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LONG-TERM CONSEQUENCES OF VENOUS THROMBOEMBOLISM IN WOMEN

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LONG-TERM CONSEQUENCES OF VENOUS THROMBOEMBOLISM IN WOMEN

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Just Do IT

To Martin, Klara and Hugo

ABSTRACT

Background: Venous thromboembolism (VTE) is the third most common cardiovascular disease with a high recurrence rate. There is a risk of chronic complications affecting health-related quality-of life (QoL).

Aim: To evaluate long-term consequences of VTE in women and to study and to explore the risk factors for recurrent event, arterial cardiovascular disease (CVD), mortality, post-thrombotic syndrome (PTS) and QoL.

Methods: We performed a cohort study inviting 1433 women with a previous episode of VTE (exposed) and 1402 women without VTE (unexposed). The cohort was derived from the 'Thrombo Embolism Hormone Study' (TEHS), a Swedish nation-wide case-control study on risk factors for VTE, recruiting women 2002 - 2009. During 2011 all women were followed up through a mailed questionnaire, including questions on life-style factors, recurrent VTE and inquiries according to the QoL instrument SF-36. To assess disease specific QoL, exposed women were also asked questions according to VEINES-Sym / VEINES-QoL. A modified Villalta scale was applied to evaluated PTS. Information on CVD and mortality was obtained through the Patient Register and the Cause of Death Register. Baseline data on life style factors, risk factors for VTE, including hormone treatment and family history of VTE and CVD were collected through a telephone interview within three months after the diagnosis of VTE for the exposed, or at time of inclusion in TEHS for unexposed. Blood samples for DNA-analyses were collected at time of inclusion in TEHS.

Result: A total of 2117 women (1087 (75%) exposed and 1030 (73%) unexposed) accepted participation in the study. During a median follow-up of 5 years (range 0.1-9.1), 10 % of exposed women had a recurrent event. The risk of recurrence was highest among women with unprovoked VTE and obesity. Women with hormone-induced VTE had a lower risk of recurrence than women with unprovoked VTE but not as low as women with surgery/cast induced VTE. Women carrying the risk alleles of *F5* rs6025/ Factor V Leiden (FVL) and *F11* rs2289252 had a significantly higher risk of recurrence compared to non-carriers (HR 1.7 (95% CI 1.1-2.6) and 1.8 (95% CI 1.1-3.0) respectively). In a subgroup analysis of women with unprovoked VTE the difference was even larger, with a cumulative recurrence rate of 21.9% (95% CI 13.7-34.0) versus 7.5 % (95% CI 3.4-16.0) for non-carriers at 5 years follow-up. The mortality rate for exposed women was 5.7/1000 person years with the corresponding mortality rate for unexposed of 2.2 /1000 person years, generating a HR of 2.4 (95% CI 1.2-4.6). None of the exposed died from VTE. During follow-up 35 (3.2%, 95% CI 2.1-4.3) among the exposed and 14 (1.4%, 95% CI 0.7-2.1) among the unexposed had any CVD event. Women with unprovoked VTE and pulmonary embolism had the highest risk of both death and CVD. The prevalence of self-reported PTS among all exposed was 20 % (95% CI 18-22). Women with proximal deep vein thrombosis had the highest prevalence (30%, 95% CI 25-35). Women reporting PTS had significantly lower QoL compared to both unexposed women and exposed women without PTS. Other factors affecting QoL were obesity, physical inactivity and recurrent VTE.

Conclusion: Among young and middle-aged women, the overall recurrence rate of VTE was low, indicating that the majority may not benefit from prolonged anticoagulation after a first episode of VTE. A combination of FVL and *F11* rs2289252 may be useful to predict the risk of recurrence. PTS, the most common complication, was seriously affecting QoL. Women with prior VTE had a two-fold increased risk of both overall mortality and CVD compared to unexposed. Obesity was a shared risk factor for recurrent VTE, PTS and reduced QoL.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

- I. **Ljungqvist M**, Sonnevi K, Bergendal A, Holmstrom M, Kieler H, Larfars G. Risk factors for recurrent venous thromboembolism in young and middle-aged women. *Thrombosis research* 2014;133(5):762-7.
- II. Bruzelius M, **Ljungqvist M**, Bottai, M, Bergendal A, Strawbridge R. J, Holmström M, Silveira A, Kieler H, Hamsten A, Lårfars G, Odeberg J. F 11 is associated with recurrent VTE in women. A prospective cohort study. *Thrombosis and Haemostasis* 2016;115(2):406-14.
- III. **Ljungqvist M**, Holmstrom M, Kieler H, Odeberg J, Larfars G. Cardiovascular disease and mortality after a first episode of venous thromboembolism in young and middle-aged women. *Thrombosis Research* 2016;138(2): 80-5.
- IV. **Ljungqvist M**, Holmstrom M, Kieler H, Larfars G. Long-term quality-of-life and post-thrombotic syndrome in women after a first episode of venous thromboembolism. *Manuscript* 2016.

CONTENTS

1	Introduction	7
2	Background	8
2.1	Venous Thromboembolism.....	8
2.1.1	Epidemiology	8
2.1.2	Etiology	8
2.1.3	Clinical Features and Treatment	8
2.2	Consequences of VTE.....	10
2.2.1	Recurrent VTE	10
2.2.2	Cardiovascular disease	10
2.2.3	Malignancy.....	11
2.2.4	Mortality.....	11
2.2.5	Health Related Quality-of-life	11
2.2.6	Post-thrombotic syndrome	12
3	Aims	14
4	Material And Methods	15
4.1	Study design and Study Population	15
4.1.1	TEHS	15
4.1.2	TEHS-follow-up.....	16
4.2	Data Collection.....	18
4.2.1	TEHS	18
4.2.2	TEHS-follow-up.....	18
4.2.3	Swedish National Registers	20
4.3	Statistical Methods	21
4.4	Ethical Considerations.....	22
5	Results	23
5.1	Paper I.....	23
5.2	Paper II	25
5.3	Paper III	27
5.4	Paper IV.....	29
6	Discussion	31
6.1	Methodological Considerations	31
6.1.1	Random error	31
6.1.2	Systematic error – selection bias.....	31
6.1.3	Systematic error – information bias.....	33
6.1.4	Systematic error – confounding	33
6.2	Main Findings.....	34
6.2.1	Recurrent VTE (I and II).....	34
6.2.2	Cardiovascular disease (III)	35
6.2.3	Mortality (III).....	37
6.2.4	Quality-of-life (IV).....	37
6.2.5	Post-thrombotic syndrome (IV)	38

7	Conclusion.....	40
8	Future Perspectives	41
9	Sammanfattning på svenska.....	42
10	Acknowledgements	44
11	References	47

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BMI	Body Mass Index
CDT	Catheter Directed Thrombolysis
CHC	Combined Hormonal Contraceptive
CT	Computed Tomography
CTEPH	Chronic Thromboembolic Pulmonary Hypertension
CVD	Cardiovascular Disease
DVT	Deep Vein Thrombosis
ECS	Elastic Compression Stockings
F2 (G20210A)	Polymorphism in prothrombin gene, <i>F2</i> rs1799963
FVL	Factor V Leiden, <i>F5</i> rs6025
HR	Hazard ratio
HT	Menopausal Hormone Therapy
INR	International Normalized Ratio
ISTH	The International Society of Thrombosis and Haemostasis
LMWH	Low Molecular Weight Heparin
MI	Myocardial Infarction
NOAC	New Oral Anticoagulants
OR	Odds ratio
PE	Pulmonary Embolism
PTS	Post-thrombotic Syndrome
PVI	Primary Venous Insufficiency
QoL	Health related Quality of Life
TEHS	Thrombo Embolism Hormone Study
VKA	Vitamin K Antagonist
VTE	Venous Thromboembolism

1 INTRODUCTION

Annually, in Europe, there will be about one million events of venous thromboembolism (VTE), defined as deep vein thrombosis and pulmonary embolism. About four to five hundred thousand will die from pulmonary embolism every year. Among survivors of pulmonary embolism two to four in every 100 patients will develop chronic thromboembolism pulmonary hypertension. Up to half of patients suffering a deep vein thrombosis will develop post-thrombotic syndrome, a chronic, burdensome complication seriously affecting health related quality of life. There is also a risk of recurrent VTE, with a cumulative recurrence rate of about 30-40 % over 10 years. VTE is a disease with a complex pathophysiology including both genetic and acquired risk factors. VTE is an important health issue in women with surgery, pregnancy and postmenopausal hormone therapy as important risk factors. One of the most frequent acquired risk factor of VTE among women in childbearing age is the use of oral contraceptives.

The ‘Thrombo Embolism Hormone Study’ (TEHS) started in 2002 in Sweden and was designed to evaluate risk factors for VTE in women. About two years later I was involved in the project including patients at Södersjukhuset in Stockholm. During the following years I was a part of the formation of the TEHS-follow-up study. Throughout the process I have learned a lot. My major learning outcome was to be introduced to epidemiological research and to understand how much time and effort that is needed to complete a project like this.

The focus of this thesis was to gain better knowledge on the consequences of VTE in young and middle aged women.

2 BACKGROUND

2.1 VENOUS THROMBOEMBOLISM

2.1.1 Epidemiology

Venous thromboembolism (VTE) is a common disease with an overall incidence of 1-2/1000 person years (1, 2). The incidence is increasing with age for both men and women with a slightly higher overall age adjusted incidence rate for men. In contrast, the incidence for women is somewhat higher during childbearing age, probably due to the use of estrogen containing contraceptives and pregnancy (3, 4). After a first episode of VTE about 25 % will suffer a recurrent event within five years (5, 6). Approximately 30-50 % of persons suffering a deep vein thrombosis (DVT) will develop post-thrombotic syndrome (PTS) and up to 5 % of patients with pulmonary embolism (PE) will suffer from chronic thromboembolic pulmonary hypertension (CTEPH) (7-9). PTS and CTEPH both seriously affecting health related quality of life (QoL) (10, 11).

2.1.2 Etiology

Already in the middle of the 19th century Rudolph Virchow proposed three main causes of VTE; stasis of the blood, changes in the vessel wall and changes in the composition of the blood (12). It is still valid that VTE is a multifactorial disease with both genetic and acquired risk factors, not seldom interacting within one patient (1, 13). Acquired risk factors for VTE includes age, trauma, surgery, estrogen, pregnancy, malignancy and a number of medical or inflammatory illnesses (14, 15). The strongest acquired risk factors for VTE are surgery and active cancer. Without prophylaxis with anticoagulants about 40-60 % of patients undergoing major orthopedic surgery in the lower limb will suffer from a VTE (16). Women using contraceptives containing estrogen have a 2 to 4 fold increased risk of VTE compared to non-users (17).

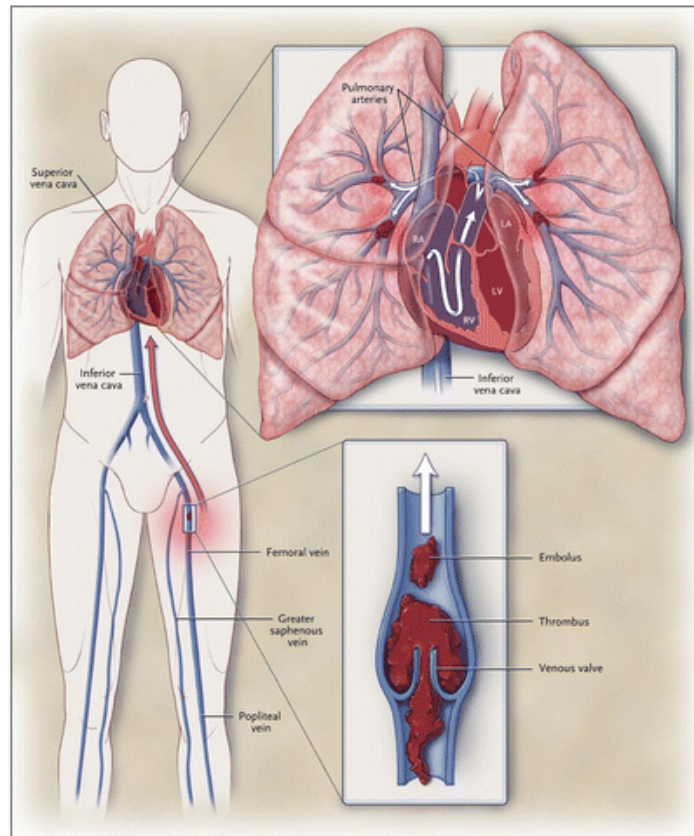
A number of inherited thrombophilias associated with an increased risk of VTE have been described (1, 18, 19). First the deficiency of the inhibitors of the natural anticoagulants, antithrombin, Protein S and Protein C were described, being rare but strongly associated with risk of VTE (20). The later described, and most common thrombophilias are the FV Leiden, *F5* rs6025 mutation (FVL) and the prothrombin G20210A, *F2* rs1799963 mutation (F2 (G20210A)) (19, 21). These thrombophilias increase the risk of VTE about 5 to 2 fold respectively, when appearing in heterozygous forms (22-24).

2.1.3 Clinical Features and Treatment

Diagnosis

The most common location of VTE is deep vein thrombosis (DVT) in the lower limb or pulmonary embolism (PE). DVT in the lower limb is divided into distal DVT, involving the calf veins and proximal DVT involving the popliteal vein and veins more proximal.

Since the clinical diagnosis of VTE is unreliable the diagnosis has to be confirmed by an objective test. The most commonly used diagnostic approach is a combination of clinical probability, D-dimer testing and objective radiological tests. Wells score is a widely used clinical pretest probability instrument, classifying patients into high and low risk groups (25). Patients with a high pretest probability of DVT is usually further examined by Doppler ultrasonography or, more seldom, venography (26). For patients with suspicion of PE the most frequently used objective test is computed tomography (CT) scan, followed by ventilation-perfusion scan for patients having contraindications for CT (27).



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Treatment

Treatment of VTE is started with parenteral, subcutaneous, injections of low molecular weight heparin (LMWH), usually already at time of clinical suspicion of VTE, awaiting the result of the objective test. Once VTE is confirmed, treatment with anticoagulation therapy is continued, either by initiation of any of the new oral anticoagulants (NOAC) or vitamin K antagonist (VKA) while continuation of LMWH until international normalized ratio (INR) is 2.0 – 3.0. Treatment should be continued for at least three months to avoid early recurrence. For patients considered having a high risk of recurrence, anticoagulant treatment should be extended (28, 29).

2.2 CONSEQUENCES OF VTE

2.2.1 Recurrent VTE

Within 5 years after a first episode of VTE about 20-25 % of patients will suffer from a recurrent event (30-32). The risk of recurrence is effectively prevented by continuing anticoagulant treatment, although at a price of an increased risk of major bleeding (33, 34). Therefore, one of the most challenging clinical issues is to identify patients having a high risk of recurrent events who therefore will gain from prolonged anticoagulation. Many of the risk factors associated with first episode of VTE do not affect or even reduce the risk of recurrent events. The risk of recurrence is lower when the first episode of VTE was provoked by a transient risk factor like surgery (35-37). Presence of heterozygous FVL or F2 (G20210A) only slightly, if at all, increases the risk of recurrent VTE (38). It is known that women have a lower risk of recurrence than men but the reason for this is so far not fully known (5, 39-41). Previous studies on the risk of recurrence after a hormone related VTE are conflicting. Some studies find similar risk for unprovoked and hormone related VTE (42, 43), whereas others find reduced risk of recurrence after VTE related to hormones (39, 44).

Patients with an unprovoked VTE have the highest risk of recurrence. There are some clinical scoring algorithms derived to help identifying patients with unprovoked VTE being at low risk of recurrence, who safely can stop anticoagulation therapy. In the 'Men continue and HERDOO2 rule' no men with idiopathic VTE and only women with no more than one risk factor (age above 65, obesity and increased D-dimer) fall into the low risk group who safely can stop anticoagulation (45). In the Vienna prediction model D-dimer, male sex and location of first VTE (PE and proximal DVT versus distal DVT) are associated with a higher risk of recurrence (46). In the DASH prediction model risk factors associated with an increased risk of recurrence are male sex, persistently increased D-dimer after anticoagulation is stopped, age below 50 years and in women VTE not associated with hormonal treatment (47).

None of the prediction models above contains any genetic markers. Nevertheless, previous studies have found that the heritability of VTE is strong (48-50). Family history of VTE has been found to be a risk factor for first VTE, even in the absence of the known thrombophilias (51, 52). Heritability has also been found to be a predictor of hospitalization for recurrent VTE in a Swedish study (53). The effect of heritability was strongest in younger patients, suggesting a genetic contribution. However, screening for the known thrombophilias has not been found to be of clinical relevance to predict recurrence, suggesting other genetic variants to be present (54).

2.2.2 Cardiovascular disease

Arterial cardiovascular disease (CVD) and VTE have traditionally been regarded as two different diseases. VTE usually arises in vessels with low blood flow and shear stress and the clot consists mostly of erythrocytes and fibrin. Arterial thrombi on the other hand develop in vessels with high shear stress and are predominantly platelet-rich (55, 56). However, since 2003 when Prandoni et al (57) reported a higher incidence of atherosclerotic plaque in patients

with unprovoked VTE there are more studies confirming the relationship between VTE and CVD (58-61). Patients hospitalized for CVD have been found to have an increased risk of subsequent VTE in one large register-based Danish study (61). However other studies found no association between VTE and CVD, or the association disappeared after adjustment for potential confounders (62, 63). Furthermore, patients with VTE seem to have an increased risk of subsequent CVD (60, 64, 65). Whether the relationship between VTE and CVD is explained by common risk factors or a causal link is still unclear (66, 67). If the association was explained by common risk factors the correlation would diminish or disappear after adjustment. In some studies, adjustment was not possible (60) but in other studies adjustment for common risk factors only modestly changed the risk estimates (68-70) suggesting common risk factors is not the full explanation.

2.2.3 Malignancy

For more than a hundred years ago Armand Trousseau first described the association between cancer and VTE (71). Hereafter, a large number of studies have found not only that patients with known malignancy have an increased risk of VTE (72-74) but also that patients with VTE have an increased risk of developing subsequent cancer (58, 74, 75). Among patients who become ill in symptomatic DVT about 20 % are diagnosed with an active cancer (15, 76). However, VTE might also be the first sign of undiagnosed cancer. Cohort studies of patients with VTE report that 6-13% of patients have been diagnosed with a new cancer four to nine years after an episode of VTE (58, 75). One meta-analysis found a 3-fold increased risk of occult malignancy in patients with VTE compared to patients without VTE (76). The risk was highest for patients with unprovoked VTE.

2.2.4 Mortality

It has been found that overall mortality is increased after VTE, especially after PE (58, 77, 78). Mortality is highest in the first year after a VTE-event and mortality rates of 20-25 % within one year have been reported (32, 79). For patients with PE early death is due to direct hemodynamic effects of PE, although studies report increased mortality even beyond the acute period. Mortality is then usually due to concomitant disease, most commonly malignant disease (32, 58, 80-83). However, even in studies including VTE-patients without malignancy, mortality is increased (32, 78). Results on whether the long-term survival after a VTE event differs from the general population are not consistent (32, 80-82).

2.2.5 Health Related Quality-of-life

While epidemiology, etiology and treatment of VTE have been described in many studies, there are only a few studies investigating how QoL is affected by VTE. Not until late 1980s and early 1990s assessing QoL became a focus of interest in health science. Now, more and more clinical trials include QoL as an important endpoint (84). When measuring QoL it is important to use both generic and disease-specific instruments (85, 86). Generic instruments are needed for comparisons between various patient groups and between studies, while disease-specific

instrument usually are better to evaluate changes over time and treatment effects in patients with the same disease (86, 87).

Previous studies assessing QoL after VTE report that VTE-patients score significantly lower on both mental and physical health status measured with 36- Item Short-Form Health Survey (SF-36). Prevalence of PTS was the predominant predictor of low QoL after DVT (10, 88-90).

2.2.6 Post-thrombotic syndrome

PTS is one of the most common complications of DVT. PTS is a chronic condition that develops in 20–50% of patients within 1–2 years after an episode of symptomatic DVT in the lower limbs (8, 58, 91, 92). Some studies have also found PTS after an asymptomatic DVT (93, 94). PTS is characterized by symptoms such as pain, swelling, heaviness, cramping and objective signs such as edema, hyperpigmentation and varicose veins. PTS is burdensome to patients as well as expensive, both in terms of direct medical costs and indirect costs such as loss of productivity (95). Women seem to be more prone to develop PTS (96). Important risk factors for PTS appear to be ipsilateral recurrence of DVT, poor quality of initial anti-coagulation for the treatment of DVT and increased body mass index (BMI) (7, 97, 98).

Diagnosis

PTS is a clinical diagnosis with no single gold standard test. There are a number of clinical tools to diagnose PTS. In order to standardize the definition of PTS ‘The International Society of Thrombosis and Haemostasis’ (ISTH) has recommended the use of the Villalta scale (99). The Villalta scale comprises of five patient-rated symptoms and six clinical-rated signs (Table 1).

Table 1. *The Villalta Scale*

Symptoms	Signs
Pain	Pretibial edema
Cramps	Skin induration
Heaviness	Hyperpigmentation
Paresthesia	Redness
Itching	Venous ectasia
	Pain on calf compression

All symptoms and signs score from 0 (absent) to 3 (severe). PTS is considered present if score ≥ 5 and severe if ≥ 15 or the presence of a venous ulcer.
Adapted from Prandoni, Villalta *et al Haematologica* 1997

Pathophysiology

The pathogenesis of PTS is not fully understood. Venous hypertension seem to play an important role, but whether PTS depends on outflow obstruction, venous valvular reflux or both is not consistent (97). Previous studies report higher incidence of PTS in patients with persistent venous obstruction suggesting outflow obstruction is a risk factor for PTS (100-102). In a clinical study, the Catheter-Directed Venous Thrombolysis Trial (CaVenT), comparing catheter-directed thrombolysis (CDT) to standard treatment with anticoagulants and compression stockings, the risk of PTS was reduced in the group of patients treated with CDT. Compared to patients receiving conventional anticoagulant treatment more patients receiving CDT had ileofemoral patency, but there was no difference between the groups regarding valvular reflux (97, 98, 103). PTS might also be due to damage in the vessel wall and both acute and chronic inflammation might be drivers of PTS (104, 105).

Treatment

Previously the most used prevention and treatment strategy for PTS has been the use of elastic compression stockings (ECS). However, since Kahn et al presented a randomized controlled trial, the SOX-trial (106), showing no effect on PTS using ECS the use of stockings has been questioned. Hence, there are very few side effects of ESC and it can be used to reduce symptomatic swelling. Though based on sparse data, physical activity might benefit some patients with PTS (97).

3 AIMS

VTE is a common condition associated with high risk of recurrence, increased mortality and risk of PTS as a chronic complication. It also affects health-related quality-of-life.

The overall objectives of this thesis were to investigate long term consequences after a first episode of venous thromboembolism in women 18-64 years of age.

The specific aims of the studies were:

- To study the risk of recurrence after a first episode of VTE and to assess the risk related to unprovoked VTE and VTE provoked by surgery/cast or hormonal treatment.
- To investigate if a genetic risk score previously found to predict a first episode of VTE could be used to predict recurrent VTE.
- To assess the risk of CVD and mortality after a first episode of VTE compared to a control group and further to assess risk factors for CVD and mortality in women with VTE.
- To examine how QoL is affected after a first episode of VTE compared to a control group and to assess risk factors for low QoL after a first time VTE. Furthermore, to investigate the incidence of, and risk factors for, PTS after a first episode of VTE.

4 MATERIAL AND METHODS

4.1 STUDY DESIGN AND STUDY POPULATION

We conducted a long-term prospective follow-up of a cohort of women exposed to a first episode of VTE and age-matched controls. The cohort was derived from the ‘Thrombo Embolism Hormone Study’ (TEHS). In the follow-up study women with a previous VTE were included as exposed and women serving as controls in TEHS were considered unexposed.

4.1.1 TEHS

TEHS is a population-based case-control study initiated by the Swedish Medical Products Agency in collaboration with Karolinska Institutet and the Royal Institute of Technology. TEHS was designed to investigate genetic and environmental risk factors for VTE in women 18-64 years of age. The study has been well described by Bergendal et al (107). A total of 1433 women with a first episode of DVT of the lower limb or a PE were included as cases at 43 hospitals distributed all over Sweden (Figure 1). In hospitals with a centralized management of VTE (n= 32) women were identified by a study coordinator at the department. In hospitals without a centralized management (n=11) potential study participant were identified through the Department of Radiology and the Department of Clinical Physiology. A copy of the radiology report was sent to the coordinating center. To make sure that it was acceptable to contact the women a research nurse contacted the clinician responsible for the potential case.

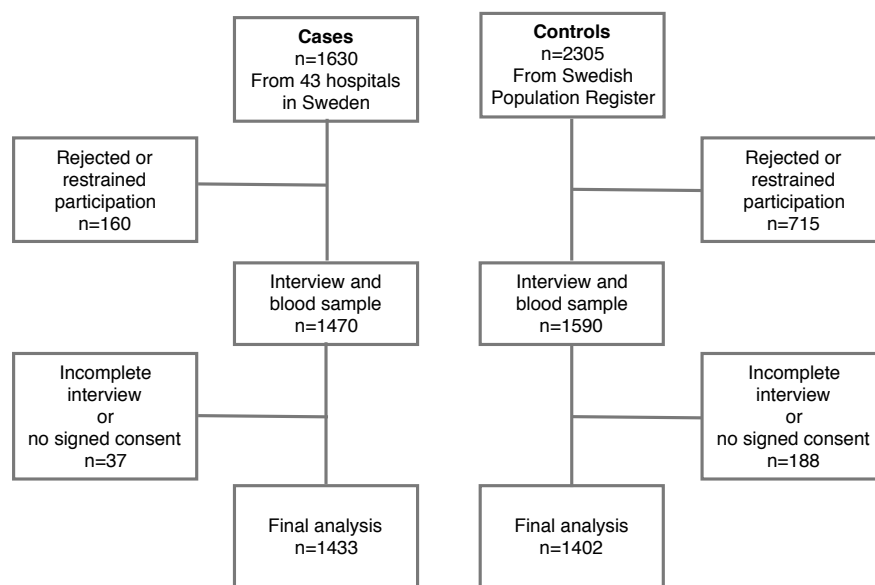


Figure 1 Flow-chart of inclusion in the 'Thrombo Embolism Hormone Study'

The VTE event had to be objectively verified by phlebography or color Doppler ultrasonography for women with DVT. For women with PE the diagnosis was verified by CT scan of the pulmonary arteries, pulmonary angiography or by perfusion and ventilation scintigraphy. Only cases initiating anticoagulant treatment were considered having a symptomatic VTE and were included in the study as such.

Controls were randomly selected from the Swedish population registry and matched to cases according to age. All participants were recruited prospectively in Sweden from 2002 to January 2009. During 2002 recruitment of patients and controls started in a pilot study and in 2003 the main study of TEHS started to recruit both patients and controls. Women with a previously diagnosed VTE event, pregnancy during the last three months or current, or a history of malignancy within the past five years were excluded.

All cases and controls were interviewed through a telephone call by a specially trained research nurse using a structured questionnaire to obtain information on acquired risk factors for VTE and baseline characteristics. Because the interview was in Swedish, non-Swedish speaking women were not eligible for the study. At time of inclusion in the study all women donated 5 mL of whole blood. DNA was prepared using QIAGEN FlexiGene DNA-kit.

4.1.2 TEHS-follow-up

In 2011 all women included in TEHS, still living in Sweden, were asked to participate in TEHS-follow-up by a questionnaire sent by mail (Figure 2). If the questionnaire was not returned within one month a reminder was sent. During the follow-up period 38 out of the 1433 women with previous VTE deceased. One women answered the questionnaire and died six months after. Among the 1402 without VTE at inclusion in TEHS, 12 deceased during follow-up.

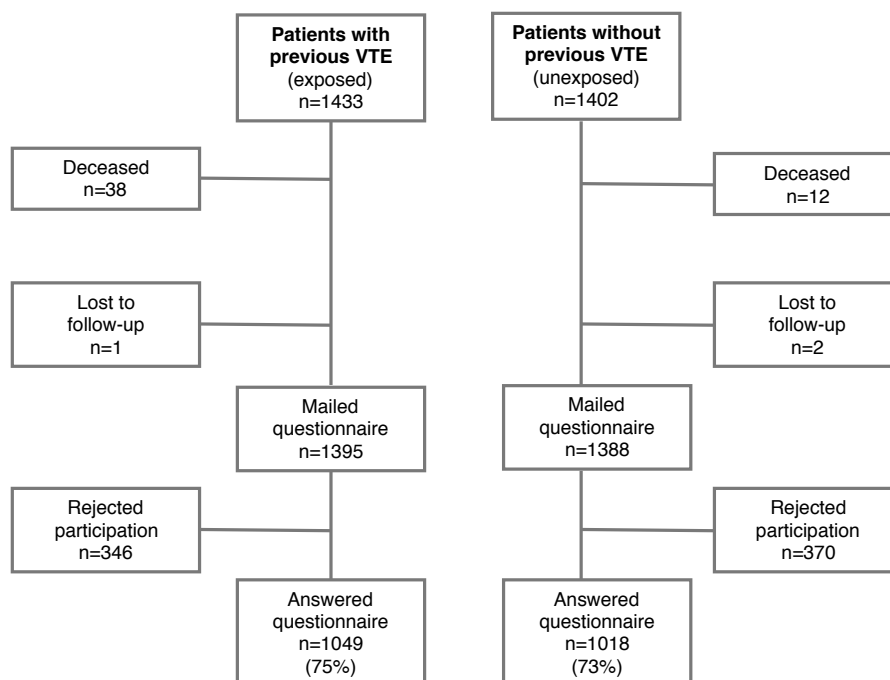


Figure 2 Flow-chart of the study cohort of TEHS-follow-up.

The questionnaire covered different medical aspects, such as current medication, duration of previous and current anticoagulant treatment, hormone therapy with combined hormonal contraceptive (CHC) or menopausal hormonal treatment (HT), recurrent events of VTE and

CVD. There were also questions regarding life style factors such as weight, smoking and physical activity. To assess QoL the questionnaire included questions according to SF-36 for all women and VEINES-QoL/VEINES-Sym for women exposed to a previous VTE. For exposed women the questionnaire also included questions according to a self reported Villalta scale, modified to suit questionnaire (8).

Information on CVD, mortality and malignancy were obtained from the Swedish Patient Register, The Cause of Death Register and the Cancer Register held by the National Board of Health and Welfare.

Figure 3 shows information on the selected study populations of the TEHS follow-up cohort included in the 4 papers. A short summary is listed below.

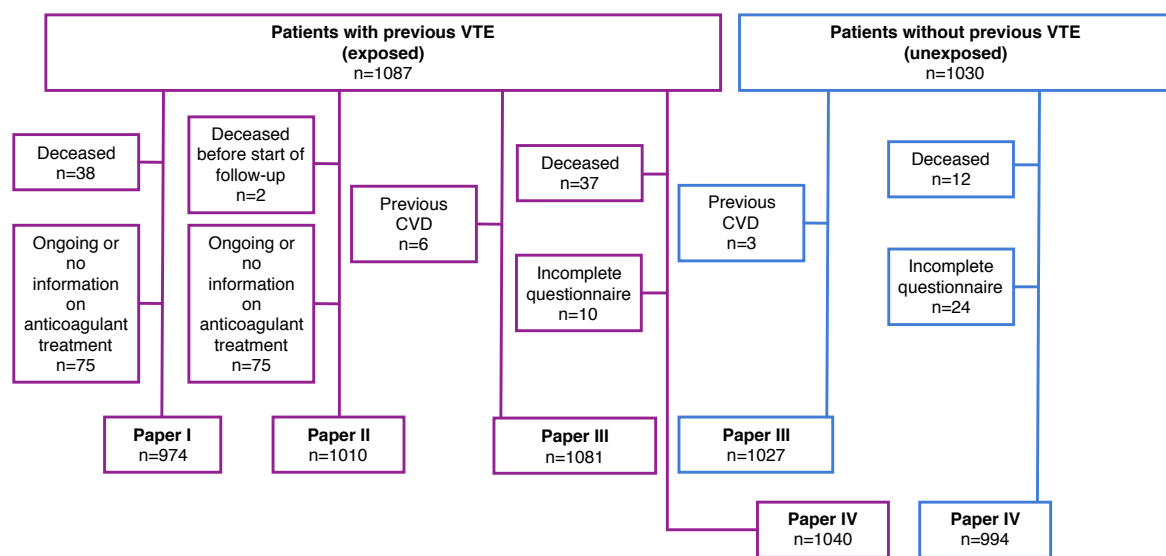


Figure 3 Flow-chart of the study cohort of TEHS-follow-up, divided by the papers included in the thesis.

Paper I included women (n=974) with a previous VTE alive at time of follow-up. Women on anticoagulant treatment (n=53) or missing information on anticoagulant treatment (n=22) were not eligible since they were not considered being at risk of recurrent VTE.

In paper II both women being alive at time of follow-up as well as women who deceased during follow-up (n=1010) with a previous VTE were included in the analysis.

Paper III included 1081 exposed women, of whom 1044 were alive, and 1027 unexposed women, of whom 1016 were alive. Women with CVD prior to inclusion in TEHS (n=9) were excluded.

In paper IV 1040 exposed and 994 unexposed alive women with complete questionnaire regarding the QoL-instruments were included.

4.2 DATA COLLECTION

4.2.1 TEHS

Baseline information was collected through a telephone interview within three months of the VTE-event for exposed women and at time of interview for unexposed. Data included questions on acquired risk factors including detailed information on hormonal intake and information on surgery and/or plaster cast. The interview contained questions on life-style factors such as weight, height, smoking and physical activity. The study participants were also asked about leg symptoms (pain, heaviness and swollen legs) and varicose veins prior to the symptoms of VTE in exposed and prior to the interview in unexposed. To monitor comorbidity, all women were asked about current and previous diseases including hyperlipidemia, hypertension, diabetes, stroke, acute myocardial infarction and angina pectoris. Women who confirmed being affected by any of these conditions were considered as having that disease. Information on the location of the VTE was obtained through a copy of the radiology report.

Genotyping

Genotyping was performed using the Illumina GoldenGate platform and read and analyzed using the Illumina BeadXpress and Illumina GenomStudio 2011.1 software at the SNP&SEQ Technology Platform, Uppsala Sweden. SNPs in *F5*rs6025 and *F2* rs1799963 were genotyped by Pyrosequencing technology (ISO standard 2004) at the Royal Institute of Technology, Stockholm (108). Genotyping was performed on plates containing both cases and controls and were completed in one stage for the Illumina platforms.

4.2.2 TEHS-follow-up

Information on the outcomes in TEHS-follow-up was obtained through the questionnaire and from Swedish Registers; the Patient Register, the Cause of Death Register and the Cancer Register.

Recurrent VTE

Information on recurrent disease was obtained both from the questionnaire as well as from the Patient Register. All possible recurrent events reported in the questionnaire or in the Patient Register were confirmed by a review of the medical record. To be considered a recurrent event it had to be radiological verified by the same methods as described in TEHS as well as be considered having indication for resumed anticoagulant treatment.

Overall Mortality and Cardiovascular disease

Information on time and causes of death for the deceased women were collected from the Cause of Death Register. Both primary and secondary causes of death were retrieved. The causes of death were classified into three groups; death by cardiovascular disease, malignancy or other cause.

The Patient Register was used to obtain information on CVD. Study endpoints in paper III were cardiovascular disease defined as myocardial infarction (ICD-10 code I20 and I25), ischemic stroke (ICD-10 code I63 and I64) or cardiovascular mortality (ICD-10 code I21, I25, I63, I46.1) and overall mortality.

Health-related Quality of Life

To assess generic QoL the questionnaire contained questions according to SF-36 (109, 110). The SF-36 consists of 36 items and assesses general wellbeing during the previous 30 days. It contains eight subscales: physical functioning, social functioning, physical role functioning, emotional role functioning, mental health, vitality, bodily pain and general health. Standard algorithms were used to calculate scores for the Mental Component Scale (mcs), a summary score that reflects scores on the vitality, social functioning, mental health, and emotional role scales, and the Physical Component Scale (pcs), a summary score that reflects scores on the physical functioning, physical role, bodily pain, and general health perceptions scales. Scores are expressed on a 0–100 scale, with higher values indicating better general wellbeing.

To assess disease-specific QoL the questionnaire of the exposed women also included questions according to VEINES-QoL/VEINES-Sym (111). The VEINES-QoL consists of 26 questions that assess venous symptoms (heavy legs, aching legs, swelling, night cramps, heat or burning sensation, restless legs, throbbing, itching, tingling, intensity of leg pain), limitations in daily activities due to chronic venous disease, psychological impact of chronic venous disease, change over the past year, and time of day when the leg problem is most intense. The VEINES-Sym is a subscale of the VEINES-QoL that measures venous symptoms (111).

Post-thrombotic syndrome

PTS was evaluated in exposed women through a self-reported Villalta-scale (8) included in the questionnaire. To assess PTS there were questions asking for five symptoms and four signs (Table 2) for both lower limbs. Each item scored one point if present. The nine items were summed into a self-reported post-thrombotic score, where PTS was considered present if score ≥ 4 points. For women with DVT the scores of the affected leg was used. For women with PE we used results from the leg with the higher score, assuming that they had a concomitant DVT in that leg.

Table 2 *Self-reported Villalta scale*

Symptoms	Signs
Spontaneous pain in calf	Newly formed varicose veins
Spontaneous pain on walking	Swelling of foot or calf
Spontaneous pain on standing	Skin changes, pigmentation, discoloration
Pain worsening during the day	Skin changes with venous ulcers
Heaviness of leg	

All symptoms and signs score 0 if absent and 1 if present. PTS was considered present if score ≥ 4 .
Adapted from Tick *et al J Thromb Haemost* 2008

4.2.3 Swedish National Registers

The National Board of Health and Welfare (Socialstyrelsen) hold a number of national registers. The registers were created for administrative purposes but researchers can apply for data needed for studies. It is mandatory by law for both public and private health care providers to report to the registers.

Patient Register

The National Patient Register started in 1967 when the National Board of Health and Welfare started to collect information regarding in-patients at public hospitals. In the beginning it covered 6 of the 26 county councils in Sweden but in 1984 it became mandatory for all county councils. From 1984 the Patient Register covers all in-patient care in Sweden. From 2001 it also includes all outpatient visits, including day-surgery, from private and public caregivers. Primary care is not covered in the Patient Register.

The Patient Register contains information on age, sex, national registration number (a unique identification number assigned to each resident in Sweden) and main and secondary diagnosis for each patient visit or hospital admission. Diagnoses are recorded at each out-patient-clinic visit or at discharge from hospital according to the current International Classification of Diseases version 10 (ICD-10). The validity and coverage of the information included in the Patient Register is high, but varies across diagnoses (112-114).

Cause of Death Register

The Cause of Death Register contains data from 1961 and is updated annually. There are some historical data from 1952-1960, but the coverage may vary during that period. The Cause of Death Register covers all deaths occurring within or outside Sweden to individuals registered in Sweden as residents or citizens. The causes of death are coded centrally at Statistics Sweden according to ICD-10. The registry has high completeness regarding cause-specific death (115, 116).

Cancer Register

The Cancer Register was founded in 1958 and covers the whole population. Every health care provider must report newly diagnosed cancer, whether it is diagnosed clinical, morphological, on laboratory examination or at autopsy. Since the mid-80s the registration, coding and major check-up and correction work is performed at one of the six regional registries associated with the regional oncological centers in Sweden. The registry contains personal information (age, sex, national registration number) and medical data including information on the tumor site, histology, stage as well as date of diagnosis, basis of diagnosis and reporting hospital (117). The coverage of the register is high, a quality study in 2009 estimated the underreporting to be approximately 4 percent (118).

4.3 STATISTICAL METHODS

Baseline data in all four studies were compared using t-test to compare means for normally distributed, continuous data and Mann-Whitney U for non-normal, continuous or ordinal data. When comparing categorical data, we used chi²-test or, when appropriate, Fischer's exact test.

The Kaplan-Meier method was used to calculate cumulative recurrence rate in paper I-II and to calculate CVD and mortality in paper III. The difference between groups were compared using log-rank-test. In paper I alive patients were followed from end of anticoagulant treatment until recurrent event. Patients were censored at date of answering the questionnaire. Paper II also included deceased women, calculating death as competing risk.

Cox proportional hazard model was used to evaluate risk between groups in paper I-III. Both crude and adjusted hazard ratios (HR) with 95 % confidence intervals (95 % CI) were reported.

Rate of recurrence in paper II and rate of CVD and mortality rate in paper III was calculated as number of events over the accumulated person-time.

In paper IV analysis of variance (ANOVA) was used to calculate the difference between exposed and unexposed regarding QoL. ANOVA was also used to assess possible predictors of QoL among women with a previous episode of VTE. Separate analyses were made for SF-36 pcs, mcs, VEINES-QoL and VEINES-Sym.

To assess predictors of PTS in paper IV logistic regression was used, comparing women with PTS to women without PTS. Risks were calculated and reported as both crude and adjusted odds ratios (OR) and their corresponding 95 % CI.

For all analyses $p < 0.05$ was considered statistically significant.

Analyses of paper I, III and IV were performed using SPSS version 20-23 (SPSS Inc., Chicago, IL, USA). Analysis of paper II was performed using Stata version 13.

4.4 ETHICAL CONSIDERATIONS

Both at time of inclusion in TEHS as well as in TEHS follow-up the study participants gave their written informed consent according to the Helsinki Declaration. All women were informed that they at any time could leave the study and have their data destroyed. Both TEHS and TEHS follow-up was approved by the regional research ethics committees in Stockholm (KI 01-255, 04-469), Uppsala (Ups 01-277), Linköping (01-453), Göteborg (M088-01), Umeå (01-198) and for TEHS-follow-up (EPN 2010/1200-31/1).

5 RESULTS

5.1 PAPER I

In paper I we studied the risk of recurrence after a first episode of VTE and in particular evaluated the risk in relation to whether the VTE was unprovoked, provoked by hormones or surgery/cast.

We included all women (n=974) exposed to a previous VTE event (i.e. cases in TEHS) alive and accepting participation in TEHS-follow-up, and followed them for a median of 5.2 years (range 0.1-9.1 years) regarding recurrent events. At time of inclusion in TEHS the mean age was 49 years and 386 (40 %) of the participants were treated with hormones. During the follow-up period 102 (10%) women had a recurrent event. For all women the recurrence rate was highest during the first year. Table 1 shows recurrence rate according to provoking factor at time of first VTE.

Table 3. Cumulative incidence of recurrent VTE according to risk factor at first episode of VTE.

	All women N=974	Women with Surgery/ Cast N= 350	Women with CHC/HT N =272	Women with unprovoked VTE N= 352
1 year	4%	2%	5%	7%
2 years	6%	3%	7%	10%
3 years	8%	3%	7%	13%
4 years	9%	3%	8%	15%
5 years	9%	4%	9%	15%
6 years	11%	6%	10%	18%

CHC/HT = treatment with either combined hormonal contraceptives or menopausal hormone treatment.

The risk of recurrence was lowest for women with a first VTE provoked by surgery/cast with a crude HR of 0.28 (95% CI 0.16-0.47) compared to women with unprovoked VTE. Women with hormonal provoked VTE had a lower recurrence rate (HR 0.57, 95% CI 0.39-0.90) compared to women with unprovoked VTE, but not as low as women with surgery/cast provoked VTE (Figure 4).

The risk of recurrence differed due to location of the initial VTE. Women with PE or proximal DVT had a higher risk of recurrence than women with distal DVT (HR 2.0, 95% CI 1.3-3.1). Women with obesity had an increased risk of recurrent VTE with a HR of 1.8 (95% CI 1.2-2.7) compared to non-obese. The impact of obesity was largest among women below the age of 50. Age did not correlate with increased risk of recurrence.

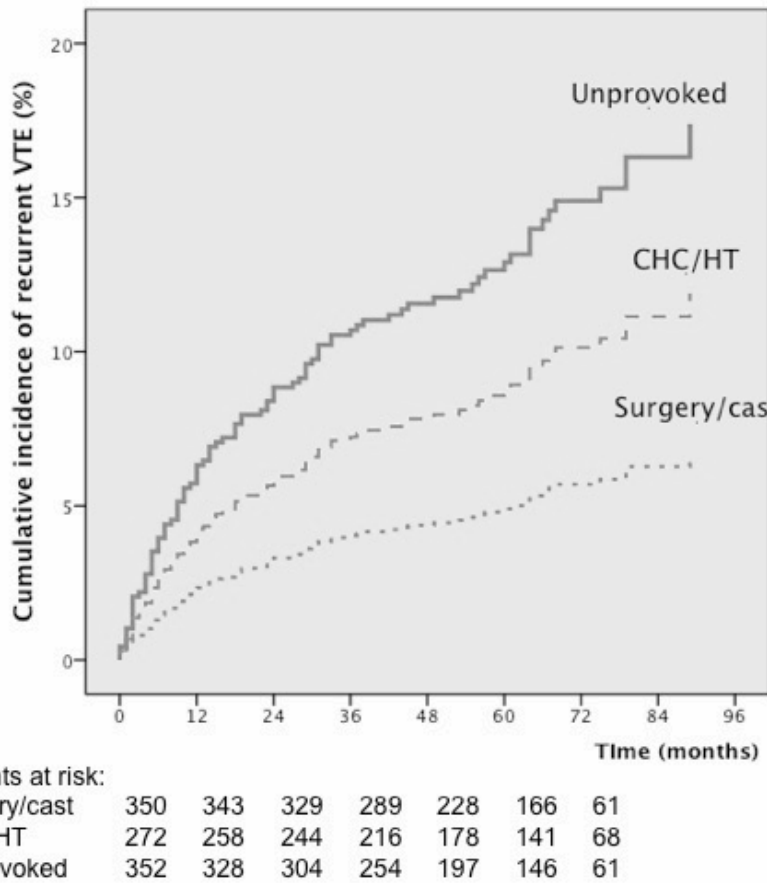


Figure 4. Cumulative incidence of recurrent VTE according to risk factor at first episode of VTE.

5.2 PAPER II

In paper II the aim was to evaluate if seven genetic variants previously found to predict first time thrombosis in the study population of TEHS was associated with recurrence. The seven genetic predictors previously identified by Bruzelius *et al* (119) were *F5* rs6025, *F2* rs1799963, *ABO* rs514659, *FGG* rs2066865, *F11* rs2289252, *PROC* rs1799810 and *KN1I* rs710446.

A total of 1010 women, of whom 38 deceased, were followed for a mean of 5 years. None of the deceased died from VTE. During the total follow-up of 5094 person-years there were 101 recurrent events.

The genetic variables *F5* rs6025 and *F11* rs2289252 were significantly associated with recurrence in the genetic model, with HR 1.6 (95% CI 1.1-2.4) and 1.8 (95% CI 1.1-2.9) respectively, compared to women with no risk-allele.

Table 4. Multivariable analysis of risk factors for recurrent VTE in a) clinical, b) genetic and c) combined model.

	HR (a)	[95% CI]	HR (b)	[95% CI]	HR (c)	[95% CI]
Clinical variables						
Proximal DVT vs distal DVT	1.54	[0.91–2.59]			1.43	[0.85–2.40]
Pulmonary embolism vs distal DVT	1.95	[1.19–3.20]			1.89	[1.16–3.10]
BMI (kg/m²)						
25–29 vs <25	1.39	[0.85–2.27]			1.41	[0.87–2.30]
30– vs <25	1.76	[1.06–2.91]			1.84	[1.10–3.06]
Provoked VTE*						
Hormonal use [†] vs Surgery and/ or cast	1.96	[1.05–3.64]			1.89	[1.02–3.54]
None vs Surgery and/or cast	2.98	[1.72–5.18]			2.89	[1.66–5.04]
Family history of VTE						
Yes vs None	1.4	[0.93–2.11]			1.33	[0.88–2.00]
Genetic variables						
<i>F5</i> rs6025 GA/AA vs GG			1.65	[1.09–2.50]	1.57	[1.02–2.41]
<i>F11</i> rs2289252 CT/TT vs CC			1.8	[1.09–2.96]	1.59	[0.96–2.62]
N	998		1004		992	

* within three month from diagnosis of first venous thrombosis; † combined contraceptives (oral, patch, vaginal devices) or oestrogen containing menopausal replacement therapy (oral and patch)

In carriers of both *F5* rs6025 and *F11* rs2289252 the cumulative recurrence rate was 2.5-fold higher compared to non-carriers (14.6% (95% CI 9.9-21.3%) vs 5.9% (95% CI 3.4-10.3%)) at 5 years follow-up (Figure 5). This difference was even larger in the subgroup analysis of women with unprovoked VTE, 21.9% (95% CI 13.7-34.0%) vs 7.5% (95% CI 3.2-16%) (Figure 6). Both Kaplan-Meier curves were statistically different using the log-rank test.

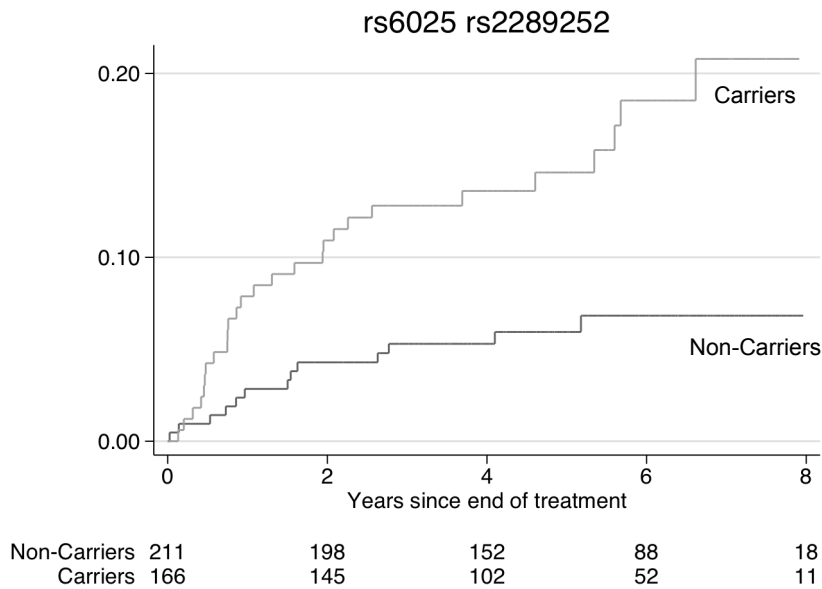


Figure 5. Cumulative recurrence comparing carriers of both risk alleles versus non-carriers of *F5* rs6025 and *F11* rs2289252.

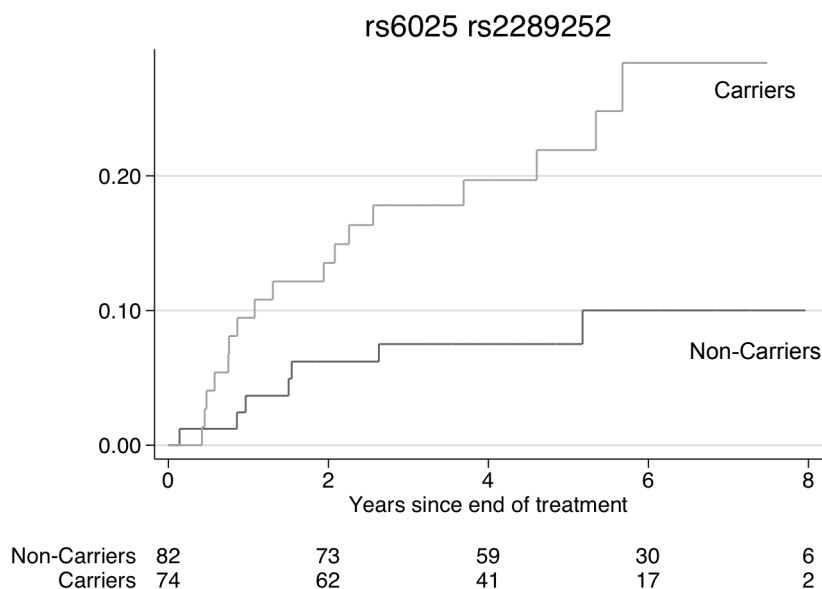


Figure 6. Sub-group analysis of women with unprovoked VTE. Cumulative recurrence comparing carriers of both risk alleles versus non-carriers of *F5* rs6025 and *F11* rs2289252.

5.3 PAPER III

The objective of paper III was to evaluate the risk of overall mortality and CVD after a first episode of VTE compared to a control population.

In the analysis we included 1081 women exposed to previous VTE and 1027 unexposed women, all with no CVD-event prior to inclusion in TEHS. During the total follow-up of 11,920 person years 49 women suffered from any CVD event, 35 (3.2%, 95% CI 2.1-4.3) among the exposed and 14 (1.4%, 95% CI 0.7-2.1) among the unexposed (Figure 7).

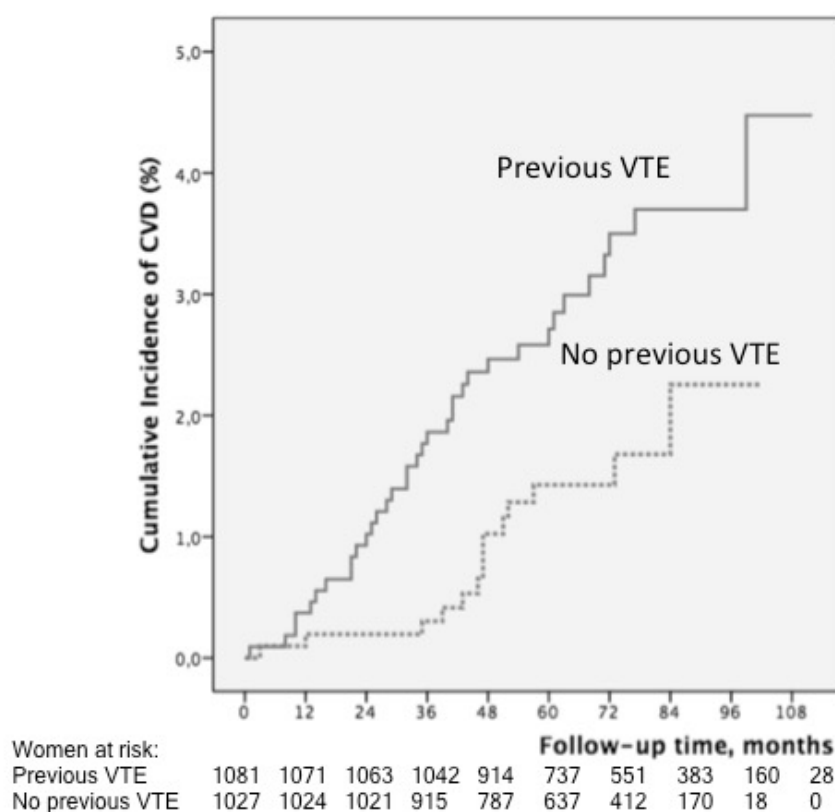


Figure 7. Cumulative incidence of cardiovascular disease.

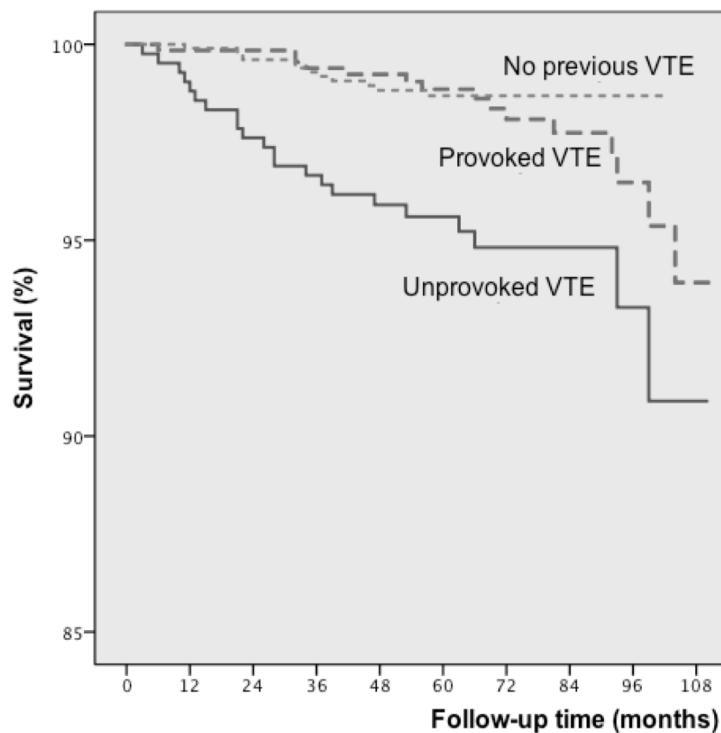
In a Cox proportional hazards model the crude HR for any CVD event was 2.2 (95% CI 1.2-4.2) comparing exposed to unexposed. The risk of CVD was highest for women with unprovoked VTE and PE. In a multivariate model adjusting for age, BMI, hypertension and smoking the estimates were only slightly affected.

The mortality rate in exposed women were 5.7 per 1000 person years and 2.2 per 1000 person years in unexposed generating a HR of 2.4 (95% CI 1.2-4.6). None of the women with known cause of death (n=45 out of 49) died from VTE. In both exposed and unexposed the major cause of death was malignancy (Table 5).

Table 5. Cause of death.

	Unprovoked VTE		Provoked VTE		Unexposed	
	N=22		N=15		N=12	
	n	%	n	%	n	%
Malignancy	10	45	4	27	6	50
Cardiovascular disease	6	27	4	27	3	25
Miscellaneous	5	23	5	33	2	17
Unknown	1	5	2	13	1	8

When analyzing women with provoked and unprovoked VTE separately the mortality rate differed between women with unprovoked VTE compared to both unexposed women and women with provoked VTE (Figure 8). Log rank test showed statistical significance difference between the Kaplan-Meier curve of women with unprovoked VTE compared to the other two curves.



Women at risk:										
Provoked VTE	660	660	660	653	582	480	360	255	117	29
Unprovoked VTE	418	414	409	401	347	274	205	42	46	7
No previous VTE	1021	1020	1018	915	790	642	417	173	20	0

Figure 8. Mortality among women with unprovoked VTE, provoked VTE and unexposed to VTE.

5.4 PAPER IV

In study IV we evaluated QoL after a first episode of VTE in 1040 women compared to 994 women with no previous VTE. Among exposed women we studied the prevalence of and risk factors for PTS.

After a median follow-up of 6 years from first VTE-event in exposed women and 5.5 years from inclusion in TEHS for unexposed there were no clinically significant difference in QoL between the groups, measured with SF-36. However, women developing PTS during follow-up had markedly impaired QoL compared to both unexposed women and women without PTS.

In exposed women PTS was the predominant predictor of QoL measured with all four QoL-instruments, SF-36 pcs, SF-36 mcs, VEINES-QoL and VEINES-Sym. Other predictors of low QoL measured with SF-36 were age, obesity and overweight, recurrent VTE and physical inactivity. VEINES-QoL and VEINES-Sym were only affected by PTS. Location of the initial thrombosis and whether it was provoked or unprovoked had no impact on QoL (Table 6).

Table 6. Predictors of QoL in exposed women, multivariate.

QoL-measure	Variable*	Parameter estimate	p-value
SF -36 pcs	PTS	-8.6	< 0.001
	Age > 50	-4.6	< 0.001
	Recurrent VTE	-4.4	< 0.001
	BMI > 30 vs < 25	-6.6	< 0.001
	BMI 25-30 vs <25	-3.4	< 0.001
	Physically inactive	-4.4	0.001
	Low education	-2.7	0.002
	Single	-1.9	0.01
SF-36 mcs	PTS	-4.9	< 0.001
	Age > 50	+2.1	0.008
	Single	-3.5	< 0.001
	Recurrent VTE	-3.1	< 0.001
	Physically inactive	-3.0	0.001
	Smoking	-2.1	0.03
	Low education	-2.3	0.03
Veins-QoL	PTS	-8.4	< 0.001
	BMI > 30 vs < 25	-0.9	< 0.001
	BMI 25-30 vs <25	-0.8	0.01
	Age > 50	-1.4	< 0.001
VEINES-Sym	PTS	-10.6	< 0.001
	BMI > 30 vs < 25	-2.0	< 0.001
	BMI 25-30 vs <25	-0.9	0.01
	Age > 50	-1.4	< 0.001

* Variables included in the model: PTS, age, recurrent VTE, BMI, physical activity, education, civil status, smoking, provoked vs unprovoked VTE and location of VTE (PE, proximal DVT, distal DVT)

Twenty per cent of women with a previous VTE had self-reported Villalta-score above 3 points at time of follow-up. Women with proximal DVT had the highest prevalence of PTS (n=98, 30%), followed by women with distal DVT (n=77, 19%) and women with PE (n=32, 11%). Obesity, proximal DVT, ipsilateral recurrence and presence of leg symptoms before first VTE were associated with an increased risk of PTS in a multivariate logistic regression model (Table 7).

Table 7. Risk factors associated with post-thrombotic syndrome in 1037 women after a first episode of VTE, presented as odds ratio (OR) and 95% Confidence Intervals (95%CI).

	Crude OR (95% CI)	Adjusted OR (95% CI)
Distal DVT	ref	ref
Prox. DVT	1.8 (1.3 – 2.6)	1.7 (1.2 – 2.7)
PE	0.7 (0.5 – 1.0)	0.6 (0.4 – 0.9)
Unprovoked	ref	ref
Surgery/ cast	0.7 (0.4 – 1.0)	0.7 (0.5 – 1.1)
Hormonal use*	0,9 (0.7 – 1.5)	1.4 (0.9 – 2.1)
BMI ≥ 30	1.9 (1.3 – 2.6)	1.7 (1.2 – 2.6)
Leg symptoms	3.1 (2.3 – 4.4)	3.1 (2.2 – 4.3)
Recurrent VTE	1.7 (1.1– 2.8)	1.4 (0.8 – 2.4)
Ipsilateral recurrence	4.1 (2.0 – 8.4)	2.9 (1.2 - 6.)
Physical activity	1.2 (0.7 – 2.1)	1.0 (0.5 – 1.8)

*treatment with either combined hormonal contraceptives or menopausal hormone treatment.

6 DISCUSSION

In these studies, on a nation-wide well-defined cohort of female patients with VTE, and a matched control population, we were able to evaluate risk factors for recurrent VTE as well as late complications as CVD and mortality and to assess QoL. The major strength of this thesis includes the large, well-defined, homogenous cohort, with detailed information on life-style factors, events taking place prior to VTE and socioeconomic factors, limited loss-to-follow-up and the prospective design. TEHS was a population-based study with standardized and detailed data collection through interviews, giving possibility to assess several risk factors at the same time. Information on the outcomes were collected both through questionnaires and Swedish National Registers. A weakness is the relatively low number of outcome events in study I-III giving rise to loss in statistical power and unable subgroup analysis.

6.1 METHODOLOGICAL CONSIDERATIONS

In observational studies both random and systematic errors can occur affecting precision, internal validity and generalizability of the study. These possible errors must be considered through-out the research process from designing the study, to finally interpreting the results.

TEHS and TEHS follow-up did not include men, children and the elderly and therefore the results cannot be generalized to the whole population. The population-based design of TEHS with 43 including hospitals spread geographically in Sweden makes the external validity good for young and middle-aged women if the internal validity is good.

6.1.1 Random error

Even though the study population is large some of the outcomes studied are rare giving rise to loss in statistical power. In all papers estimates are presented with their 95 % CIs demonstrating the precision. In general, we have good statistical power for the variables studied. However, in some analysis, especially in subgroup analyses, the CIs are wide, demonstrating statistical uncertainty.

6.1.2 Systematic error – selection bias

Selection bias might occur if the association of exposure and outcome differs in those participating and not participating in the study. In this thesis selection bias might have occurred at different stages. First, when the cohort was recruited in TEHS, the participation rate was lower among controls compared to cases. Selection bias can occur if controls participating in the study to a higher degree had a medical history and therefore were more prone to participate.

Secondly the loss to follow-up in TEHS-follow-up might give rise to selection bias. A total of 25% refrained from participating in the follow-up. Selection bias can occur when loss to follow-up is associated with either the exposure or the outcome. In TEHS-follow-up the proportion of non-participants was almost similar among exposed and unexposed. When comparing the original cohort of TEHS to the cohort of TEHS-follow-up, women lost to follow-up were younger and more often smokers both among exposed and unexposed. The

only difference noted was that among exposed women there were a higher proportion of married women among those attending TEHS follow-up while there was no difference among unexposed (Table 8). Since the baseline characteristics between women attending TEHS-follow-up and women lost-to-follow-up does not differ between exposed and unexposed the main effect of loss to follow-up should be loss in statistical power.

Table 8 Baseline characteristics of the study cohort of TEHS-follow-up and the study participants from TEHS lost to follow-up.

	Exposed				Unexposed			
	TEHS- follow-up		Lost-to follow-up		TEHS follow-up		Lost-to- follow-up	
	n	%	n	%	n	%	n	%
Age (mean, years)	47		44		48		44	
BMI (mean, kg/m ²)	27		27		25		25	
BMI < 25	447	41	150	43	601	59	204	54
BMI 25-30	370	34	114	32	300	29	116	31
BMI > 30	263	24	88	25	120	12	57	15
FV Leiden	252	24	57	18	76	7.7	32	9.7
FII GA20210A	56	5.5	19	5.8	29	3	8	2.5
Current smoker	267	25	110	31	197	19	108	28
Hypertension	231	21	74	21	204	20	82	21
Diabetes mellitus	44	4	17	5	25	2.5	13	3.4
Married/cohabitant	785	73	238	68	789	78	292	77
Physical activity								
No	80	7	27	8	34	3	17	5
Light	422	40	158	46	355	35	129	35
Regular	576	53	161	71	633	62	222	61
Education								
< 10 years	178	17	59	17	125	13	51	14
10-12 years	354	34	135	39	315	32	129	35
> 12 years	505	49	151	44	542	55	194	52
Leg symptoms	400	37	142	40	286	28	104	27
Pulmonary Embolism	311	29	122	35				
Deep Vein Thrombosis								
Proximal	288	27	73	21				
Distal	479	44	158	45				
Unprovoked VTE	413	39	142	41				
Provoked VTE*								
Surgery/cast	366	34	117	34				
Hormonal use†	292	27	83	24				

* within three month from diagnosis of first venous thrombosis; † combined contraceptives (oral, patch, vaginal devices) or oestrogen containing menopausal replacement therapy (oral and patch)

Thirdly selection bias might have occurred when selecting the study populations in the different studies. In paper I and II all women with continuous anticoagulation were not included as they were considered not being at risk of recurrent events. Possible consequences of this will be discussed further under each paper.

6.1.3 Systematic error – information bias

Information bias or misclassification can occur when information on outcome or exposure is obtained differently in the different study groups. Misclassification may be either differential, if the probability of being misclassified differs between the study groups or non-differential. The result of non-differential misclassification is a dilution of the effect, bias towards the null, while differential misclassification might exaggerate or underestimate the effect.

Misclassification of exposure in TEHS-follow-up

The prospective study design with collection of information on VTE before the development of the outcome has minimized information bias due to recall bias. Misclassification of VTE is unlikely since the diagnosis was based on both radiological report as well as clinical decision to start anticoagulant treatment. The validity of diagnosing DVT with Doppler ultrasonography and phlebography and PE with CT scan or ventilation-perfusion scintigraphy together with high clinical suspicion of PE is high. During follow-up some of the women included as controls in TEHS, and therefore considered unexposed might have been diagnosed with VTE, hence being misclassified. Although since the incidence of VTE is about 1 to 2 per 1000 persons each year the expected number of misclassified unexposed women were low. To avoid immortal time bias, all controls of TEHS were included as unexposed in TEHS follow-up.

Misclassification of outcomes in TEHS-follow-up

The outcomes in TEHS follow-up were collected through self-reported information in the questionnaire as well as through register data minimizing misclassification. When answering the questionnaire, it was not apparent to the women what associations to be studied. In study I and II Information on recurrent events were based on data both in the questionnaire as well as data from the Patient Register. All possible recurrent events recorded either in the Patient Register or in the questionnaire were verified by reviewing medical charts minimizing recall bias and misclassification. In order to minimize surveillance bias in paper III only hard endpoints with clear diagnostic definitions, like myocardial infarction (MI) and ischemic stroke, were used. Information on PTS in paper IV was based on self-reported, modified, Villalta scale instead of Villalta scale evaluated and diagnosed by a physician. This may lead to misclassification bias discussed more in detail further on.

6.1.4 Systematic error – confounding

Confounding means that the effect of the exposure is mixed with the effect of another factor, leading to bias. To be a confounding factor the factor has to be associated with both the exposure and the outcome but not be an intermediate step in the causal pathway between exposure and outcome. There are three ways to prevent confounding while planning the study:

randomization, matching and restricting. Once the data is collected confounding can be controlled for by multivariable regression analysis. In the original recruitment to TEHS, the controls were matched to cases by age. In TEHS follow-up the age-matching disappeared due to loss-of-follow-up. However, the skewed age distribution was taken care of when analyzing the data, by adjusting for age. TEHS was restricted to women aged 18-64.

In the analysis of all four studies we have been able to adjust for potential confounding factors. This was possible as we collected detailed information about the study participants during the interview of TEHS as well as in the questionnaire of TEHS-follow-up. There is a potential risk of recall-bias as well as interviewer bias for confounding factors collected at time of inclusion in TEHS. Information on exposures in TEHS, i.e. potential confounders in TEHS-follow-up, were collected retrospectively and recall bias might occur if women with VTE remember exposures better. Interviewer bias can occur if the interviewer is aware of the association between exposure, confounder and outcome and thereby asks the questions differently to exposed and unexposed. To minimize the risk of bias the interview was made by a trained nurse, aware of this problem. The interviewer used a structured questionnaire including a catalogue with pictures of different contraceptives and a life time calendar sent to the women to complete before the interview. To further diminish the risk of recall bias, the interview focused on the three-month period prior to the index event. In study III most confounding factors are life-style factors that may change over time. We measured life-style factors both at inclusion in TEHS as well as at time of follow-up. A sensitivity analysis was made adjusting for the same confounding factors measured at time of follow-up instead of inclusion in TEHS without any major changes in the risk estimates.

6.2 MAIN FINDINGS

6.2.1 Recurrent VTE (I and II)

It is an important clinical challenge to evaluate the risk of recurrence in a patient with VTE and thereby safely be able to withhold anticoagulant treatment to patients with low risk of recurrence. For patients with unprovoked VTE there is a need for more precise prediction models. In none of the available models, HER DOO2, Vienna Prediction Model or DASH, screening for thrombophilia added any predictive value (45-47).

In TEHS-follow-up the overall risk of recurrence for all women were low, with an annual incidence rate of about 2 %. Obesity, having an unprovoked VTE and the location of the primary VTE were the strongest clinical risk factors for recurrent events. The risk of recurrence for women with hormone-associated VTE were lower than for women with unprovoked VTE but not as low as for women with surgery/cast provoked VTE. Data concerning the risk of recurrence after hormone associated VTE are scarce. Many of previous studies are small cohorts or subgroup analysis reporting lower risk of recurrence in hormone users compared to unprovoked VTE, albeit not statistically significant (42, 43). There are not many studies including provoked VTE as well, and none is comparing hormone-associated VTE to surgery/cast associated VTE (39, 120). The results of TEHS follow up are in line with a cohort

study by Eischer et al in 2014, including only women with a first episode of unprovoked or hormone-associated VTE (121).

The findings of a low risk of recurrence in women with hormone-associated VTE strongly suggest that long-term anticoagulant treatment safely can be withheld in this group of patients, as in patients with surgery/cast associated VTE. Nevertheless, women with unprovoked VTE had a higher risk of recurrence, especially in the first year after cessation of anticoagulant treatment, indicating the need for an instrument to divide these women into high and low risk of recurrence.

In paper II we evaluated whether a genetic risk score, previously used in the same population to predict first episode of VTE (119), could be used to better predict recurrence. We found that *F11* rs2289252 doubled the risk of recurrence, which was higher than for both *F5* rs6025 and known family history. The association of *F11* rs2289252 and first time VTE has been reported previously and has partly been explained by increased levels of FIX (122-124). There are growing evidence that FIX may be a key player in the pathogenesis of thromboembolic disease (125, 126). The risk of recurrence was 2.5 times higher at 5 years follow-up for carriers of both *F5* rs6025 and *F11* rs2289252 than non-carriers. In the sub-group analysis of patients with unprovoked VTE this difference was even more pronounced. The risk of recurrence in women carrying both *F5* rs6025 and *F11* rs2289252 were slightly higher than for women carrying ≥ 5 of the 7 SNPs predicting first time VTE. Considering this, using the sum of genetic variants like in the MEGA follow-up (127) might underestimate the risk in patient with few risk alleles, albeit the alleles being high risk variants. Our results in paper II indicate that a combination of common genetic variant may be useful to predict risk of recurrence in women, especially after an episode of unprovoked VTE.

The strength of paper I and II is the well-defined cohort of a homogenous population. The use of both the questionnaire and the Patient Register to obtain recurrent events as well as verifying them by reviewing the medical charts reassured that all events were identified and clinically relevant. Hence, when interpreting the data a few limitations has to be taken into account. Patients with ongoing anticoagulant treatment were excluded as they were not considered being at risk of recurrence. Therefore, patients considered having high risk of recurrence already at time of first VTE, and thereby prescribed indefinite anticoagulation, were not included in our study. The low number of recurrent event left us with reduced statistical power.

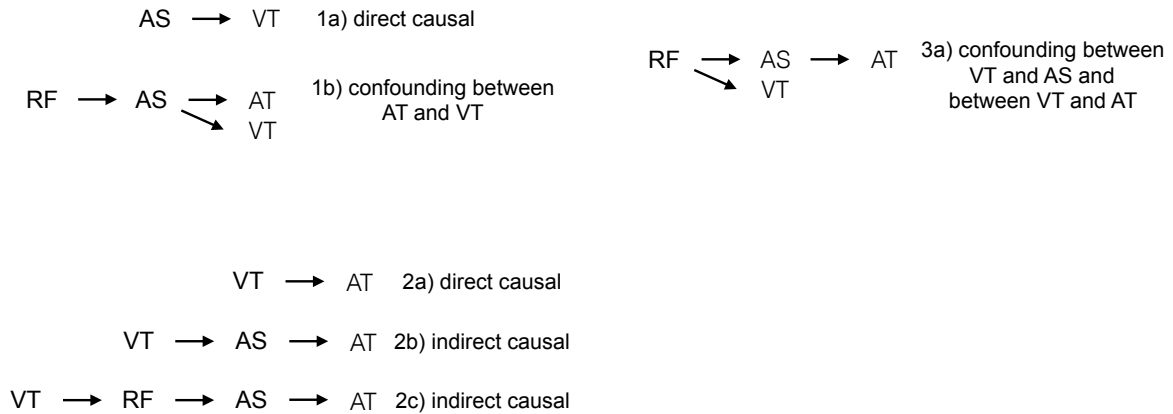
6.2.2 Cardiovascular disease (III)

Growing evidence during the recent years have suggested that there might be a potential link between venous and arterial thrombosis. It is still unclear though whether this link is real or due to bias, chance or confounding.

In paper III we evaluated the risk of arterial CVD in 1081 women with a first episode of VTE compared to 1027 women with no prior VTE. We found a 2-fold increased risk of CVD in

exposed women. The estimates were only modestly changed when adjusting for cardiovascular risk factors suggesting common risk factors is not the full explanation.

According to Lijfering *et al* (66) there might be three possible options how VTE and CVD are relate, illustrated by the directed acyclic graphs (DAGs) in figure 9.



AS, atherosclerosis; AT, arterial thrombosis; RF, risk factors; VT, venous thrombosis.

Figure 9. Directed acyclic graphs illustrating three hypotheses of how venous and arterial thrombosis may be associated. Lijfering VM *et al* *Seminars in thrombosis and hemostasis* 2011. Figure is reused and modified with the kind permission of dr Lijfering.

The two diseases might be associated through arteriosclerosis (Figure 9, 1b) though arteriosclerosis enhances inflammation and coagulation. The association might also be causal (Figure 9, 2 a-c). One possible explanation might be that arterial thrombosis is caused by the inflammatory process initiated by the venous thrombus. This explanation would explain why some studies found an increased risk of CVD during the first year after VTE (60, 69). Another explanation may be that treatment with vitamin K antagonists lead to increased arterial calcification. The association might also be explained by confounding by common risk factors for both diseases (Figure 9, 1 b and 3a). Then, adjusting for risk factors for CVD would lead to a decreased relative risk. Increased age and obesity are the only robustly reported common risk factors for VTE and CVD (66, 128).

In TEHS-follow-up the risk of CVD was unchanged after adjustment for measured cardiovascular risk factors at baseline. However, cardiovascular risk factors might change over time. To overcome this, we performed a sensitivity analyses adjusting for the same factors at time of follow-up instead, with no major change in risk estimates. Nevertheless, there might still be residual confounding due to unknown or unmeasured confounding factors, for instance genetic factors. In TEHS, we previously found that the only shared genetic risk factor, among common genetic variants associated with CVD, was the ABO locus (129) In TEHS follow-up the risk of CVD between exposed and unexposed started to diverge after one year (Figure 8) and the Kaplan Meier curves were parallel thereafter which might support that the VTE event itself or the treatment therefore may have a causal effect of CVD.

Strengths of this study include the use of hard endpoints obtained by the Swedish National Registers with high coverage and validity of the defined diagnoses reducing misclassification. By collecting detailed information on cardiovascular risk factor both at time of inclusion in TEHS and TEHS follow-up it was possible to adjust for potential confounding factors. The limitations that one should bare in mind interpreting the results are that there are few outcome events giving rise to statistical uncertainty. Unfortunately, the study design did not allow us to adjust for the effect of anticoagulant treatment or other pharmacological treatments.

6.2.3 Mortality (III)

Overall survival is affected by VTE, especially in patients with underlying malignancy.

In TEHS only women with good life-expectancy were included, since women with malignant disease were not eligible and the prevalence of comorbidities were low. Hence, women exposed to VTE had a two-fold increased risk of overall mortality compared to unexposed women. None of the women with known cause of death (n=45/49) died from VTE. In ten of the thirteen women with cardiovascular death the cause of death was based on autopsy results. This reduced misclassification bias, due to PE-related death misdiagnosed as CVD. Overall, the mortality rate was low compared to previous studies on patients with VTE (32, 78). The difference may be explained by TEHS not including patients with known malignancy and being restricted to patients below 65 years of age. The mortality rate in the control population was comparable to the mortality rate reported by the National Board of Health and Welfare for the general population of women 18–64 years of age in Sweden (115). The elevated risk of mortality was seen in women with unprovoked VTE. Death due to malignancy was twice as common among exposed women compared to unexposed. In a stratified analysis of women with provoked and unprovoked VTE it is found that the elevated risk of cancer-associated mortality occurred among women with unprovoked VTE. This might reflect that unprovoked VTE not seldom is the first manifestation of cancer, not diagnosed at time of inclusion in TEHS.

Among the strengths of this study are the long-term follow-up of a well-defined cohort. The use of National Registers with good validity to retrieve mortality data and a high proportion of women with cardiovascular deaths based on autopsy results minimized misclassification. A limitation of the study may be that the follow-up time for unexposed are shorter compared to exposed introducing selection bias. Restricting the analysis for the first two years may be a way to overcome this, however the low number of events did not allow us to do that without losing power.

6.2.4 Quality-of-life (IV)

The limited number of studies on QoL after VTE have found that the main predictor of QoL after VTE is occurrence of PTS.

In paper IV we found that on average there were no difference in QoL in exposed and unexposed women. However, in consistence with previous studies (79, 88, 89), women developing self-reported PTS had markedly lower QoL compared to both unexposed women and exposed women without PTS. Age, obesity, inactivity and recurrent VTE also affected general physical health in our study. Having a proximal DVT did not affect QoL, as opposed

to a study by Kahn *et al* (10). Even though we have a larger number of study participants compared to the study by Kahn the proportion of patients with proximal DVT is lower. Although, we have 90 % power to detect a difference of 3 points in our study population, it might be that we missed significant associations of smaller magnitude. Since the study by Kahn *et al* included older patients as well as patients with malignancy the affection of QoL in patients with proximal DVT might be due to a higher incidence of comorbidities rather than the proximal location itself. We found no differences in age, BMI or prevalence of malignancy during follow-up between patients with proximal or distal DVT except that more women with distal DVT had a surgery/cast provoked DVT than women with proximal DVT.

To our knowledge, only few studies have examined the effect of socioeconomic factors on QoL after VTE. Short education was an independent predictor of SF-36 pcs, while VEINES-QoL was only affected in the univariate model. A Norwegian study on women with pregnancy-related VTE has reported that low education was an independent risk predictor for VEINES-QoL scores < 25th percentile as compared with the ≥50th percentile (130).

Physically active women reported better QoL measured with SF-36 pcs. The results from previous studies on physical activity and QoL in VTE-patients are inconsistent. One randomized trial could not show any effect on QoL with physical activity, while two smaller studies report improved QoL by exercise (131-133).

The major strengths of the study on QoL is the large population-based, homogenous cohort with detailed information on life-style factors and socioeconomic factors. QoL was comprehensively assessed by the use of both generic and disease specific QoL instruments. There are some limitations that one has to consider when interpreting the results. The loss to follow-up might have introduced selection bias. Women not accepting participation in the follow-up study are younger (mean age 44 vs. 47 years for exposed and 44 vs. 48 years for unexposed) and more often smokers (31% vs. 25% for exposed and 28% vs. 19% for unexposed) than participants. It should be noted though that the loss to follow-up was similar among exposed and unexposed women except slightly more married women among the exposed participating in the follow-up (73% vs. 68% for exposed and 78% vs. 77% for unexposed).

6.2.5 Post-thrombotic syndrome (IV)

PTS is the most common complication of VTE. In TEHS follow-up the prevalence of self-reported PTS was 20% for the total cohort. Compared to other studies that is a low prevalence of PTS (8, 58, 134). One explanation may be the large proportion of women with distal DVT in our cohort. However, analyzing women with proximal DVT separately still reveals a low prevalence of PTS. Since age has been reported to affect PTS (8, 96, 135) the restriction of TEHS including only women below 65 years of age may explain the low occurrence of PTS. The prevalence of PTS may also be low due to high quality of anticoagulation therapy in Sweden (136, 137).

Our findings that obesity, proximal DVT and recurrent ipsilateral DVT increased the risk of PTS has been reported previously (8, 96, 97). Women in TEHS reporting leg symptoms like heaviness, pain and swollen legs prior to the onset of VTE-symptoms have a 3-fold increased risk of PTS. There is no gold standard test for PTS, hence the diagnosis is clinical (99). The recommended tool to use, the Villalta scale, comprises of 5 subjective symptoms and 6 objective signs, neither being specific to PTS. The signs and symptoms reported could therefore be due to primary venous insufficiency (PVI) (134). It might be that women in TEHS reporting leg symptoms prior to VTE are misdiagnosed with PTS rather than PVI. It could also be that these women have PVI prior to VTE and that venous insufficiency plays a role in the occurrence of PTS. Previous findings from the REVERSE study and the MEGA study support this theory (134, 138).

The size of the cohort and comprehensive information on life-style factors and leg symptoms prior to VTE are the major strengths of this study. There are however some limitations that has to be mentioned. We used a modified Villalta scale to assess PTS, not being validated in our setting. The same modified scale has however been used and validated in the MEGA study (8) with good correlation to the original Villalta scale (kappa 0.88, 95 % CI 0.79-0.96). A study from Norway validating a modified Villalta scale reported that patients usually overestimated their signs compared to professional health care givers. We did not use the Villalta scale to grade the severity of PTS but rather to assess whether women had developed PTS or not. The prevalence of PTS was rather low indicating that overestimation of PTS may not have been a problem. Another limitation that may introduce bias is loss to follow-up. The prevalence of PE as primary VTE was lower among women accepting participating in TEHS-follow-up than those lost to follow-up. Therefore, the prevalence of PTS in the whole cohort might be overestimated. However, a sensitivity analysis stratified by location of thrombosis showed no major difference in the risk estimates for the predictors of PTS.

7 CONCLUSION

- The overall risk of recurrence in young and middle-aged women was low and in the majority of patients prolonged anticoagulation therapy can be safely withheld. Women with hormone associated VTE had a lower risk of recurrence than women with unprovoked VTE but not as low as after surgery/cast provoked VTE.
- The risk of recurrence was for the first time found to be associated with the risk allele *F11* rs 2289252. Especially among women with unprovoked VTE the combination of the *F5* rs6025 and *F11* rs2289252 contributed to the risk of recurrent VTE and might be of clinical relevance for risk prediction.
- Compared to a control population women with previous VTE had an increased risk of future arterial cardiovascular disease and overall mortality. The risk was highest among women with unprovoked VTE and pulmonary embolism. The elevated risk of cardiovascular disease was only modestly changed after adjustment for cardiovascular risk factor suggesting there might be a causal effect.
- About a fifth of women developed PTS as a chronic complication of VTE. Women with ipsilateral recurrence and reporting leg symptoms prior to VTE had the highest risk of PTS. Women developing PTS had severely impaired health related QoL while QoL in women with VTE not developing PTS was similar compared to a control population.

8 FUTURE PERSPECTIVES

The best way to avoid VTE complications in women is to prevent the first occurrence of VTE. The major risk factors for incident VTE in women in young and middle age is the use of estrogen containing hormones and surgery. Better ways to identify women at high risk of incident VTE is warranted to be able to prescribe hormones safer and more frequent use, or prolong, thromboprophylaxis among these women.

In the future there is a need for a better understanding of the epidemiology and risk factors for recurrent VTE. For patients it is important to have a good, validated tool to be able to balance the risk of major bleeding with continues anticoagulant treatment against the risk of recurrence after stopping anticoagulation. Since women are found to have a lower risk of recurrence than men there might be a need for a separate risk score for women.

Obesity is a risk factor not only for primary VTE but also for recurrence, PTS and lower QoL. The mechanism how obesity predisposes VTE is however not fully understood. Studies on the influence of weight-loss on risk of VTE, recurrence, PTS and QoL would be interesting to learn more of the mechanism of obesity and VTE and in a better way tailor preventive actions.

There is a need for a better understanding of the relation between venous and arterial thrombosis. Hence, if the relationship is causal than every VTE patient might benefit from arterial thrombosis prevention while only patients with cardiovascular risk factors should be directed if the association is due to common risk factors. The findings that statins and low-dose aspirin might protect against recurrent VTE is interesting and deserves further studies.

There is a need for a better PTS risk prediction tool including both clinical and biomarker information. Since inflammation might be a part of the development of PTS it would be interesting to study the influence on anti-inflammatory agents, prolonged treatment with LMWH and the influence on treatment with NOACs on the risk of PTS.

9 SAMMANFATTNING PÅ SVENSKA

Bakgrund

Venös trombos är ett samlingsnamn för blodproppar i kroppens vener. De vanligaste lokalisationerna för venös trombos är i benen eller i lungan. Venös trombos är en vanlig och potentiellt livshotande sjukdom som årligen drabbar 1-2 per 1000 personer. Venös trombos förekommer hos både män och kvinnor, men kvinnor drabbas oftare i yngre ålder delvis beroende av behandling med p-piller och graviditet. Även östrogenbehandling i klimakteriet ökar risken för venös trombos.

Efter ett första insjuknande i venös trombos finns risk för återfall i sjukdomen. Den risken kan minimeras genom att äta blodförtunnande läkemedel, en behandling som dock innebär en ökad risk för allvarlig blödning. Det är därför viktigt att kunna bedöma risken för återfall, för att kunna hitta de patienter som har låg risk att drabbas av återfall och därmed säkert kan avstå från långtidsbehandling med blodförtunnande läkemedel. Risken för återfall är lägre hos kvinnor än män, orsaken till det är inte helt klarlagd. Efter en venös trombos kommer ett flertal att drabbas av posttrombotiskt syndrom (PTS). PTS är ett kroniskt tillstånd med symtom i form av smärta, tyngdkänsla, svullnad, klåda och kramp i det drabbade benet. Bland de få studier som studerat livskvalitet efter en venös trombos ses att förekomst av PTS är det som har störst påverkan på livskvalitet efter en venös trombos. Andra långtidskomplikationer efter venös trombos verkar vara en ökad risk för hjärtkärlsjukdom och ökad dödlighet.

Syfte

Målet med avhandlingen är att få bättre kunskap om långtidskomplikationerna efter en venös trombos hos kvinnor mellan 18-64 år genom att studera:

- Risken för återfall i venös trombos och studera om återfallsrisken skiljer sig för de kvinnor som haft en blodpropp utlöst av hormoner, kirurgi eller gips jämfört kvinnor med idiopatisk (utan känd orsak) trombos.
- Om förekomst av vanliga genetiska förändringar kan användas för att hitta kvinnor med ökad risk för återfall i trombos.
- Risken för hjärtkärlsjukdom och död hos kvinnor efter en venös trombos jämfört med risken hos kvinnor som inte haft venös trombos.
- Livskvalitet efter en venös trombos jämfört med den hos kvinnor utan trombos samt studera förekomst av PTS efter en förstgångstrombos och hur det påverkar livskvaliteten.

Metod

Studierna i denna avhandling är gjorda på en grupp av kvinnor hämtade från en svensk studie, 'Thrombo-Embolic Homonal Study' (TEHS). Avsikten med TEHS var att studera medfödda och förvärvade riskfaktorer för venös trombos hos kvinnor. Mellan 2002 och 2009 inkluderades 1433 kvinnor med en förstgångs venös trombos och 1402 jämnåriga kvinnor

utan venös trombos. Kvinnor som hade eller haft cancer senaste 5 åren, tidigare trombosjukdom eller graviditet senaste 3 månaderna exkluderades från studien.

Under 2011 tillfrågades alla kvinnor som deltog i TEHS och som levde i Sverige om deltagande i uppföljningsstudien, TEHS-follow-up. Kvinnorna tillfrågades skriftligt, genom att en enkät skickades till dem per brev. För de kvinnor som accepterade deltagande i TEHS-follow-up (1087 kvinnor med trombos och 1030 kvinnor utan trombos) och för de som avlidit under uppföljningsperioden (38 kvinnor med trombos och 12 kvinnor utan trombos) inhämtades data om hjärtkärlsjukdom och återfall även från svenska hälsoregister. Enkäten innehöll frågor för att mäta livskvalitet och förekomst av PTS.

Resultat

Studien visar att den sammanlagda risken för återfall i studiepopulationen är låg. Kvinnor med idiopatisk trombos och kvinnor med fetma har högst risk för återfall. Återfallsrisken för de kvinnor som drabbats av en hormonutlöst trombos är lägre än för kvinnor med idiopatisk trombos men inte lika låg som för kvinnor med trombos utlöst av kirurgi eller gips. Kvinnor med förekomst av 2 genetiska förändringarna (*F5* rs6025 och *F11* rs2289252) har ökad risk för återfall vilket talar för att kombinationen av dessa gener möjligen kan användas för att hitta de kvinnor som har ökad risk för återfall.

Andelen kvinnor med venös trombos som drabbats av hjärtkärlsjukdom eller död under uppföljningstiden var låg, men jämfört med kvinnor utan trombos var risken fördubblad.

Omkring en femtedel av kvinnorna hade utvecklat PTS under uppföljningstiden. Dessa kvinnor hade en tydligt sänkt livskvalitet. För kvinnor med trombos utan PTS skiljde sig inte livskvaliteten jämfört de kvinnor som inte haft trombos.

Slutsats

Sammanfattningsvis har denna avhandling bidragit till att öka kunskapen om långtidskomplikationer efter venös trombos hos unga och medelålders kvinnor. Efter en venös trombos är den vanligaste komplikationen PTS, ett kroniskt tillstånd med påverkan på framtida livskvalitet. För majoriteten av kvinnor är risken för återfall i trombosjukdom låg och långtidsbehandling med blodförtunnande medicin är inte motiverat.

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