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VENOUS THROMBOEMBOLISM IN WOMEN

AN ASSESSMENT OF HORMONAL, GENETIC
AND OTHER RISK FACTORS

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"Här ligger jag och duger"

Bob Hansson
ABSTRACT

Background Venous thromboembolism (VTE) occurs in 1-2 in 1000 individuals per year. VTE is found in both sexes, but women have a higher incidence at younger ages, particularly during the childbearing years. Although several acquired and genetic risk factors for venous thrombosis have been identified, the modes and consequences of combinations of these risk factors are not fully understood.

Aim The overall aim of this thesis was to clarify risk factors for VTE in women.

Methods The ThromboEmbolismHormoneStudy (TEHS) is a nation-wide population-based case-control study that included 1470 cases and 1590 controls. All participants were recruited prospectively in Sweden from 2003 to 2009. Reports on acquired risk factors for thrombosis were collected through telephone interviews of the participants and genetic risk factors were identified by DNA analyses on blood samples.

Results In Study I we found that risks associated with recognized acquired and genetic risk factors for VTE generally were of similar magnitude in pre-and postmenopausal women. The acquired, transient risk factors were stronger than the genetic factors and the combination of surgery and plaster cast yielded a 50-fold increased risk for VTE in both pre- and postmenopausal women.

In study II current use of combined hormonal contraception (CHC) was associated with a five-fold increased risk of VTE, adjusted odds ratio (ORadj)=5.3, 95% confidence interval (CI)=4.0-6.9. In adjusted analyses combinations with desogestrel had the highest risk (OR=11.4, 95% CI=6.0-22.0) followed by drospirenone, etonogestrel, norgestimate, levonorgestrel and norethisterone (OR=2.0, 95% CI=1.1-3.8). Current use of progestogen-only contraception (POC) was not associated with increased risks of VTE (ORadj=0.9, 95% CI=0.7-1.2). In stratified analyses (by dose) current users of “high dose” POC had an increased risk of VTE (ORadj=2.2, 95% CI=1.3-4.0).

In study III for the group of propionic acid derivatives, most women used ibuprofen (92%); of the women who used acetic acid derivatives, almost all used diclofenac (97%). In adjusted analyses overall use of NSAIDs was not associated with increased risks of VTE (ORadj=1.0, 95% CI=0.8-1.2). The adjusted OR was 0.9 for propionic acid derivatives (95% CI=0.72-1.10), 1.2 for acetic acid derivatives (95% CI=0.8-1.7) and 1.8 for coxibs (95% CI=0.7-4.3). For users of acetic acid derivatives and coxibs, the adjusted ORs increased by cumulative dose, suggesting a dose–effect relationship for these drugs.

Conclusion Menopausal status has only a minor impact on the risk levels with regard to recognized risk factors for VTE. The risk of VTE associated with the use of CHC varies depending on the type of progestogen used, even after adjustment for individual factors such as smoking and body mass index (BMI). Except for high-dose preparations, POC seems to be a safer alternative to CHC, with no obvious increased risks for VTE. There is no apparent risk of VTE associated with the use of propionic acid derivatives in young and middle-aged women.

Key words venous thromboembolism, combined oral contraception, progestogen-only contraception, NSAID, premenopausal, postmenopausal, risk factor, SNP
LIST OF PUBLICATIONS


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<tr>
<td>APC</td>
<td>Activated protein C</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CHC</td>
<td>Combined hormonal contraception</td>
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<td>COC</td>
<td>Combined oral contraceptives</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>DVT</td>
<td>Deep vein thrombosis</td>
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<td>fVL</td>
<td>Factor V Leiden</td>
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<td>MHT</td>
<td>Menopausal hormone therapy</td>
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<td>NOAC</td>
<td>Novel oral anticoagulants</td>
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<td>NSAID</td>
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<td>OC</td>
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<td>PE</td>
<td>Pulmonary embolism</td>
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<td>PGM</td>
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<td>POC</td>
<td>Progestogen-only contraception</td>
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<td>POP</td>
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<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<td>VTE</td>
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1 INTRODUCTION

Venous thromboembolism (VTE) is a disease that includes both deep vein thrombosis (DVT) and pulmonary embolism (PE), the latter being a potentially fatal condition. VTE is a multicausal disease, i.e. any single risk factor may predispose to VTE but is not sufficient to trigger thrombosis on its own. Instead several risk factors act together to compose a causal mechanism (1-2). Established risk factors include both genetic and acquired conditions.

This study, designed to evaluate risk factors for VTE in women, started in January 2003. Shortly thereafter I joined the research group. Little did I know then about the enormous amount of time and effort that is required to drive to completion an epidemiologic project of this character. Today, 10 years later, I cannot but agree with the words “Patience is bitter, but its fruit is sweet.” The three publications listed in this thesis are based on the data collected in the ThromboEmbolismHormoneStudy (TEHS). The publication listed as related work, was written during the data collection process.

The overall aim of this thesis was to shed further light on risk factors associated with VTE in women.
2 BACKGROUND

In 1961, a case report was published describing a nurse who suffered from PE soon after she started taking the first marketed combined oral contraceptive Enovid (3). Ever since this event, numerous studies have been conducted to study the association between hormone use and VTE and combined hormonal contraception (CHC) is now a well established risk factor for VTE (4-9). However, most users of CHC will never experience a thrombosis. It is therefore of utmost importance to determine which other factors that are implicated in thromboembolic events. CHCs are not only used to prevent unwanted pregnancies but also to treat dysmenorrhea and menorrhagia (10-11). Another group of medication commonly used to treat these conditions is the non-steroidal anti-inflammatory drugs, NSAIDs (12). Only a few studies have investigated the association between NSAIDs and VTE, and these studies have not shown consistent results (13-18). Investigating the risk for VTE associated with different exposures in women is a challenge because hemostasis in women is particularly affected by physiological changes in hormone status (e.g. pregnancy and menopausal transition)(19).

To gain fuller understanding how combinations of factors affect the risk for VTE in women, a population-based case-control study, the TEHS, was initiated by the Swedish Medical Products Agency (MPA) in collaboration with Karolinska Institutet (KI) and the Royal Institute of Technology (KTH).

This thesis illustrates the planning and performance of TEHS, together with a presentation of the initial results and their interpretation.

2.1 DEFINITIONS

Epidemiology is the study of the distribution and determinants of disease frequency in human populations. Clinical pharmacology is the study of the effects of drugs in humans. Hence, pharmacoepidemiology is the study of the use and the effect of drugs in large number of people. Put differently, pharmacoepidemiology is simply using the epidemiological toolbox in clinical pharmacology. For ethical reasons it is not possible to design an experimental study aiming primarily at studying adverse effects of an intervention, such as drug exposure. Further, serious adverse drug effects are so rare that they will not be discovered in clinical studies of ordinary size. Accordingly, adverse drug effects are best investigated through observational studies. The two major types of analytic observational study are the cohort design and the case-control design. The results from pharmacoepidemiological studies supplement information available from premarketing trials and, in contrast to spontaneous reports, can contribute to quantification of the incidence of adverse effects. Moreover, observational studies may be performed in patient groups not studied prior to marketing such as children, pregnant women or the elderly(20).
2.2 PLANNING AND PREPARING

Around the millennium the MPA decided to perform a study on risk factors for VTE in women. The underlying motive for study was the numerous spontaneous reports on VTE in women using menopausal hormone therapy (MHT) or CHC. To accomplish the study objective a project leader was appointed and a reference group formed. At that time the pharmacovigilance work at the MPA was decentralized with seven surveillance centers located in the departments of clinical pharmacology at the Swedish university hospitals. These centers also constituted a case-control study network that had been applied to identify participants in a previous case-control study of pancreatitis (21). Originally, this study was also planned to take place in the network setting. To co-finance this extensive study contact was established with pharmaceutical companies that marketed CHC and MHT products. All hospitals in Sweden were contacted to make an inventory of the number of possible cases diagnosed at each hospital and to form an impression of how the management of VTE cases was organized. Further, visits to the larger hospitals were made to provide information about the study and assign a local study coordinator. The Tax Agency was contacted to obtain regular extracts from the Swedish Population Register of randomly selected female controls. The controls were frequency-matched on birth year to the cases. Initially, Skåne, the most Southern part of Sweden, was not included in the study. Consequently controls were not sampled from that region during the first period of the study. A pilot study was performed 2002 to test the multi-center set-up for data collection used in the previous case-control study of pancreatitis. After the pilot study it was decided to establish one coordinating centre at the MPA. The coordinating center was manned with one coordinator and three research nurses who performed the telephone interviews. All data from the interviews were entered into a paper form and then manually transferred into a data base.

A detailed description of the method can be found in the methods section of this thesis.

2.3 CHOICE OF STUDY DESIGN

The case-control design refers to an observational analytic epidemiologic study in which subjects are selected based on whether they have (cases) or do not have (controls) a well-defined condition (outcome). The cases and controls are then compared regarding the proportion in each category that has the exposure or characteristic of interest. For a case-control study to provide reliable support of whether there is an association between an exposure and an outcome, comparability of cases and controls is essential. The selection of controls is crucial, in the sense that the controls must be selected to represent the population of individuals who would have been identified and included as cases if they had also developed the outcome of interest. The case-control design is particularly useful when studying multiple possible causes of a single outcome in that the same cases and controls may be used to examine several exposures. Further, the design with an initial selection of cases guarantees a
sufficient number of cases even when the outcome is rare. Hence the design makes it possible to study rare conditions, with a much smaller sample size than would be needed in a cohort study. Because the TEHS was initiated to gain a better understanding as to how combinations of risk factors affect the risk of VTE, the case-control design was deemed to be most suitable (22-23).

Measure of association

The odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular risk factor (exposure), compared to the odds of the outcome occurring in the absence of that risk factor (exposure) (24). The OR is used as the measure of association in case-control studies because in these studies investigators have a numerator (cases) but the whole study population is not available as denominator, so “true” rates and relative risks cannot be determined. In case-controls studies the OR is mostly considered a good proxy for the true relative risk in case-control studies when the outcome (disease) is rare, the “rare disease assumption” (25).

2.3.1 Sample size

To decide upon sample size we assumed a significance level of 5% (two-sided), a power of 80% and an incidence of VTE of 1/1000. By including 1500 cases and 1500 controls it would be possible to detect a relative increase in risk for VTE of at least two, for any combination of risk factors with a prevalence of at least 1.6%.

2.4 OUTCOME

2.4.1 Descriptive epidemiology of VTE

The incidence of diagnosed VTE is 100-200 per 100,000 individuals per year. VTE is rare in children and young individuals, the annual incidence, expressed in numbers per 100 000, rises exponentially with age from a rate of less than 5 in children younger than 15 years of age to 450-600 in individuals over the age of 80 years (26-27).

Approximately one third of patients with symptomatic VTE have apparent PE and two thirds have DVT. The conditions are strongly related signs of DVT have been reported in 32% of patients with PE and of these a DVT could be detected with ultrasonography in 60% of the cases (28). PE is a potentially life-threatening condition with an acute mortality of 1-2% (1, 29-31).

No consistent differences in the overall incidence of VTE between men and women have been found though some studies have described a higher incidence of first VTE in women of childbearing age. This sex difference in the incidence of VTE is presumably related to underlying hormonal exposures including pregnancy, puerperium and use of CHC or MHT (32-36).
2.4.2 Pathogenesis and clinical aspects of VTE

Venous thromboembolism—the entity

DVT and PE represent different manifestations of the same clinical entity, namely VTE. DVT is the formation of a venous clot (thrombosis) that often occurs in the deep veins of the leg, thighs or pelvis. Less common locations include veins in the arm and the mesentery or cerebral sinus. PE occurs if parts or all of a thrombus dislodge from the vessel wall (embolisation), is transported to the lung and get stuck within the pulmonary arteries (1, 14, 29, 37).

Pathogenesis

Three important underlying mechanisms for occurrence of thrombosis are stasis of the blood, changes in the vessel wall and changes in the composition of blood (triad of Virchow)(38). Venous thrombi are mainly composed of fibrin and erythrocytes mixed with variable amounts of platelets and leukocytes. They arise initially as small fibrin deposits and may grow by apposition leading to increased occlusion of the vessel. Most thrombi form in regions of slow or disturbed blood flow, but may also develop in vessels exposed to trauma or inflammation. Imbalance in the coagulation system such as a hyperactive clotting system, resistance to natural anticoagulants or an inhibited fibrinolytic system predispose to venous thrombosis (39).

Symptoms

Some VTEs may be subclinical, whereas others present as sudden PE or symptomatic DVT. If symptomatic, the most common clinical symptoms of DVT in extremities are pain, swelling and discoloration of the affected limb. Clinical examination may discover unilateral edema, warmth, tenderness and superficial venous dilation. The signs and symptoms of PE include pleuritic chest pain, sudden shortness of breath, cough and even anxiety. If massive embolism takes place PE may present itself as cardiovascular collapse causing shock or death (40-42). Major complications of VTE are the post-thrombotic syndrome and chronic pulmonary hypertension conditions that are sources of morbidity, diminished quality of life and loss of functional status (43-44).
Figure 1

Clinical symptoms of deep vein thrombosis

Diagnosis

Objective tests to confirm diagnosis of VTE is required as the clinical diagnosis of venous thromboembolism is imprecise. Furthermore, many of the clinical signs are unspecific and may be found in patients without VTE. Clinical probability scores (e.g. Wells score) are now widely used to rule out VTE in patients with a joint negative test of d-dimer and low clinical probability. Hence, clinical examinations and probability scores are used in conjunction with objective diagnostic tests for exclusion or confirmation of VTE diagnosis (45-46). The most common radiological diagnostic methods used for DVT includes venography and venous ultrasonography. Ultrasonography remains the non-invasive investigation of choice for the diagnosis of clinically suspected DVT. For PE pulmonary angiography is the reference method, but because this method is more invasive than the others it is not often used. The ventilation-perfusion scintigraphy was previously widely used, but has become more rarely performed today and instead computed tomography pulmonary angiography is the diagnostic examination of choice in patients with suspected PE (41, 47-48)
Treatment of VTE

Treatment of VTE may commence with low molecular weight heparin (LMWH) in the bare suspicion of VTE. Subcutaneous injections are often given during the diagnostic procedure to avoid unnecessary delay. When diagnosis is confirmed treatment with an oral anticoagulant (vitamin K antagonist or a so called NOAC), is usually initiated. Recommended duration of treatment is three to six month after diagnosis, but in patients with high risk of recurrence secondary prevention treatment is recommended with long-term or infinite anticoagulation treatment option (49).

2.5 RISK FACTORS FOR VTE

Numerous risk factors, both genetic and acquired, are suggested to be associated with VTE and it is beyond the scope of this thesis to describe all of them. Focus is therefore on the risk factors that were particularly addressed in the underlying case-control study or which had some bearing on defining the study population in the TEHS. Because the TEHS was designed to investigate risk factors of VTE in women with special focus on the use of CHC and HT we included women aged 18-64 years. Pregnancy and malignancy are not only well recognized risk factors for VTE, but are also known circumstances that affect the use of CHC and MHT and therefore women with these conditions were not included in the study (50).

Pregnancy and puerperium

The risk of VTE is increased during normal pregnancy, especially during the third trimester and in the postpartum period (six weeks after delivery). VTE occurs in 0.5-2.2 women per 1000 pregnancies (51-52). Pregnancy induces a hypercoagulable state
characterised by increased levels of clotting factors and acquired resistance to activated protein C. Another contributing cause of VTE during pregnancy is the hormonal influence on vascular tone and compressive effect of the growing uterus which causes decreased venous flow in the legs (53-59).

**Malignant disease**

Risk of VTE is high in patients with cancer and varies with cancer type. In a recent meta-analyses the authors reported that the overall risk of VTE among patients with cancer was estimated to be between 13 and 68 per 1,000 person-years depending on the type of cancer, stage of disease and treatment modality (60).

### 2.5.1 Acquired risk factors

*Female sex hormones, hormonal contraception and menopausal hormone therapy*

Exposure to endogenous sex hormones varies throughout a woman’s lifetime. Estrogen, essential for menstruation and reproduction is the main female sex hormone. The biologically active estrogen during the fertile period in a woman’s life is mainly 17-β-estradiol (E2) which is secreted from the granulosa cells in the ovaries. After menopause endogenous ovarian estrogen (E2) levels decrease naturally. When ovaries lose their function, estrone (E1) becomes the predominant form of estrogen in women. Estrone is formed in various peripheral tissues, but mostly within adipose tissue. Estradiol is more potent than estrone. Progesterone is produced predominantly in the ovaries (by the corpus luteum) during the second half (luteal phase) of the menstrual cycle.

**Figure 3** Schematic presentation of endogenous estradiol levels throughout a woman’s life
Combined hormonal contraception

More than 100 million women use hormonal contraception world-wide with CHC being the most common contraceptive method used (61). Because use of CHC is a risk factor for VTE numerous studies have been conducted over the past five decades to examine the relation between CHC use and risk for VTE, and a recent review reported ORs ranging from 3 to 5 (4, 62). CHCs contain two female synthetic steroid hormones, an estrogen (most often ethinylestradiol, EE) and a progestogen (most often 19-nortestosteron derivates). Ethinyl estradiol is regarded a more potent estrogen than estradiol (E2).

The increased risk of VTE was initially attributed entirely to the estrogen component. To decrease the risk of thrombosis the estrogen dose in COCs has been gradually reduced. A lowering of the estrogen dose from 100 μg to 50 μg was associated with a decreased risk of venous thrombosis and with the shift to COCs containing <50 μg, there was a further decrease in the risk of venous thrombosis (63-64). In two recently published studies estrogen doses of 20 μg led to an additional lowering of the risk (65-66).

In parallel with lowering of the estrogen dose, the chemical composition of the progestogen component has changed and CHCs containing new types of progestogens were introduced in the 1980s. In the mid-nineties several observational studies reported differences in risk of VTE in users of COC with the same amount of estrogen but different types of progestogen (67-69). The newer types of progestogens (e.g. desogestrel, gestodene, and norgestimate) were found to be associated with a greater venous thromboembolic risk than the older progestogens (e.g. levonorgestrel, lynestrenol, and norethisterone) (70-71). This finding attracted enormous attention and has been referred to as the “1995 pill scare” i.e because of intense and heated discussions in the media women terminated use not only for these newer preparations but for all types of CHC. In some countries consequences such as unplanned pregnancies and an increase of legal abortions were reported (72-74).

The impact of different types of progestogens on the risk of VTE is still debated, but most observational studies points to increased risks associated with the newer progestogens compared with levonorgestrel or norgestimate (65-66, 75). Because CHC with similar doses of estrogen but with different types of progestogen was observed to express differences in VTE risk the thrombogenicity was ascribed to the “total estrogenicity” of the product. It has been suggested that the newer types of progestogen exerts a weaker anti-estrogenic activity as compared with levonorgestrel and therefore they counteract the thrombotic effects of estrogen less efficiently (76).

Several haemostatic parameters are affected during COC use. Changes occur in levels of coagulation factors as well as in the anticoagulant and fibrinolytic system (77).
One of the changes in the anticoagulant system associated with increased risk of VTE in users of CHC is resistance to activated protein C, acquired APC resistance. (Activated protein C, APC, is a potent natural anticoagulant that acts by cleaving which inactivates the activated forms of factor V and VIII). Higher APC resistance has been demonstrated in users of all types of COC compared with non-users. Users of COC including desogestrel were more APC resistant than users of levonorgestrel containing COC (78-79).

Progestogen only contraception POC

In eight studies included in a recent meta-analysis the adjusted relative risks for a venous thromboembolic event for users of POC in comparison with non-users varied from a decreased risk of about 30% to an almost doubled risk. The summary measure for the adjusted relative risk was 1.03. A slightly higher risk was found for injectable progestin formulation with an RR of 2.67 (80). As most of the few available studies show no significant increased risks of VTE and because progestogens only have minor effects on the coagulation system POC is generally thought to have little risk for VTE (81-83). Consequently, POC is recommended for women at high risk of VTE such as hereditary thrombophilia (84) even though studies of POC and risks of VTE in high-risk women are lacking.

Menopausal hormone therapy

MHT is used to treat climacteric symptoms that are due to decreased endogenous estrogen levels. Oral MHT contains either conjugated equine estrogens or 17-β- Estradiol alone or in combinations with various progestogens. Studies have shown an approximately 2-to 3-fold increased risk of VTE in users compared with non-users (5, 85-86). The risk of VTE in association with the use of MHT seems to depend on the dose of estrogen, the type of concomitant progestogen and the way in which estrogen is administered (87). According to a meta-analysis, the transdermal route of administration seem to be the safest alternative. Differential effects on haemostatic variables may explain the differences in thrombotic risk between oral and transdermal estrogen use. For instance activation of the coagulation cascade as well as APC resistance has been shown to be increased in women who use oral estrogen to treat climacteric symptoms but not in women who use transdermal estrogen (88). The orally administered estrogen may exert a prothrombotic effect through hepatic induction related to higher concentrations of estrogens in the liver due to a hepatic “first pass” effect which is avoided when using the transdermal route (89-90).

Non-steroidal anti-inflammatory drugs NSAIDs

Safety concerning risk of thrombosis has been raised for these drugs, in particular the cyclooxygenase (COX)-2 selective inhibitors (91-93). Lately arterial and venous thrombosis have been proposed to share common risk factors and increased risk of VTE in individuals exposed to traditional risk factors for arterial thrombosis has been reported (94) Therefore, the use of NSAID use may also affect the risk of VTE.
However, only a few studies have investigated the association between NSAID use and VTE risk, and the reported findings are inconsistent (13-18).

*Immobilization and surgery*

In a meta-analysis the risk of VTE associated with immobilized (medical bedridden) patients was increased two-fold compared with patients with a normal walking (95). Immobilization in connection with long distance flights has been associated with a two-fold increased risk of VTE(96) The increased risk, in addition to stasis because of sitting cramped for long periods has been ascribed to hypoxia in the airplane cabin and dehydration. Both stasis and dehydration will occur in connection with other circumstances (e.g. immobilization that is due to hospitalization). The risk of VTE in patients undergoing general surgical procedures and with no thromboprophylaxis varies between 15-40%, but may be higher after major orthopedic surgery (97-98). The risk of VTE associated with surgical procedures varies with the type and duration of surgery and also with patient characteristics. Gynecological and orthopedic surgery are associated with high risks of VTE (99).

*Smoking*

Data on smoking and risk of DVT are inconsistent. Some studies have found increased risks of VTE associated with smoking, whereas others could not support such an association. Overall, the risk increase reported for smoking as an independent risk factor for VTE is moderate with an OR ranging from 1.2-1.4 (100-102).

*Body weight, Body Mass Index BMI*

Relative to those with normal weight (BMI <25 kg/m2), those who are overweight (BMI ≥ 25 or obese BMI ≥30) have an increased risk of venous thrombosis (103). For the obese the risk of VTE appears to be at least double that for normal weight subjects OR 2.33 (95% CI, 1.68–2.34) according to a meta-analysis (104). Several mechanisms underlying the association between overweight and the risk of VTE have been proposed. The suggested mechanisms related to obesity include general low-grade inflammation, increased prothrombotic factors, lack of exercise and venous stasis (105-106).

2.5.2 Genetic risk factors

Several inherited forms of abnormalities in the coagulation system have been linked to increased risks for VTE. Up to 50 % of all patients with a first event of VTE have a detectable inheritable thrombophilia which affects either the procoagulant or the anticoagulant pathways (107).

In the TEHS, we included information concerning the presence of seven single nucleotide polymorphisms (SNPs), which were all somehow related to hemostasis. Two of the SNPs, factor V Leiden (fVL) and the prothrombin gene mutation (PGM), were selected because they are well established risk factors for VTE. The rest were
selected because they were considered candidate genes in venous or arterial thrombotic
disease at the time when the TEHS study was launched (PAI-1, FXIII, MTHFR,
GPIIIa, eNOS).

**Single Nucleotide Polymorphisms**

When a variant nucleotide at a single nucleotide position in a DNA-sequence is found
in more than 1% in a population it is called “Single Nucleotide Polymorphisms” (SNP). A
SNP located either in the coding region of a gene or in a region regulating the
expression of the gene can have an effect on the phenotype, such as an increased risk of
disease. The product of an exchange in amino acids might be a dysfunctional protein.
Likewise, an altered nucleotide sequence in non-coding gene regulatory region might
be dysfunctional regulation of protein expression, leading to either reduced or increased
protein levels of the corresponding gene product.

**Factor V Leiden (G1691A; Arg506Gln)**

The factor V Leiden is a confirmed risk factor for VTE. The point mutation of the
coagulation factor V gene, which was discovered in 1994, causes resistance to the
natural anticoagulant protein C. The mutation replaces the amino acid arginine with
the amino acid glutamine at protein position 506 (Arg506Gln) (108). This process
affects one of the sites where APC cleaves the factor V protein and results in
impaired inactivation of factor V. FVL in the heterozygous form is the most common
form of hereditary thrombophilia in individuals of Caucasian origin. It is present in
up to 15% of the general population and in about 20% of unselected patients with a
first VTE (109-111). The mutation results in a mild chronic hypercoagulable state and
heterozygous carriers have an approximate five-fold risk of VTE. For homozygous
carriers up to 50-fold increased risk has been reported (112).

**The prothrombin gene mutation (G20210A)**

The PGM is the second most common form of hereditary thrombophilia in individuals
of Caucasian origin. The mutation was discovered in 1996 and is present in about 2-3%
of healthy individuals and in approximately 6% of unselected patients with a first-time
VTE (113). The point mutation is associated with elevated prothrombin levels and
hence an increased risk of VTE. The relative risk of VTE associated with PGM
heterozygosity is approximately two to three-fold (114).

**Factor XIII (G163T; Val34Leu)**

The fXIII-A Val34Leu polymorphism influences the structure of fibrin clots and an
inverse association of factor XIII (Val 34 Leu) with VTE has been reported, but
results overall have been inconclusive (115).

**Plasminogen activator inhibitor-1 (PAI-1; −675 4G/5G)**

Plasminogen activator inhibitor, type 1 (PAI-1), inhibits tissue plasminogen activator
(tPA). The polymorphism has been suggested to be involved in the regulation of PAI-1
synthesis resulting in increased plasma levels of PAI-1 and impaired fibrinolysis. The association between the 4G allele and the risk for venous thrombotic events has not been consistently shown (116-117).

*Methyl tetrahydrofolate reductase MTHFR (C677T; Ala222Val)*

The C677T mutation alters the protein structure resulting in reduced enzymatic activity which has been suggested to result in increased plasma homocystein levels. Conflicting results have been reported regarding the risk of VTE being associated with this SNP (118-119).

*Glycoprotein IIIa (GPIIIa; C1565T; Leu33Pro; also known as PIa1/PIa2)*

The platelet membrane glycoprotein (GP) IIIa plays a key role in platelet function, the mutation leads to altered protein structure. Enhanced platelet aggregation has been reported (120).

*Endothelial nitric oxide synthase (eNOS; G894T; Glu298Asp)*

Nitric oxide (NO) is produced mainly by the endothelial-type NO synthase (eNOS) in the vasculature. The SNP results in altered protein structure, an event known to result in reduced enzymatic activity, and hence reduced basal production of NO (121).
3 AIMS
The overall aim of this thesis was to gain additional knowledge on the risk factors associated with VTE in women
The specific aims of the individual studies were to investigate associations between VTE and:

Study I recognized risk factors by menopausal status

Study II use of CHC and POC in relation to type or dose of progestogen, duration of use and carriership of genetic hemostatic variations.

Study III use of NSAIDs by chemical subgroups and cumulative doses

The aim of the related publication (IV) was to assess the current knowledge concerning POC and risk of VTE
4 METHODS

4.1 ETHICAL PERMISSION

The study was approved by the regional research ethics committees in Stockholm (01-255, 04-469), Linköping, (01-453), Göteborg (M088-01), Uppsala (01-277) and Umeå (01-198), Sweden. Both written and oral information on the study was given. All women gave their written, informed consent to take part in the study and all participants were informed that their blood samples, and test results will be stored for 15 years and that they have the right to be informed of their test results and to demand that data should be destroyed.

4.2 STUDY DESIGN AND STUDY POPULATION IN STUDIES I-III

The TEHS is a nationwide, population-based case-control study that included 1470 cases and 1590 controls. All participants were women and recruited prospectively in Sweden from 2003 to 2009. As cases we included women between 18 and 65 years with an incident and first time DVT or PE. These women were recruited from 43 larger or medium-sized hospitals spread geographically in throughout Sweden (figure 5). Both in- and outpatients were included.

Controls were continuously and randomly selected from the Swedish population register and frequency matched to cases in a 1:1 ratio based on birth year. Index date was the date of diagnosis for women with VTE (cases) and the day of enrolment for women without VTE. Women who had previously experienced a thrombosis (venous or peripheral arterial), were or had been pregnant during the last three months before the index date or had a current malignancy (except for basal skin carcinoma) were not included. Women who have previously been diagnosed with cancer were included only if they had been free of disease and treatment for at least five years before the index date. Information on acquired risk factors for thrombosis was collected through telephone interviews with the participants. Experienced research nurses performed the interviews using a structured questionnaire. Genetic risk factors were identified by means of DNA-analysis on blood samples. Because the telephone interview was conducted in Swedish, only Swedish speaking women were included in the study.

4.2.1 Identification and selection of cases

Women with a first time DVT located in the leg or the pelvis or with PE were included in the study. The thromboembolic diagnoses were based on objective radiological findings. For women with a thrombosis of the leg or the pelvis the thrombosis was verified by means of venography or colour Doppler ultrasonography. PEs were verified by CT scans of the thorax, pulmonary angiography or perfusion- ventilation scintigraphy. To further confirm the diagnosis, treatment with anticoagulantia should have been initiated. We performed a pre-study inventory by contacting all hospitals (n=43) asking for their management of VTE At the 32 hospitals with a centralized management of VTE at Departments of Internal Medicine or Haematology, a study coordinator assigned to each department identified eligible women. At the 11 hospitals
with no centralized management of patients with VTE, the study coordinator identified potential study participants through registers of referrals kept at each Department of Radiology and Department of Clinical Physiology. For women who were identified as potential study participants a copy of the referral for radiological examination was sent to the coordinating centre. At the centre, the research nurses contacted the clinician responsible for the potential case to make sure that there were no hindrances to contact the participants. If there were no obstructions, and if the criteria for inclusion were fulfilled, the participants were temporarily registered in a database at the centre. All women eligible for inclusion then received both written (by mail) and oral (by phone) information about the study. If individuals consented to take part in the study they were registered in the database as a case. After consent the interview was scheduled and the participants were instructed to donate a blood sample at the nearest health care provider. For the blood sample a “blood sample kit” that included the bar code and test tubes was sent to the participants with instructions to bring the kit to the nearest health care provider. The samples were then sent by overnight mail to the laboratory at the MPA in Uppsala, Sweden.

4.2.2 Identification and selection of controls

Controls were continuously and randomly selected from the Swedish Population Register. The controls were frequency matched to cases according to birth year, with an aim to have a similar age distribution between cases and controls. Data on the randomly selected controls were temporarily stored in a database at the coordination centre. Selected controls were then contacted and enrolled according to a similar procedure as was employed for the cases.

4.2.3 Data collection-information on exposures

Acquired risk factors

The telephone interview included questions on previous and current diseases, surgery, fractures, medication, lifestyle factors, socioeconomics and reproductive health. Events taking place within three months before the index date were especially penetrated. For CHC and MHT lifetime exposure was mapped. To support the memory, a lifetime calendar and a catalogue with pictures of all oral contraceptives and hormones available in Sweden since 1960 were sent to the participants before the interview (appendix).

Genetic risk factors

Participants were instructed to mail the blood sample to the laboratory at the MPA in Uppsala. At the laboratory DNA was prepared from 5 to 7.5 ml of blood using QIAGEN FlexiGene DNA kit. The amount of DNA prepared varied from 100-300 ug. When the Completed DNA-preparations were transported on a regular basis to the Royal Institute of Technology in Stockholm for genotyping. Initially, target genes were amplified by PCR-technique and the genotyping was made by means of pyrosequencing (122). Pyrosequencing is a method of real-time sequencing based on the conversion of pyrophosphate groups that are released during DNA elongation into
measurable light. This process relies on the fact that the generated light is directly proportional to and reflects the nucleotides incorporated into DNA by DNA polymerase at each given moment in time. The results of the genetic analyses were entered into a database at the laboratory and transferred to the database at Karolinska Institutet.

**Figure 4 Pyrosequencing output Factor V Leiden (G1691A)**

C/C

C/T

T/T

**4.2.4 Statistical analyses**

In studies I-III we used unconditional logistic regression models to calculate odds ratios (ORs) with their corresponding 95% confidence intervals (CIs) as the measure of association between the studied exposures and risk of VTE.

In study I statistical analyses were performed in two models. In model 1 adjustment was made for age. In model 2 adjustments were made for age BMI, smoking, use of hormones, exercise, surgery, cast, bedrest and carriernesship of the prothrombin gene mutation and/or factor V Leiden.

In study II analyses of risks of VTE associated with use of CHC or POC adjustments were made for smoking, BMI and immobilization. In this study we stratified analyses according to type of progestogen. Analyses were not adjusted for age as this was not possible due to lack of either exposed cases or controls. We performed crude analyses for VTE risk associated with CHC and POC use according to carriernesship (homozygotes and heterozygotes) of studied SNPs.

In study III associations between NSAID exposure and VTE were tested in three models. In model 1, we adjusted for the matching factor age, and in model 2, we also adjusted for immobilization, chronic disease, use of CHC, HT, smoking and body mass index (BMI). Finally, in model 3, we included additional information concerning carriernesship of the prothrombin gene mutation (PGM) and/or
factor V Leiden (fVL). The statistical analyses were performed using STATA 10.0 software (Collage Station, TX, USA) For studies I-III the selection of independent (explanatory) variables included in multivariate analyses was made a priori on the basis that they all posed potential risk factors for VTE.

**Figure 5 Flow-chart for inclusion in Thrombo-Embolism-Hormone-Study (TEHS)**
5 RESULTS

This presentation is a summary of the results. For a complete presentation, the reader is invited to visit the result section in each study presented in full at the end of this thesis.

5.1 PAPER I

Risk factors for venous thromboembolism in pre- and postmenopausal women

Of the 1433 women with VTE and 1402 control subjects a total of 1441 women were classified as premenopausal and 1100 women were classified as postmenopausal, 294 women could not be classified by menopausal status. In adjusted analyses 218 observations were lost due to missing values. The acquired, transient risk factors were stronger than the genetic ones. In adjusted analyses recent plaster cast were associated with high risks of VTE in both pre- and postmenopausal women. Surgery increased the risk for VTE particularly in postmenopausal women. In both premenopausal and postmenopausal women surgery and cast in combination was associated with very high risks of VTE Of the cases who reported surgery 160 (51%) reported that they had received thromboprophylaxis. (Median 8 days, min=1 day and max=42 days). The corresponding figures for plaster cast were 118 (32%) with a median length of treatment of nine days, (min=1 day and max=30 days).

Table 1

<table>
<thead>
<tr>
<th>Risks of VTE among 1441 premenopausal women</th>
<th>Risks of VTE among 1100 postmenopausal women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bedrest/minor trauma</strong></td>
<td><strong>Bedrest/minor trauma</strong></td>
</tr>
<tr>
<td>1.4 (1.1-2.0)</td>
<td>1.7 (1.2-2.4)</td>
</tr>
<tr>
<td><strong>Surgery only</strong></td>
<td><strong>Surgery only</strong></td>
</tr>
<tr>
<td>5.9 (3.0-11.6)</td>
<td>10.6 (4.5-25.2)</td>
</tr>
<tr>
<td><strong>Cast only</strong></td>
<td><strong>Cast only</strong></td>
</tr>
<tr>
<td>28.6 (11.8-68.9)</td>
<td>13.3 (5.7-31.0)</td>
</tr>
<tr>
<td><strong>Surgery and cast</strong></td>
<td><strong>Surgery and cast</strong></td>
</tr>
<tr>
<td>53.3 (16.3-174.4)</td>
<td>54.1 (16.6-176.2)</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td><strong>Smoking</strong></td>
</tr>
<tr>
<td>1.4 (1.0-1.9)</td>
<td>1.3 (0.9-1.9)</td>
</tr>
<tr>
<td><strong>Hormone therapy</strong></td>
<td><strong>Hormone therapy</strong></td>
</tr>
<tr>
<td>3.7 (1.9-7.5)</td>
<td>2.2 (1.5-3.2)</td>
</tr>
<tr>
<td><strong>CHC</strong></td>
<td><strong>CHC</strong></td>
</tr>
<tr>
<td>8.5 (5.8-12.4)</td>
<td>-</td>
</tr>
<tr>
<td><strong>POC</strong></td>
<td><strong>POC</strong></td>
</tr>
<tr>
<td>1.0 (0.6-1.6)</td>
<td>0.8 (0.2-3.7)</td>
</tr>
<tr>
<td>The prothrombin mutation</td>
<td>The prothrombin mutation</td>
</tr>
<tr>
<td>3.3 (1.6-6.7)</td>
<td>1.9 (0.9-4.0)</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>Factor V Leiden</td>
</tr>
<tr>
<td>3.3 (2.3-4.8)</td>
<td>3.8 (2.4-6.0)</td>
</tr>
</tbody>
</table>

We found that risks associated with recognized acquired and genetic risk factors for VTE generally were of similar magnitude in pre- and postmenopausal women. The acquired, transient risk factors were stronger than the genetic ones and the combination of surgery and cast yielded a 50-fold increased risk for VTE in both pre- and postmenopausal women. The fact that the highest risks were found among the acquired, transient risk factors is important to acknowledge as these factors in contrast to the more persistent ones at least in theory are avoidable.
5.2 PAPER II

Hormonal contraception and the impact of progestogens, duration of use and hemostatic mutations on risks of venous thromboembolism: a case-control study

In the analyses we included 1850 (948 cases, 902 controls) women 18-54 years of age. Current use of CHC was reported by 311 cases (32.8%) and 107 (11.9%) controls. For POC the corresponding figures were 145 (15.3%) and 177 (19.6%) respectively. The mean age was 39.4 years for cases and 40.0 years for controls. Current use of CHC was associated with a five-fold increased risk of VTE, adjusted OR 5.3, (95% CI 4.0-6.9). In adjusted analyses combinations with desogestrel had the highest risk (OR 11.4, 95% CI 6.0-22.0) followed by drospirenone (OR 8.4, 95% CI 4.2-17.0), etonogestrel (OR 6.3, 95% CI 1.7-24.0), norgestimate (OR 4.7, 95% CI 1.2-18.0), levonorgestrel (OR 4.4, 95% CI 3.0-6.4) and norethisterone (OR 2.0, 95% CI 1.1-3.8). Risks for VTE associated with use of combined hormonal contraception (CHC) by type of progestogen with users of levonorgestrel as reference category is shown in the forest plot below (figure 7). Carrier of factor V Leiden or PGM in women using CHC was associated with an approximately 20-fold risk increase of VTE when compared with non-carriers not using CHC.

Figure 7

Forest plot showing OR for VTE associated with use of combined hormonal contraception (CHC) by type of progestogen using levonorgestrel as reference category
Current use of POC was not associated with increased risks of VTE (adjusted OR 0.9, 95% CI 0.7-1.2). In stratified analyses by dose, the “very low dose” category entailed a decreased risk (a OR 0.6, 95% CI 0.4-1.0), “low and medium doses” were not associated with VTE risk, whereas current users of “high dose” had an increased risk (adjusted OR 2.2, 95% CI 1.3-4.0). When using “extra low dose” progestogen users as reference category we found that both “low and medium dose” POCs were associated with slightly, but imprecise increased risks (Figure 8). Women who were users of POC and carriers of FVL had a five-fold risk of VTE compared to non-carriers with no use of POC.

**Figure 8**
*Forest plot showing OR for VTE associated with use of progestogen only contraception (POC) by type of progestogen using of users of “extra low dose” as reference category*
5.3 PAPER III

Non-steroidal anti-inflammatory drugs and venous thromboembolism in women

Only women with complete information on all variables were included in the final analyses (1196 cases and 1248 controls). Cases were more obese, more often smokers and users of CHC or HT than controls. The cases had also to a greater extent been exposed to recent surgery, trauma, plaster cast or bedrest. Chronic inflammatory disease, asthma, COPD, renal disease and diabetes were also more common among cases than among controls. In the group propionic acid derivatives, most women used ibuprofen (92%), and of the women who used acetic acid derivatives, almost all used diclofenac (97%). Among coxib users, celecoxib (53%) was most the commonly used substance followed by rofecoxib (29%), etoricoxib (15%) and valdecoxib (3%). In adjusted analyses (model 2) overall use of NSAIDs was not associated with increased risks of VTE (table 2).

The adjusted OR (model 2) was 0.9 for propionic acid derivatives (95% CI 0.7–1.1), OR 1.18 for acetic acid derivatives (95% CI 0.8–1.7) and OR 1.8 for coxibs (95% CI 0.7–4.3). We found no dose–effect relationship for users of propionic acid derivatives. For users of acetic acid derivatives and coxibs the ORs increased by cumulative dose suggesting a dose–effect relationship for these drugs (table 3).

Table 2
OR and 95% confidence intervals (95% CIs) of VTE among 2444 women 18-64 years of age associated with use of NSAID Only women with complete information on all variables were included in the analyses

<table>
<thead>
<tr>
<th>All NSAIDS</th>
<th>Cases n=1196 (%)</th>
<th>Controls n= 1248 (%)</th>
<th>Model 1* OR (95% CI)</th>
<th>Model 2** OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No use</td>
<td>740 (61.9)</td>
<td>797 (63.9)</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Any use</td>
<td>456 (38.1)</td>
<td>451 (36.1)</td>
<td>1.0 (0.9-1.3)</td>
<td>1.0 (0.8-1.2)</td>
</tr>
</tbody>
</table>

*Adjusted for age. **Adjusted for age, use of menopausal hormone therapy, combined hormonal contraceptives or other NSAIDs in the table, Body Mass Index, smoking, chronic disease, immobilization.
Table 3
ORs and 95% confidence intervals (95% CIs) of a first episode of VTE among 2444 women 18–64 years of age associated with type of NSAID and the cumulative dose. Only women with complete information on all variables were included in the analyses.

<table>
<thead>
<tr>
<th></th>
<th>Cases n=1196 (%)</th>
<th>Controls n= 1248 (%)</th>
<th>Model 1* OR (95% CI)</th>
<th>Model 2** OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Propionic acid derivatives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No use</td>
<td>828 (69.2)</td>
<td>849 (68.0)</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Cumulative dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>20 (1.7)</td>
<td>28 (2.2)</td>
<td>0.6 (0.4-1.1)</td>
<td>0.8 (0.4-1.6)</td>
</tr>
<tr>
<td>&gt;25&lt;sup&gt;th&lt;/sup&gt;≤75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>69 (5.8)</td>
<td>47 (3.8)</td>
<td>1.5 (1.0-2.2)</td>
<td>1.2 (0.7-1.8)</td>
</tr>
<tr>
<td>&gt;75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>26 (2.2)</td>
<td>22 (1.8)</td>
<td>1.1 (0.7-2.0)</td>
<td>0.7 (0.3-1.4)</td>
</tr>
<tr>
<td>Unspecified dose</td>
<td>253 (21.2)</td>
<td>302 (24.2)</td>
<td>0.8 (0.7-1.0)</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td><strong>Acetic acid derivatives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No use</td>
<td>1086 (90.8)</td>
<td>1172 (93.9)</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Cumulative dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>11 (0.9)</td>
<td>13 (1.0)</td>
<td>1.2 (0.5-2.5)</td>
<td>0.7 (0.3-1.9)</td>
</tr>
<tr>
<td>&gt;25&lt;sup&gt;th&lt;/sup&gt;≤75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>48 (4.0)</td>
<td>21 (1.7)</td>
<td>2.4 (1.5-4.0)</td>
<td>1.7 (0.9-3.2)</td>
</tr>
<tr>
<td>&gt;75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>30 (2.5)</td>
<td>13 (1.0)</td>
<td>3.0 (1.6-5.8)</td>
<td>2.0 (0.9-4.2)</td>
</tr>
<tr>
<td>Unspecified dose</td>
<td>21 (1.8)</td>
<td>29 (2.3)</td>
<td>0.8 (0.5-1.4)</td>
<td>0.7 (0.4-1.4)</td>
</tr>
<tr>
<td><strong>Coxibs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No use</td>
<td>1174 (98.2)</td>
<td>1240 (99.4)</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Cumulative dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>3 (0.3)</td>
<td>2 (0.15)</td>
<td>1.5 (0.3-9.2)</td>
<td>0.4 (0.0-3.6)</td>
</tr>
<tr>
<td>&gt;25&lt;sup&gt;th&lt;/sup&gt;≤75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>8 (0.7)</td>
<td>2 (0.15)</td>
<td>4.9 (1.1-22.8)</td>
<td>2.3 (0.5-11.5)</td>
</tr>
<tr>
<td>&gt;75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>10 (0.8)</td>
<td>2 (0.15)</td>
<td>5.5 (1.2-25.0)</td>
<td>3.6 (0.7-17.4)</td>
</tr>
<tr>
<td>Unspecified dose</td>
<td>1 (0.1)</td>
<td>2 (0.15)</td>
<td>0.4 (0.0-3.4)</td>
<td>0.6 (0.0-8.0)</td>
</tr>
</tbody>
</table>

Cumulative dose was defined as the total intake of daily doses (DDDs) within 90 days before diagnosis for cases and index date for controls. Quartiles were based on the distribution of the cumulative doses among the controls.*Adjusted for age. **Adjusted for age, use of menopausal hormone therapy, combined hormonal contraceptives or other NSAIDs in the table, Body Mass Index, smoking, chronic disease, immobilization
6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Validity
External validity has to do with the generalizability of the study results. Internal validity has to do with the accuracy of the results. In epidemiological studies, the internal validity can be affected by two types of error, systematic error (bias) and random error. Bias is often classified into three categories: selection bias, information bias, and confounding(22-23).

Selection bias
Selection bias occurs when the association between exposure and outcome differs between those who participate and those who do not participate in a study. For instance, if doctors are more prone to investigate VTE in young women with a swollen leg who use CHC than in women who do not because CHC is a well known risk factor for VTE, then selection bias could have occurred. Also, if women with VTE and using CHC to a higher degree accepted to participate in TEHS than women who did not use VTE. It should, however, be noted that the recruitment procedure after identification was similar for cases and controls and it was not apparent for the participants that the association between CHC, MHT, or NSAIDs and VTE should be studied, which should minimize the risk of selection bias. Yet selection bias cannot be ruled out considering the lower participation rate in controls than in cases. In TEHS, the reported use of CHCs and MHT in the controls were in line with the use of these in the general population as recorded in the Swedish Prescribed Drug Register. This finding runs counter to a selection by hormonal exposure.

Information bias
Information bias occurs when non-comparable information is obtained from different study groups. Interviewer bias refers to the situation in which an interviewer interprets the information differently; for instance if the interviewer knows about the association between CHC and risk of VTE he or she may ask about that exposure in a different way for cases than for controls. To avoid interviewer bias the interviewer should preferably be blinded to the outcome status of the study participant. This approach was not possible in TEHS as the index date for cases was the date for diagnosis, and the questionnaire included questions regarding the diagnosis. To minimize interviewer bias the interviewers in the TEHS used a structured questionnaire from which all questions were read during the interview. Moreover, interviewers were experienced research nurses well aware of these pitfalls. Recall bias pertains to the situation when study subjects themselves report exposures or characteristics differently, depending on their exposure or outcome status. In the TEHS, exposure ascertainment was made retrospectively and women with VTE might have remembered exposure to CHC better.
than women without VTE especially if they are aware of the association. To minimize recall bias all women in TEHS received a catalogue with pictures of different hormonal contraceptives and lifetime calendar to fill in before the interview. For other exposures we focused on the three month period before the index date to diminish the risk of recall bias.

Misclassification

Inaccuracies in the collection of data are inevitable and can lead to misclassification of subjects for either exposure or outcome. The misclassification can be either differential or non-differential depending on whether the inaccurate information is equally distributed in cases and controls. Non-differential misclassification will minimize the differences between cases and controls resulting in an underestimate of the true association. Differential misclassification can either enhance or diminish an existing effect or create a non-existing effect (22-23).

Misclassification of exposure in TEHS

Misclassification of exposure is unavoidable in the retrospective data collection on exposure because of the inherent risk of recall bias. Recall and interview bias predominantly affects exposures with a known association with the outcome. To minimize this risk all women received a catalogue and a lifetime calendar to enhance remembering the use of CHC and MHT. For many of the other exposures in the interview the association with VTE was not apparent and any existing misclassification is therefore most likely non-differential (random error) and such error will, if anything, reduce the association and bias the results towards the null. Misclassification may also occur when creating variables or defining exposure for statistical analyses. In study I we defined menopausal status using an algorithm for women who did not label themselves as postmenopausal. This type of procedure may result in misclassification. To evaluate the effect of potential misclassification a sensitivity analysis was performed using the cut-off age of 50 years for menopause instead of 55 as used in the main analyses. The lower cut-off to define menopause only changed the risk estimates slightly. In study II current use of CHC was categorized according to type of progestogen. As it has been shown that interview data on contraceptive use was in good agreement with the information obtained from pharmacy records we believe that the risk of recall bias on exposure to CHC was a minor problem (123). In study III we defined NSAID-use according to ATC-categories and dose, and misclassification may have occurred due to difficulties in remembering type of substance and dose. To minimize errors use of reported drugs were penetrated rigorously.

Misclassification of outcome in the TEHS

Misclassification of outcome for cases is not likely as all diagnoses were verified by an objective radiologic examination and required initiated anticoagulant treatment. The validity for diagnosing VTE with Doppler, venography and CT is high. For both cases
and controls the risk of a previous silent thrombosis cannot be excluded, and even though previous thrombosis increases the risk of recurrence this knowledge refers to VTE events that have been symptomatic and hence diagnosed. We do not know whether this applies to asymptomatic VTE. If so, the risk of recurrence was low in this data and thus potential misclassification will not have any major effect on the results.

Confounding

The term confounding refers to a situation in which a measure of the effect of an exposure is distorted because of a non-causal association between a given exposure and other variables that affect the outcome. It occurs when the effect of the studied exposure is mixed with the effect of another known, or unknown variable. To be counted as a confounder a variable must fulfill three criteria as 1) must be associated with the studied exposure, 2) must be a risk factor for the outcome under study and 3) it cannot be an intermediate factor in the pathway between exposure and outcome. Identified confounders can be controlled in the design of the study or during the analyses stage, or both (22-23).

In the TEHS women were matched on the confounding factor age and we adjusted for potential confounders in the statistical analyses. In studies of associations it is important to take the temporal factor in consideration, i.e. the exposure has to precede the disease. Prothopatic bias refers to the situation in which the initial symptoms of the studied outcome constitutes the reason for the initiation of the pharmacological treatment that is the exposure of interest. In Study III we cannot exclude the risk of women starting use of NSAID because of early symptoms of VTE, to minimize this risk we performed sensitivity analyses in which we assessed exposure during the one month before the index date instead of the default three-month period.

Matching

In TEHS controls were frequency-matched for birth year to the cases. Thus the frequency of the confounder (age) is equal in both groups. Consequently, matching on age was not done per individual, but the overall distribution of age was made equal among cases and controls. We used a 1:1 match, which provides the most statistically efficient design, when the efforts to obtain information from cases and controls are of similar magnitude. As age was used for matching the effect of age on risk of VTE could not be studied(124)

Ethical considerations

Identifying potential cases by means of their radiological referral implies that we knew of their condition before we had the possibility to contact the women and ask for their consent to participate. We considered the benefit of this study, designed to identify women at risk of VTE, was larger than the potential harm caused by us having knowledge of the diagnosis. All women who denied participation were deleted from our data. The case-control design also involves ethical considerations as the participants comprising the control group are free of the studied outcome. Analyzing genetic risk
factors for the outcome in the control group may cause more harm than good and therefore the risk-benefit of such analyses should be thoroughly evaluated. Because two of the SNPs analyzed in TEHS were known risk factors for VTE, we believe that both the controls and the cases who gained knowledge of the presence of this genetic predisposition are better prepared to avoid a future VTE by taking preventative measures. In addition, participants were informed that those who tested positive for the homozygous form of fVL would be informed of their carriership and that contact with a medical specialist for further information of risk of thrombosis would be arranged.

6.2 GENERAL DISCUSSION

The role of epidemiological studies when assessing drugs is sometimes questioned. However, an RCT is not feasible in some situations for ethical reasons and also observational designs are better suited to study actual use of drugs. Naturally epidemiological studies must be well designed, cautiously performed and the results interpreted and reported with reference to potential limitations (125). When these requirements are met, the epidemiological studies play an important role in the understanding of the benefits and risks with various treatments. When we designed and performed the TEHS our ambition was to comply with these standards.

Communication of risk in the mass media is important particularly regarding the correct interpretation of a relative risk in order to avoid unnecessary anxiety. It is obvious that the results of a study, which relate to public health and affect many persons may have a large impact on both current and future treatment. An example is the “pill scare”, which has appeared several times during the last decades, when new reports on risks with CHC have been published. The “pill scare” has affected both the public and the profession and for the profession the controversy has mainly concerned whether the increased risk of VTE associated with CHC containing desogestrel and drospirenone as compared to levonorgestrel found in several case-control studies is valid. Also, it has been discussed whether the finding of increased risks for VTE with the newer CHCs is due to poor design and attrition of susceptibles. In the TEHS we adjusted for individual confounding factors and controls were randomly selected from the same population as the cases. In the analyses according to type of progestogen we did not take duration of use into account. As desogestrel had been on the market for 10 years at the time when TEHS started and there were already reports as early as in 2002 on increased risks with drospirenone, we believe that the potential prescription bias towards prescribing the newer CHCs to risk patients was not an issue in TEHS.

In study I the highest risks for VTE were found for the acquired, transient risk factors. This is an important finding as these factors in contrast to the genetic risk factors are theoretically avoidable. This observation raises the question of whether thromboprophylactic routines are inadequate to reduce occurrence of VTE. Potential reasons for insufficient thromboprophylaxis are difficult to identify, but during the past decade, hospital stays have been shortened and more surgical procedures are performed in outpatients wards. Though, these changes with more out-patient services might
increase mobilization after a hospital stay, they may also reduce the possibility of adequate thromboprophylaxis

Women seem more prone to adverse drug reactions (ADRs) than men(126-127). The reasons for this are several, one of which may be gender related differences in drug exposure. For instance, some drugs have a greater bioavailability in women for a given dose of a drug, for other substances, the response may be more pronounced in women despite equal drug plasma concentrations(128).

Beside pharmacokinetic and pharmacodynamic parameters, differences in perception and reporting of ADRs may differ between men and women and thereby influence these data(129-130). Another side is the use of gender-specific treatments which could also affect the occurrence of adverse effects. NSAIDs are more frequently prescribed to women than to men and might also due to their approval for dysmenorrhea be used as OTC by more women than men and also more frequently. Accordingly, it is of great importance to assess the potential for ADRs for these drugs in women. Gender-specific treatment is certainly valid for CHC and MHT. Moreover, most users of hormonal contraception are young, healthy women taking these compounds not to treat disease, but to prevent unwanted pregnancies. It is therefore of great importance to continuously acquire new knowledge to make use of hormonal birth control as safe as can be.
7 CONCLUSIONS

- Menopausal status has only minor influence on the risk levels in association with recognized risk factors for VTE.

- Acquired, transient risk factors such as surgery and plaster cast are associated with a 50-fold increased risk of VTE in women. This is important as these factors in at least in theory are avoidable.

- The increased risk of VTE associated with use of CHC varies according to type of progestogen included, even after adjustment for individual factors such as smoking and BMI. Combinations desogestrel and drospirenone confer the highest risk of VTE, whereas preparations with levonorgestrel or norethisterone have lower risks.

- Except for high-dose preparations, POC seems to be a safer alternative to CHC, with no increased risks for VTE.

- We found no increased risks of VTE in association with use of NSAIDs. Users of high cumulative doses of acetic acid derivatives and coxibs had the highest risks, suggesting a relationship with cyclooxygenase selectivity and dose.

- There seems to be no risk of VTE associated with use of propionic acid derivatives (ibuprofen and naproxen) among young and middle-aged women.
8 FUTURE PERSPECTIVES

Future studies should focus on use of POC. It seems as the safest contraceptive method for women, but the number of studies is still limited, especially in women with other risk factors such as thrombophilia or previous VTE. POC is not as well studied as COC considering other potential adverse effects and this too needs to be investigated.

More studies on the association between NSAIDs and VTE are warranted as results so far are inconclusive. The overall risk as well as potential differences in risk between types and doses needs to be elucidated.

VTE is multicausal and in this thesis we have explored the risk for VTE in analyses adjusted for other risk factors as potential confounders. COC use among carriers of factor V Leiden is associated with high risk of VTE. It would also be of interest to further investigate the risk of VTE in individuals with other combination of risk factors to see whether some combinations interact, additively or multiplicatively and thus are associated with a higher risk for VTE than others.

Patterns of drug use and comorbidity may differ between individuals who get prescribed drugs and those who use OTC drugs. In the era of register based research in pharmacoepidemiology it is of importance to take this into consideration when designing future studies.

Future studies should also focus on developing contraceptives for men.
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10 APPENDIX

TROMBOSSTUDIEN

Livstidskalender

Livstidskalender och bildkatalog som skickades hem till studiedeltagarna
Livstidskalender och bildkatalog

I telefonintervjun kommer vi att fråga om händelser som kan ligga långt tillbaka i tiden och vara svåra att komma ihåg om man inte är förberedd. Till exempel vill vi veta när Du fick din första menstruation. Vi kommer också att fråga om eventuella graviditeter, sjukhusvistelser (benbrott och operationer), tidigare p-piller användning och behandling med hormoner vid klimakteriebesvär.


Om Du har fött barn föreslår vi att Du i kalendern noterar barnets/barnens födelsevikt och i vilken graviditetsvecka eller månad det/de föddes.

De flesta frågor Du kommer att få besvara rör Dig själv. Eftersom det finns en ärftlig benägenhet att få blodpropp kommer vi också att ställa några frågor om Dina närmaste släktingar. Vi vill således gärna veta om Dina föräldrar och syskon haft blodpropp eller andra hjärt- eller kärlsjukdomar och om Dina mor- och farsföräldrar eller föräldrars syskon haft blodpropp. Om Du inte redan vet detta har Du kanske möjlighet att ta reda på det före intervjun.

Här nedan visas ett exempel på hur man kan fylla i livstidkalendern

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**P-piller**

Anovlar och Anovlar Mite, 1964-1982


Desolett, 1988-

Follimin, 1976-

Follinett, 1972-

Follinyl, 1964-1981

Lyndiol and Lyndiol Mite, 1964-1972
Menokvens, 1964-1972

Neo-Delpregnin, 1971-1977

Mercilon, 1999-

Neovlar 21 och 28, 1972-1982

Noracyclin N, 1964-1972

Orthonett Novum, 1981-

Ovisec, 1964-1998

Ovulen, 1964-1972

Piloval, 1964-1985

Primovlar 21, 1964-1982

Restovar, 1982-

Sequens, 1964-1972

Synfase, 1987-

Trimiron 1999-
Trionetta, 1982-

Trinordiol, 1982-

Trinordiol 28, 1985-

Trinovum, 1987-

Trinovum 28, 1987-

Yasmin, 2001-
Minipiller

Exlutena, 1975-

Follistrel, 1975-

Mini-Pe, 1972-
Hormonmediciner

**Tabletter med östrogen**

- **Activelle**, 1999-
- **Cyclabil**, 1977-
- **Etivex**, 1964-1992
- **Femanest**, 1997-
- **Femasekvens**, 1997-
- **Femanor**, 1997-
- **Indivina**, 2000-
- **Kliogest**, 1984-
Linoral, 1964-1988

Oestriol NM Pharma, 2000-

Premarina, 1975-

Livial, 2000-

Ovesterin, 1972-

Premelle, 1996-
Progynon, 1972-

Promarit, 1964-1972

Triovex, 1964-1972

Trisekvens, 1986-

Trivina, 1994-

Vallestril, 1964-1972
Plåster

Estalis, 1999-

Estracomb, 1993-

Estraderm, 1988-

Evorel, 1994-

Femseven, 1999-

Menorest, 1996-
**Lokalbehandling**

Dienoestol, 1956-2000  
Ovesterin, 1983-  
Vagifem, 1994-
Gulkroppshormoner

Gestapuran, 1972-

Primolut-Nor, 1972-

Provera, 1987-
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