE	IR (95% CI)*	HR (95% CI)†
Provoked VTE					
MI -	Obesity -	392 287	314	0.8 (0.7 – 0.9)	Reference
MI -	Obesity +	45 290	75	1.7 (1.3 – 2.1)	1.56 (1.21 – 2.01)
MI +	Obesity -	8 707	25	2.9 (1.9 – 4.2)	1.48 (0.98 – 2.24)
MI +	Obesity +	1 688	9	5.3 (2.8 – 10.2)	2.78 (1.43 – 5.41)
Unprovoked VTE					
MI -	Obesity -	392 287	275	0.7 (0.6 – 0.8)	Reference
MI -	Obesity +	45 290	65	1.4 (1.1 – 1.8)	1.57 (1.20 – 2.06)
MI +	Obesity -	8 707	11	1.3 (0.7 – 2.3)	0.76 (0.41 – 1.39)
MI +	Obesity +	1 688	10	5.9 (3.2 – 11.0)	3.59 (1.90 – 6.79)

CI, confidence interval

* Per 1000 person-years.

† Age as time scale, adjusted for sex.

MI +/- indicates incident MI/no incident MI during follow-up, respectively. Obesity +/- indicates BMI $\geq$/< 30 kg/m^2 at baseline.

Table 5 Measures of interaction on an additive scale between previous myocardial infarction (MI) and obesity exposure: The Tromsø Study

	RERI (95% CI)	AP (95% CI)
Overall VTE	1.44 (-0.05 – 2.94)	0.46 (0.17 – 0.74)
PE	1.19 (-1.22 – 3.61)	0.34 (-0.15 – 0.83)
DVT	1.63 (-0.26 – 3.52)	0.56 (0.23 – 0.89)
Provoked VTE	0.74 (-1.20 – 2.67)	0.27 (-0.27 – 0.81)
Unprovoked VTE	2.26 (-0.05 – 4.58)	0.63 (0.35 – 0.91)

CI, confidence interval; RERI, relative excess risk attributable to interaction; AP, proportion attributable to interaction

Figure 1 Inclusion of study participants from the fourth (1994–1995), fifth (2001) and sixth (2007–2008) surveys of the Tromsø study
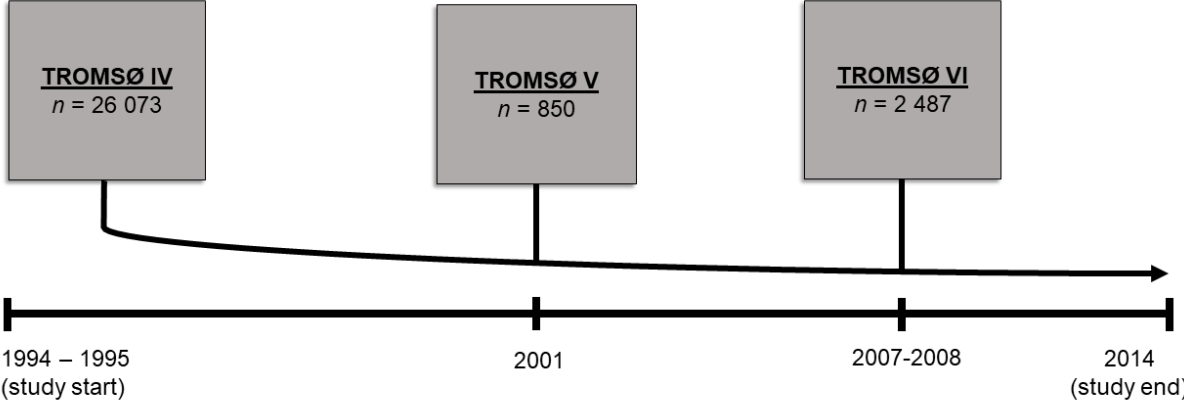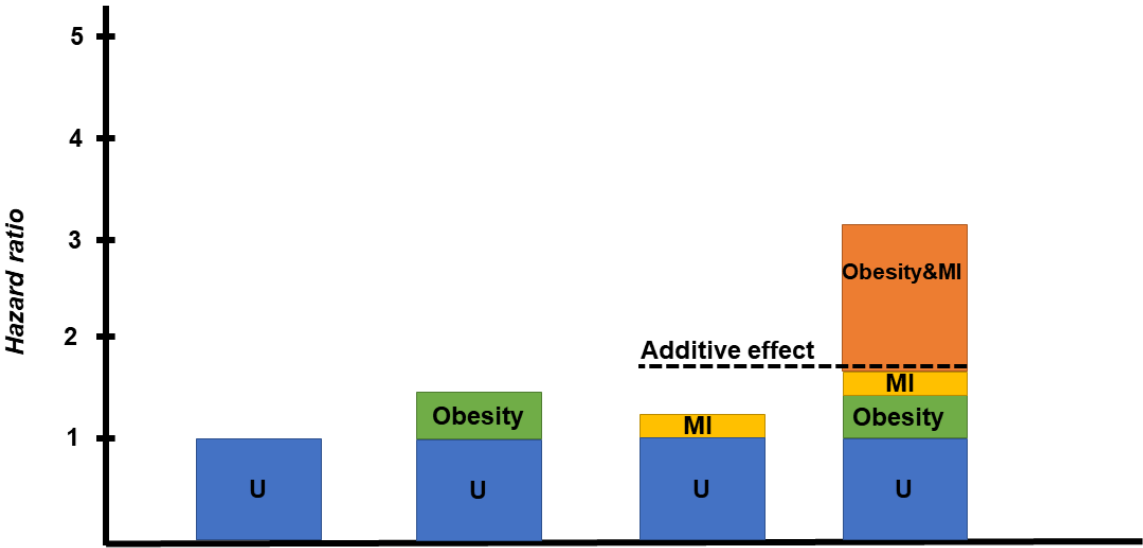
Figure 2 Hazard ratio of overall venous thromboembolism with contributions from different exposure categories marked green (Obesity), yellow (Myocardial infarction, MI) and orange (Obesity&MI)

U, common reference category; dotted line, additive effect Obesity&MI