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VENOUS THROMBOEMBOLISM IN WOMEN

AN ASSESSMENT OF HORMONAL, GENETIC AND OTHER RISK FACTORS

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Front cover pictures “Beata” one of the Lisa Larson (f.1931) stoneware figures in the collection ABC-Flickor (ABC Girls). Photo by Emrik Meshesha

”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 (OR_{adj})=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 (OR_{adj}=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 (OR_{adj}=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 (OR_{adj}=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

I. Bergendal A, Bremme K, Hedenmalm K, Lärfars G, Odeberg J, Persson I, Sundström A, Kieler H. *Risk factors for venous thromboembolism in pre-and postmenopausal women* Thromb Res. 2012 Oct;130(4):596-601

II. Bergendal A, Persson I, Odeberg J, Sundström A, Holmström M, Schulman S, Björgell O, Kieler H. *Hormonal contraception and the impact of progestogens, duration of use and hemostatic mutations on risks of venous thromboembolism: a case-control* study
In manuscript

III. Bergendal A, Adami J, Bahmanyar S, Hedenmalm K, Lärfars G, Persson I, Sundström A, Kieler H. *Non-steroidal anti-inflammatory drugs and venous thromboembolism in women* Pharmacoepidemiol Drug Saf. 2013 Mar 19.

IV. Related publication

Bergendal A, Odlind V, Persson I, Kieler H. *Limited knowledge on progestogen-only contraception and risk of venous thromboembolism.* Acta Obstet Gynecol Scand. 2009;88(3):261-6.

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LIST OF ABBREVIATIONS

APC	Activated protein C
BMI	Body mass index
CHC	Combined hormonal contraception
COC	Combined oral contraceptives
COPD	Chronic obstructive pulmonary disease
DVT	Deep vein thrombosis
fVL	Factor V Leiden
MHT	Menopausal hormone therapy
NOAC	Novel oral anticoagulants
NSAID	Non-steroidal anti-inflammatory drugs
OC	Oral contraceptives
PE	Pulmonary embolism
PGM	Prothrombin gene mutation
POC	Progestogen-only contraception
POP	Progestogen-only pill
SNP	Single nucleotide polymorphism
VTE	Venous thromboembolism

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).

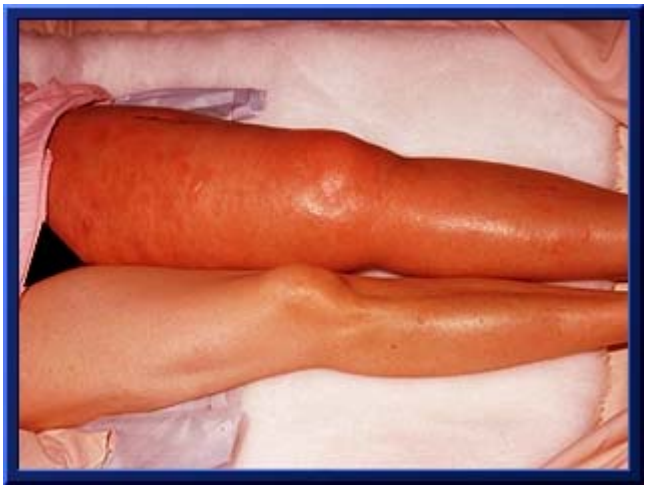
Pathogenesis

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).

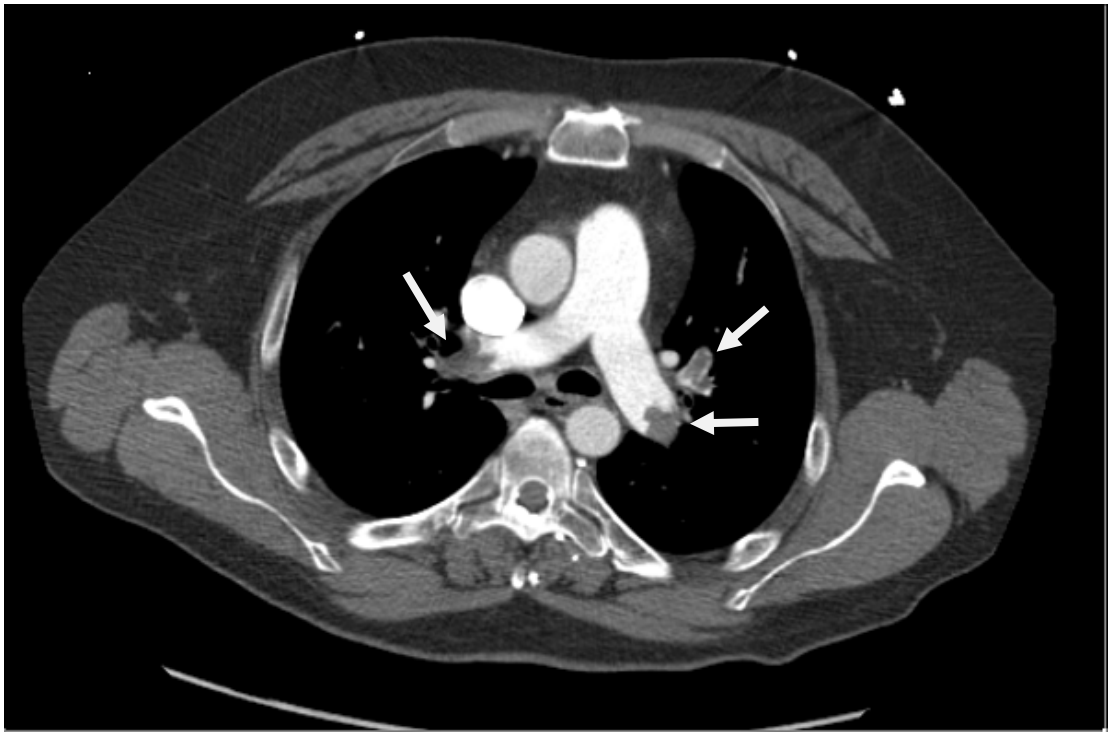
Figure 1
Clinical symptoms of deep vein thrombosis



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 conjuction 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 angiograpy is the diagnostic examination of choice in patients with suspected PE (41, 47-48)

Figure 2 Pulmonary CT angiographic image showing PE in central pulmonary arteries



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 aged18-64 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

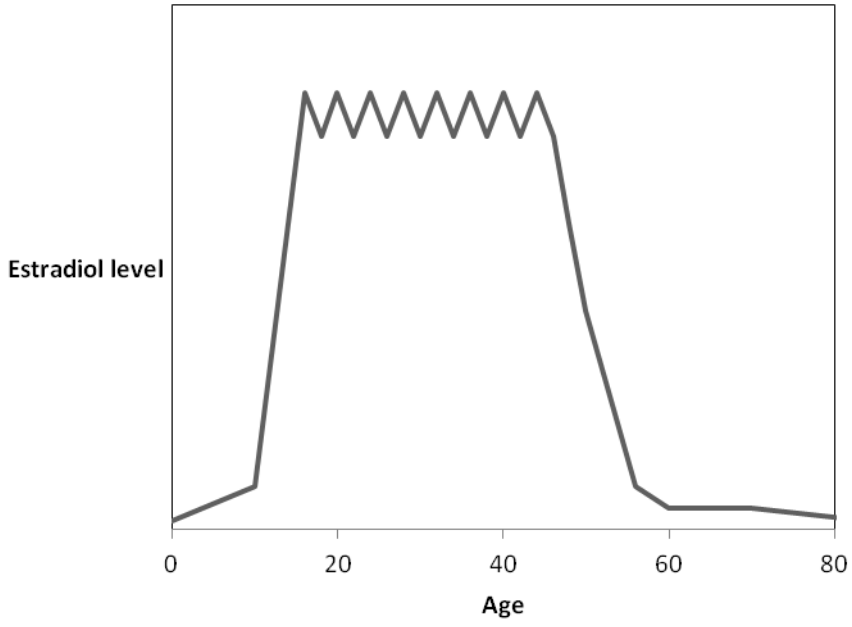
Risk of VTE is high in patients with cancer and varies with cancer type. In a recent meta-analyses the authors reported that the overall risk of VTE among patients with cancer was estimated to be between 13and 68 per 1,000 person-years depending on the type of cancer, stage of disease and treatment modality (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 womans 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 3to5 (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 ug to 50 ug was associated with a decreased risk of venous thrombosis and with the shift to COCs containing <50 ug μg, there was a further decrease in the risk of venous thrombosis (63-64). In two recently published studies estrogen doses of 20 ug 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 thrombogenecity 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 othopedic 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 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 PlA1/PlA2)

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

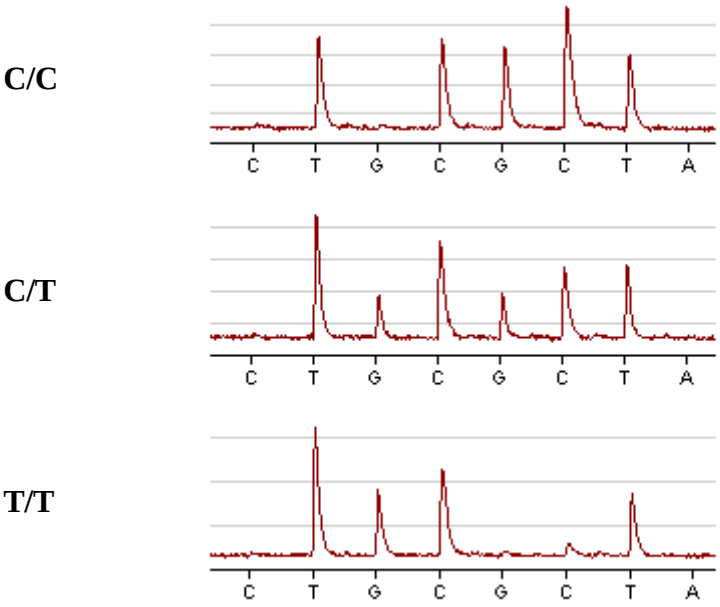
The telephone interview included questions on previous and current diseases, surgery, fractures, medication, lifestyle factors, socioeconomics and reproductive health. Events taking place within three months before the index date were especially penetrated. For CHC and MHT lifetime exposure was mapped. 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)*



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.

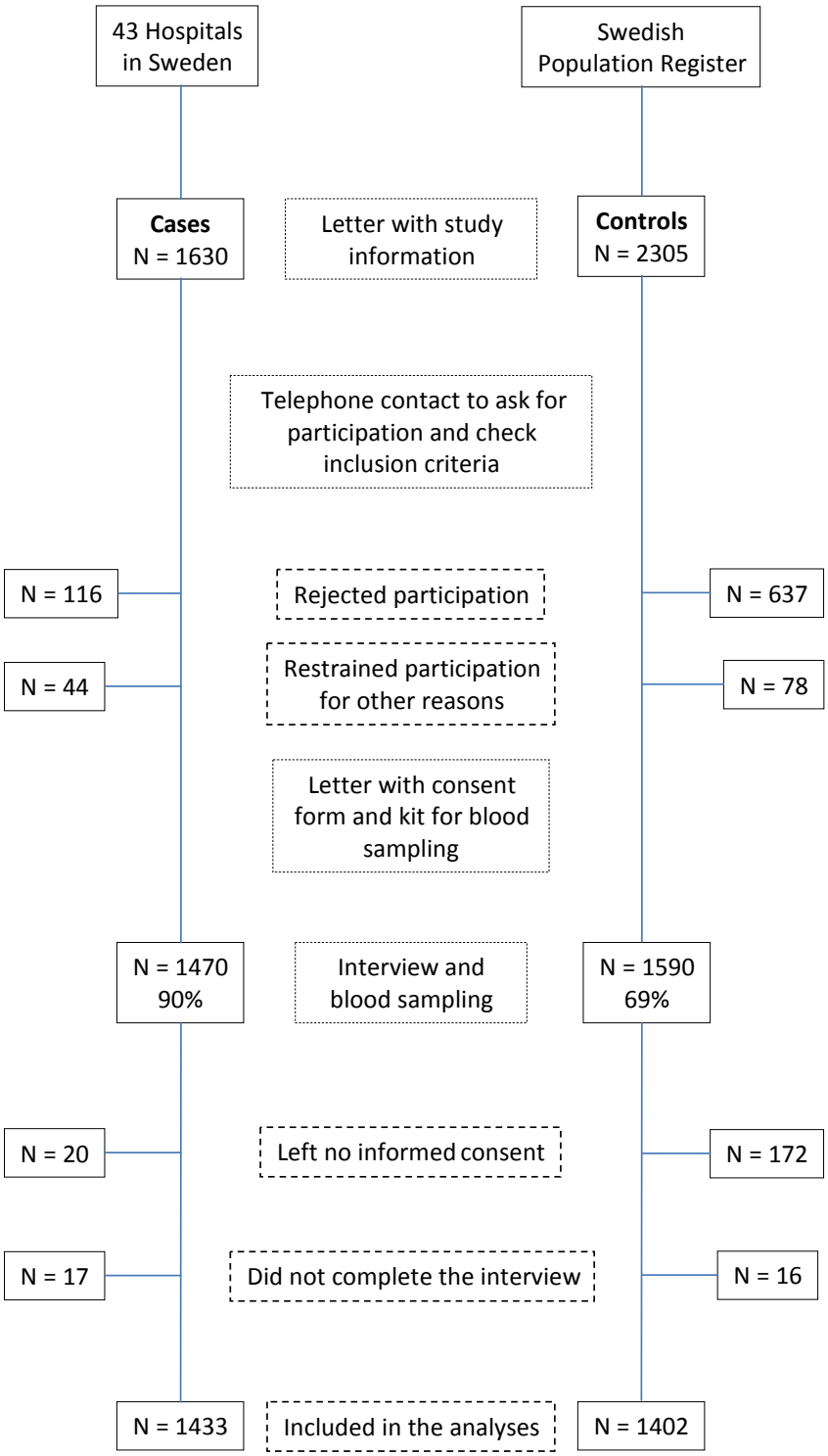
In study I statistical analyses were performed in two models. In model 1 adjustment was made for age. In model 2 adjustments were made for age BMI, smoking, use of hormones, exercise, surgery, cast, bedrest and carriership of the prothrombin gene mutation and/or factor V Leiden.

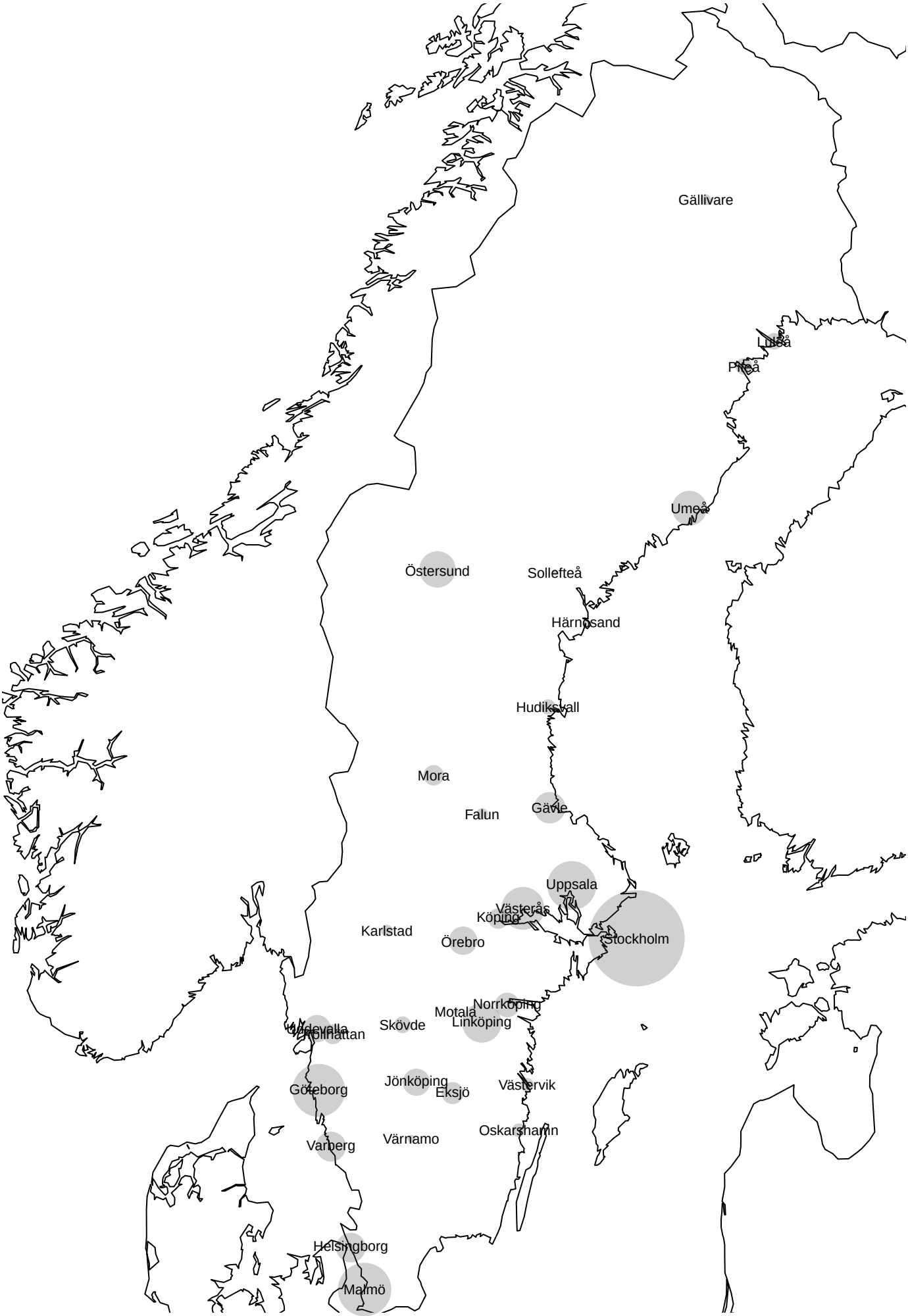
In study II analyses of risks of VTE associated with use of CHC or POC adjustments were made for smoking, BMI and immobilization. In this study we stratified analyses according to type of progestogen. Analyses were not adjusted for age as this was not possible due to lack of either exposed cases or controls.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




Gällivare
Luleå
Piteå
Umeå
Östersund
Sollefteå
Härnösand
Hudiksvall
Mora
Falun
Gävle
Uppsala
Västerås
Köping
Karlstad
Örebro
Stockholm
Norrköping
Motala
Linköping
Skövde
Trollhättan
Jönköping
Eksjö
Västervik
Göteborg
Oskarshamn
Värnamo
Varberg
Helsingborg
Malmö

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

Risks of VTE among 1441 premenopausal women	
Bedrest/minor trauma	1.4 (1.1-2.0)
Surgery only	5.9 (3.0-11.6)
Cast only	28.6 (11.8-68.9)
Surgery and cast	53.3 (16.3-174.4)
Smoking	1.4 (1.0-1.9)
Hormone therapy	3.7 (1.9-7.5)
CHC	8.5 (5.8-12.4)
POC	1.0 (0.6-1.6)
The prothrombin mutation	3.3 (1.6-6.7)
Factor V Leiden	3.3 (2.3-4.8)

Risks of VTE among 1100 postmenopausal women	
Bedrest/minor trauma	1.7 (1.2-2.4)
Surgery only	10.6 (4.5-25.2)
Cast only	13.3 (5.7-31.0)
Surgery and cast	54.1 (16.6-176.2)
Smoking	1.3 (0.9-1.9)
Hormone therapy	2.2 (1.5-3.2)
CHC	-
POC	0.8 (0.2-3.7)
The prothrombin mutation	1.9 (0.9-4.0)
Factor V Leiden	3.8 (2.4-6.0)

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.

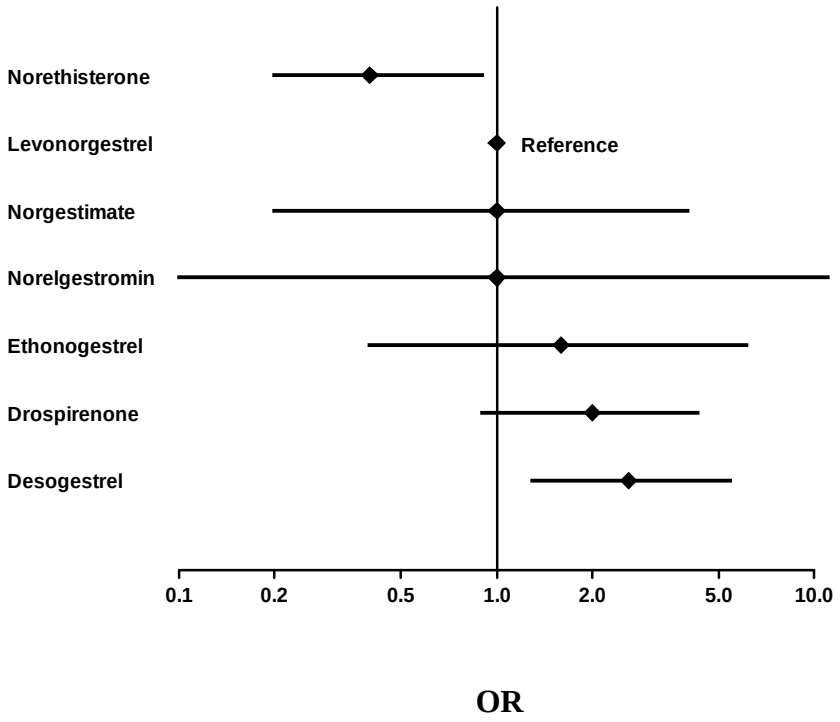
5.2 PAPER II

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 95 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

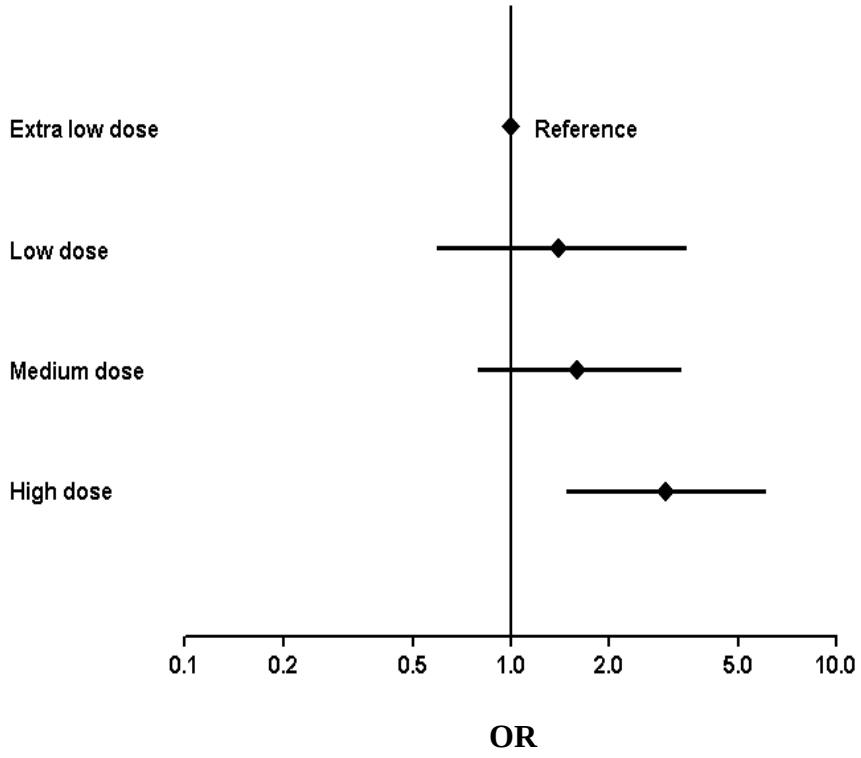
Forest plot showing OR for VTE associated with use of combined hormonal contraception (CHC) by type of progestogen using levonorgestrel as reference category



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

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

*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

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

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 life time 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.

9 ACKNOWLEDGEMENTS

Stor tack till alla kvinnor som deltagit i TEHS.

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R you a magician?

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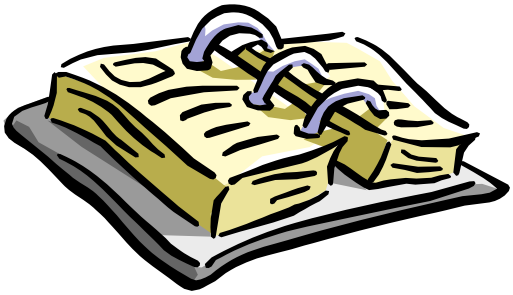
10 APPENDIX

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.

Som ett stöd för minnet bifogar vi en livstidskalender. Kalendern är bara till för Dig och Du avgör själv om och hur Du vill använda den. I kalendern kan Du anteckna händelser som kan göra det lättare att svara på våra frågor. Vi bifogar också en bildkarta med namn och bilder på de vanligaste p-piller och hormonpreparat vid klimakteriebesvär så Du lättare kan komma ihåg namnen på de preparat du har använt.

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.

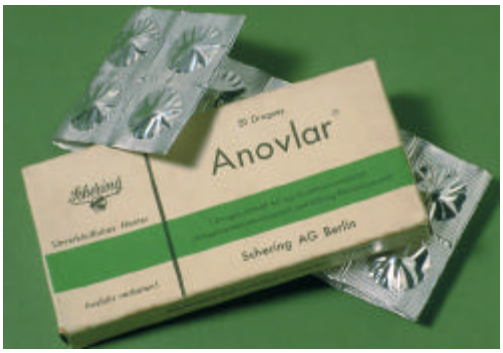
Här nedan visas ett exempel på hur man kan fylla i livstidkalendern

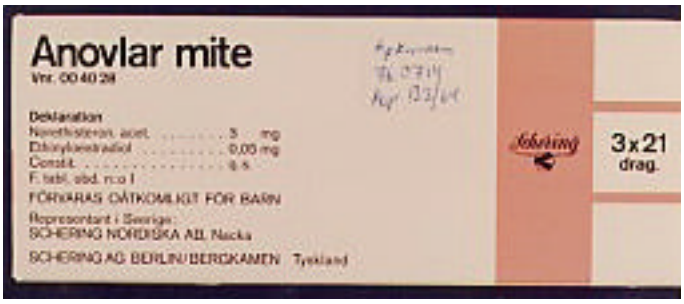
Ålder	År	Jan	Feb	Mar	Apr	Maj	Juni	July	Aug	Sept	Okt	Now	Dec
14	1960			Första									
19	1965					Student		Sjuksköterskeutbildning 1965-					
24	1970				Giftemål								
25	1971			Hemmafru oktober-mars									
26	1972	24/1, Peter, 3400 g, född i normaltid											
28	1974						15/6, Annika, 3600g, född i						
29	1975	P-piller, Conlumin till december											
34	1980		Sjuksköterska, Enköping lasarett										
35	1981					Minipiller till jul 1983, MiniPe							
37	1983			Spiral till januari 1994									
40	1986				Opererad för struma, Levaxin 0,1 mg dgl								
44	1990								Sjuksköterska,				
50	1996										Sista mens		
51	1997		Trisekvens till i										

Bildkatalog

I katalogen finns det bilder på ett urval av de under åren mest sålda p-piller, minipiller och hormonmediciner. Bilderna kan hjälpa Dig att komma ihåg namnet/namnen på de preparat Du har använt. Först visas p-piller, därefter minipiller och slutligen hormonmediciner. Hormonmedicinerna är uppdelade i östrogentabletter, -plåster, lokalbehandling och gulkroppshormoner. Preparaten är ordnade alfabetiskt och under förpackningarna står årtalen som de såldes. Några förpackningar har under åren ändrat utseende och alla visas inte här.

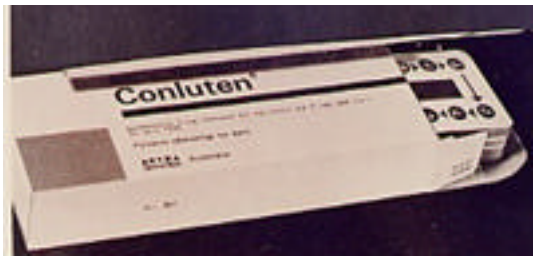
P-piller



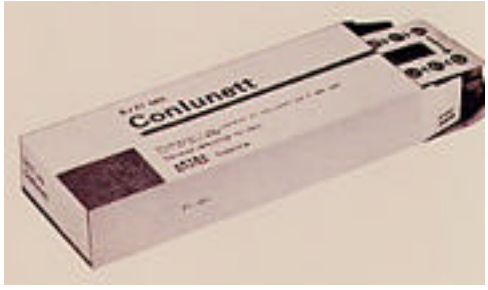


Anovlar och Anovlar Mite, 1964-1982

Conlumin, 1973-1992



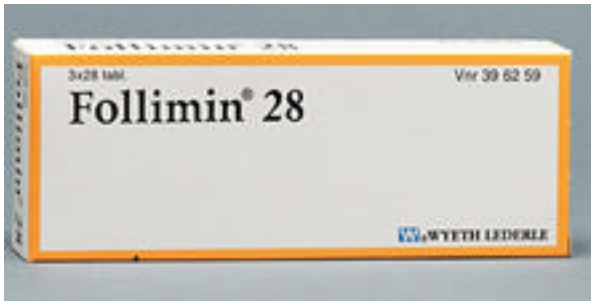
Conluten, 1964-1972



Conlunett 21, 1964-1998

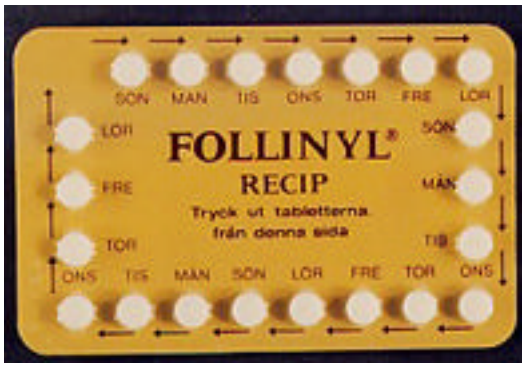
Delpregnin, 1964-1975

Desolett, 1988-

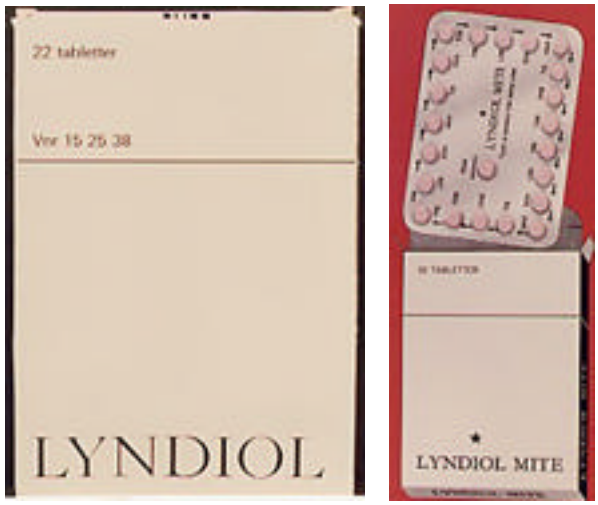


Follimin, 1976-

Follinett, 1972-

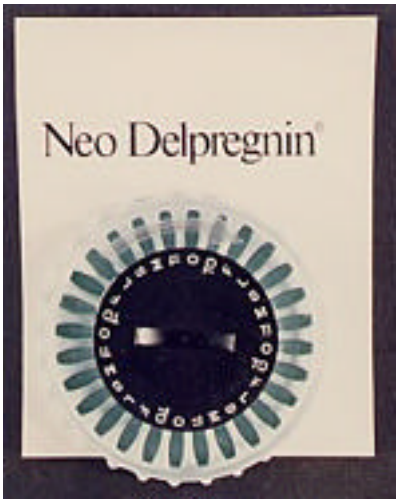


Follinyl, 1964-1981



Lyndiol and Lyndiol Mite, 1964-1972

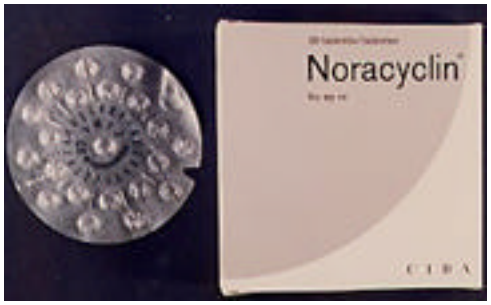
Menokvens, 1964-1972



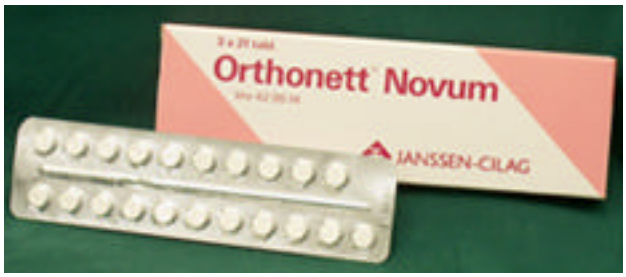
Neo-Delpregnin, 1971-1977

Mercilon, 1999-

Neovlar 21 och 28, 1972-1982



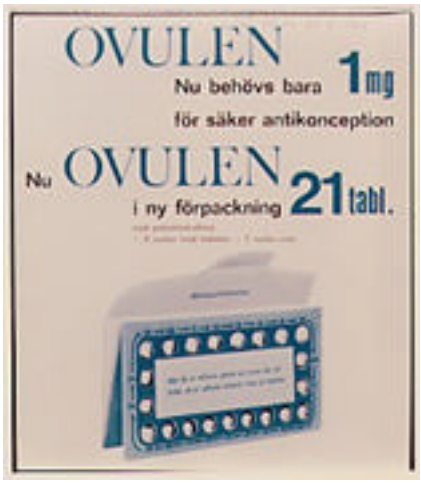
Noracyclin N, 1964-1972



Orthonett Novum, 1981-

Ortho-Novin, 1964-1973 och Ortho-Novin Mite, 1964-1974

Ovisec, 1964-1998



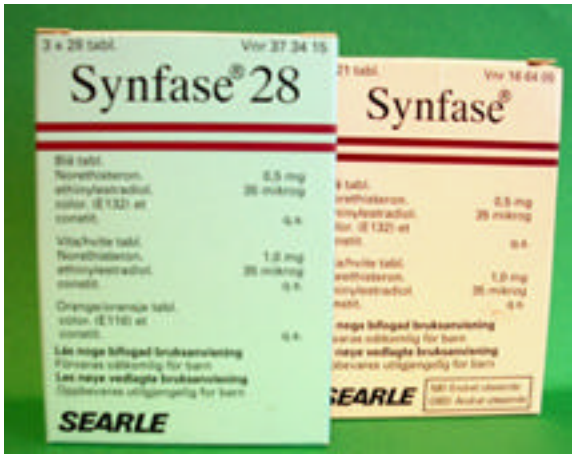
Ovulen, 1964-1972

Piloval, 1964-1985

Primovlar 21, 1964-1982

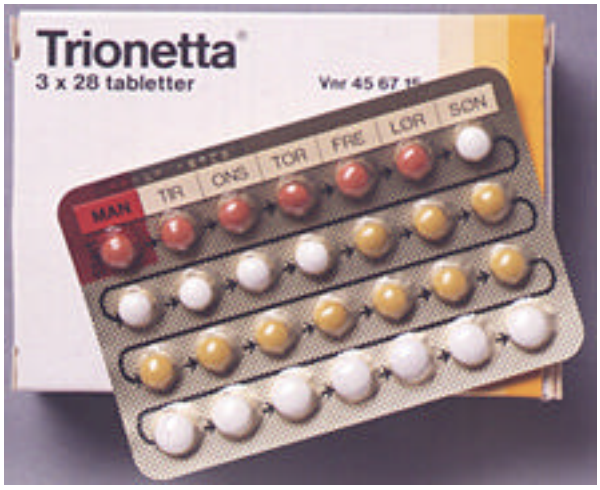
Restovar, 1982-

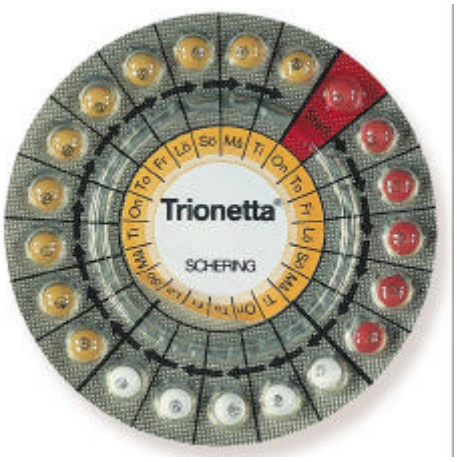
Sequens, 1964-1972



Synfase, 1987-

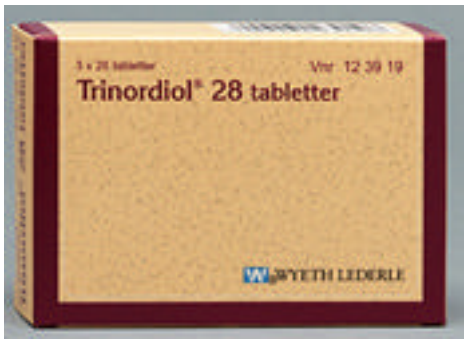
Trimiron 1999-



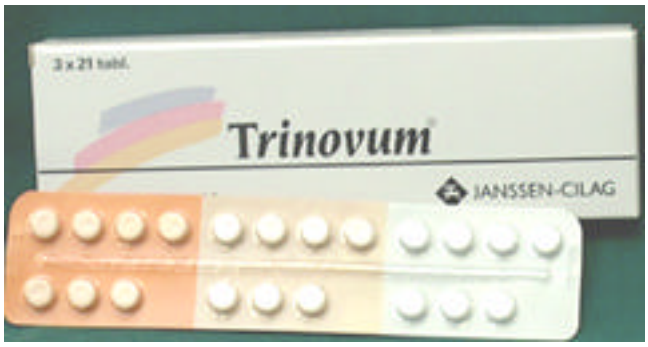


Trionetta, 1982-

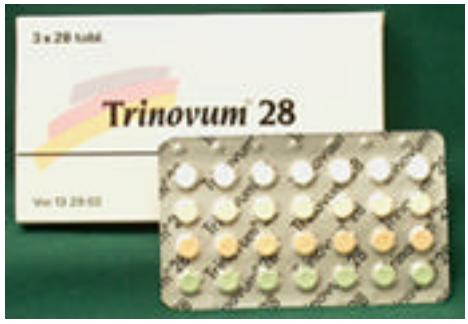
Trinordiol, 1982-



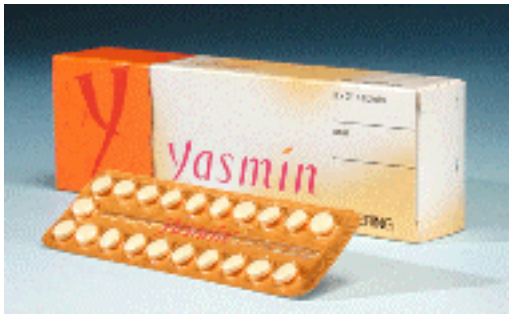
Trinordiol 28, 1985-



Trinovum, 1987-

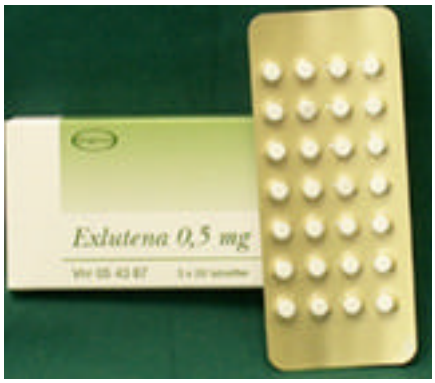


Trinovum 28, 1987-



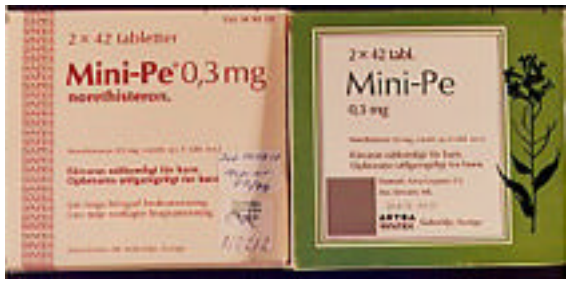
Yasmin, 2001-

Minipiller



Exlutena, 1975-

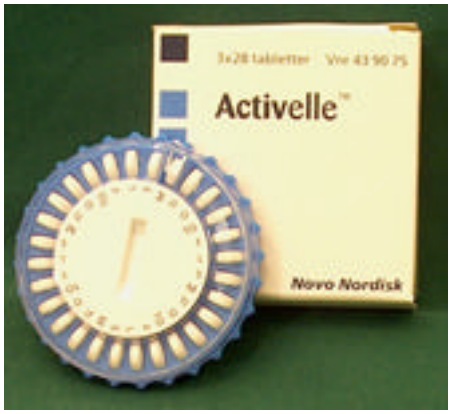
Follistrel, 1975-



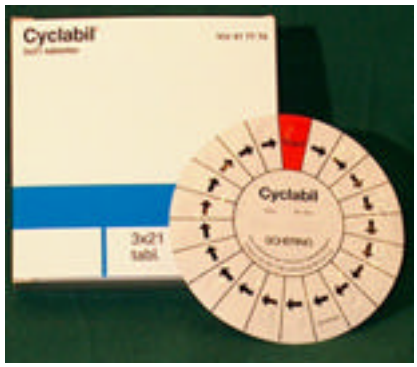
Mini-Pe, 1972-

Hormonmediciner

Tabletter med östrogen

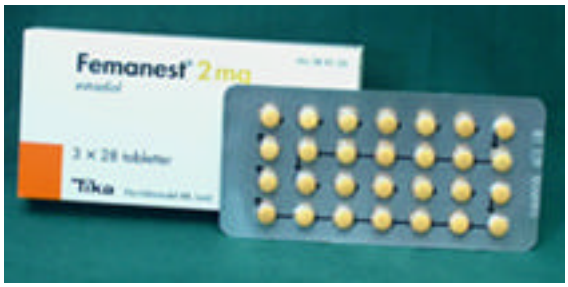


Activelle, 1999-

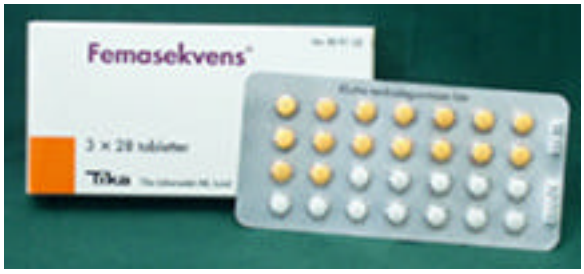


Cyclabil, 1977-

Etivex, 1964-1992

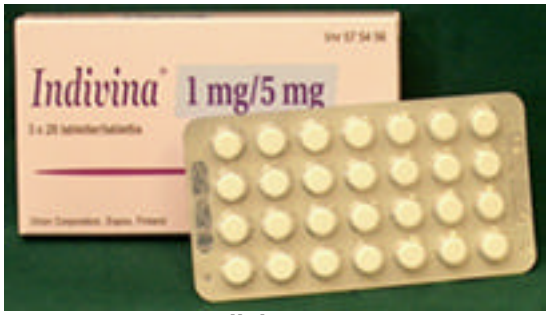


Femanest, 1997-

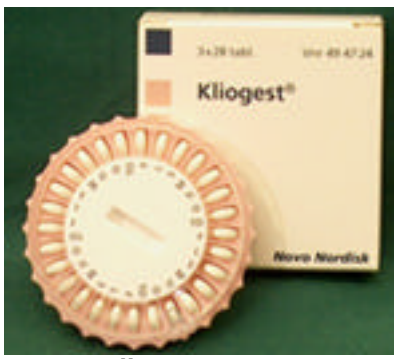


Femasekvens, 1997-

Femanor, 1997-

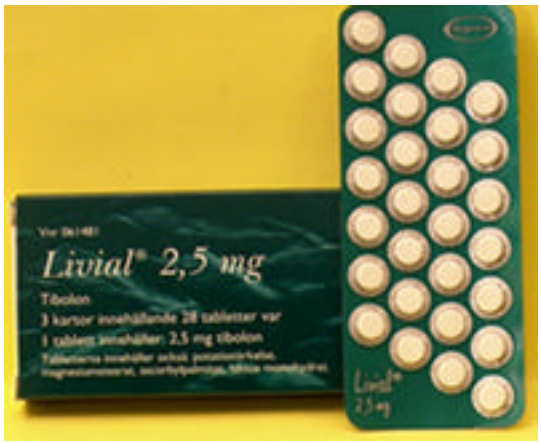


Indivina, 2000-



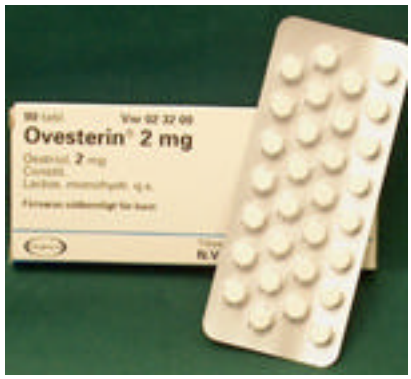
Kliogest, 1984-

Linoral, 1964-1988

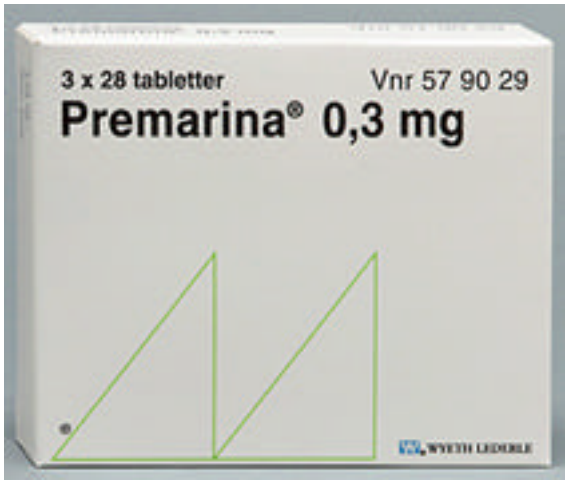


Livial, 2000-

Oestriol NM Pharma, 2000-



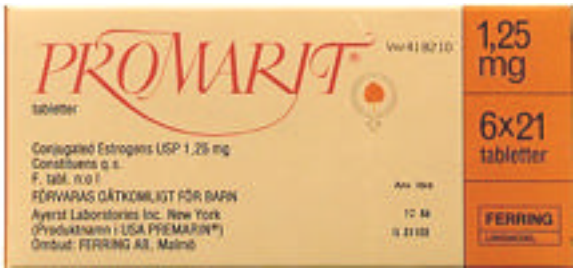
Ovesterin, 1972-



Premarina, 1975-

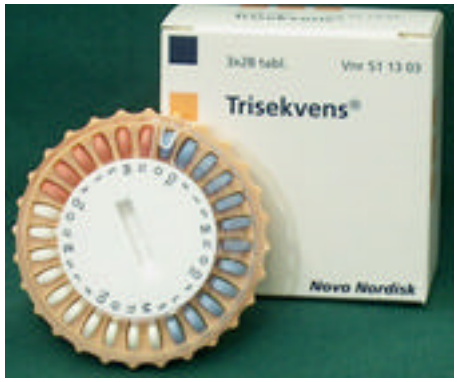
Premelle, 1996-

Progynon, 1972-

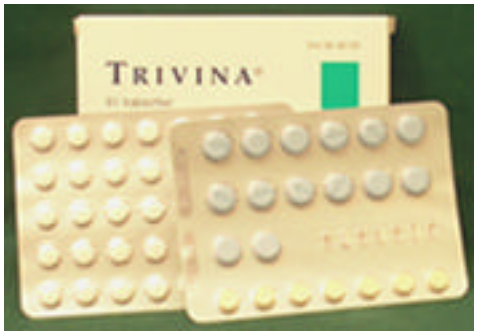


Promarit, 1964-1972

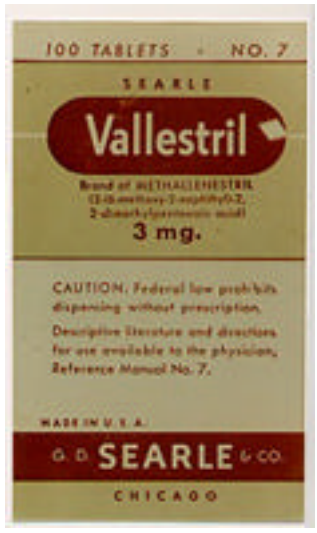
Triovex, 1964-1972



Trisekvens, 1986-

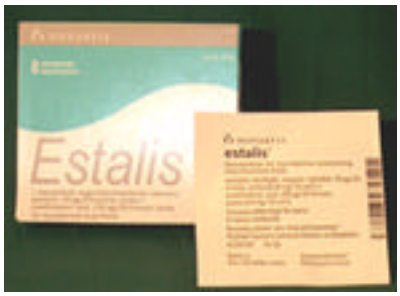


Trivina, 1994-

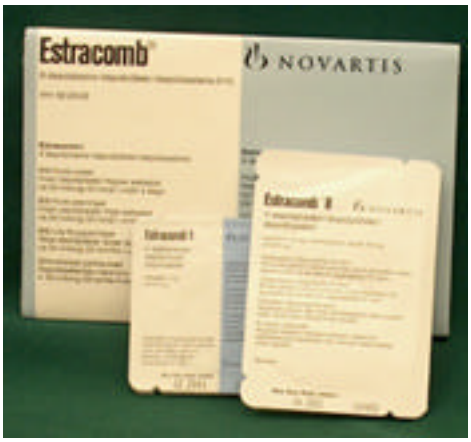


Vallestril, 1964-1972

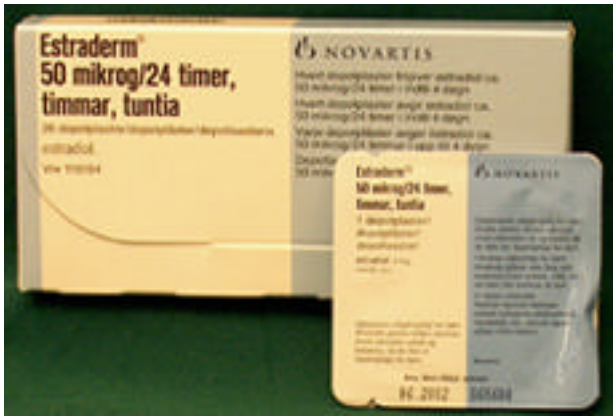
Plåster



Estalis, 1999-

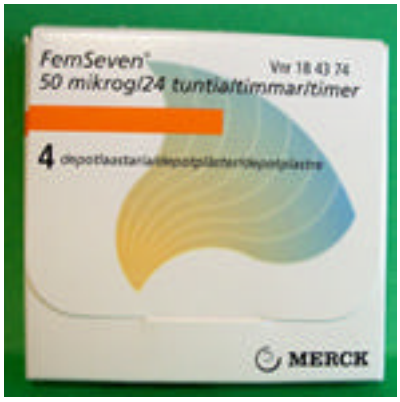


Estracomb, 1993-



Estraderm, 1988-

Evorel, 1994-

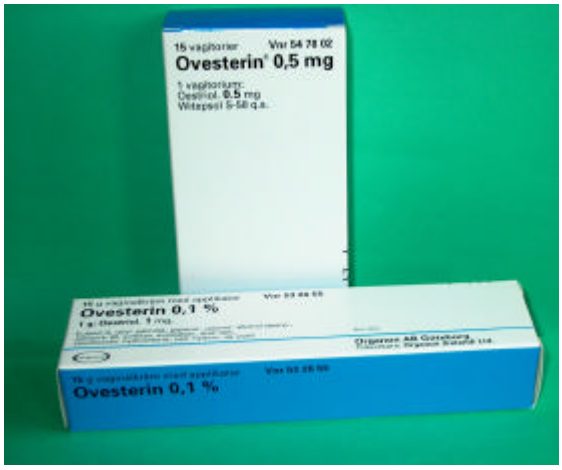


Femseven, 1999-

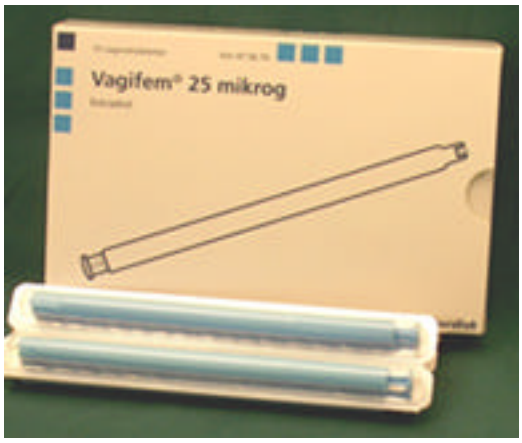
Menorest, 1996-

Lokalbehandling

Dienoestol, 1956-2000

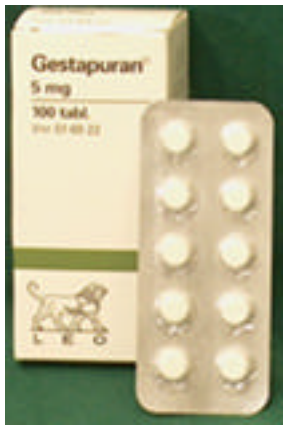


Ovesterin, 1983-

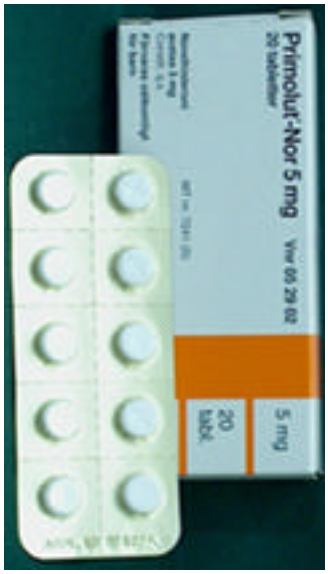


Vagifem, 1994-

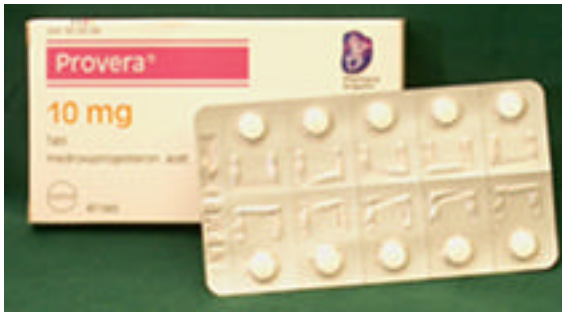
Gulkroppshormoner



Gestapuran, 1972-



Primolut-Nor, 1972-



Provera, 1987-

11 REFERENSER

1. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet. 1999 Apr 3;353(9159):1167-73.
2. Martinelli I. Risk factors in venous thromboembolism. Thromb Haemost. 2001 Jul;86(1):395-403.
3. Jordan WM AJ. Pulmonary Embolism. The Lancet. 1961 18 November;278, originally Volume 2(7212):1146-47.
4. Hannaford PC. Epidemiology of the contraceptive pill and venous thromboembolism. Thromb Res. 2011 Feb;127 Suppl 3:S30-4.
5. van Hylckama Vlieg A, Middeldorp S. Hormone therapies and venous thromboembolism: where are we now? J Thromb Haemost. 2011 Feb;9(2):257-66.
6. Farmer RD, Lawrenson RA, Thompson CR, Kennedy JG, Hambleton IR. Population-based study of risk of venous thromboembolism associated with various oral contraceptives. Lancet. 1997 Jan 11;349(9045):83-8.
7. Bloemenkamp KW, Rosendaal FR, Buller HR, Helmerhorst FM, Colly LP, Vandenbroucke JP. Risk of venous thrombosis with use of current low-dose oral contraceptives is not explained by diagnostic suspicion and referral bias. Arch Intern Med. 1999 Jan 11;159(1):65-70.
8. Lidegaard O, Edstrom B, Kreiner S. Oral contraceptives and venous thromboembolism: a five-year national case-control study. Contraception. 2002 Mar;65(3):187-96.
9. Jick H, Kaye JA, Vasilakis-Scaramozza C, Jick SS. Risk of venous thromboembolism among users of third generation oral contraceptives compared with users of oral contraceptives with levonorgestrel before and after 1995: cohort and case-control analysis. BMJ. 2000 Nov 11;321(7270):1190-5.
10. Wong CL, Farquhar C, Roberts H, Proctor M. Oral contraceptive pill as treatment for primary dysmenorrhoea. Cochrane Database Syst Rev. 2009(2):CD002120.
11. Farquhar C, Brown J. Oral contraceptive pill for heavy menstrual bleeding. Cochrane Database Syst Rev. 2009(4):CD000154.
12. Lethaby A, Duckitt K, Farquhar C. Non-steroidal anti-inflammatory drugs for heavy menstrual bleeding. Cochrane Database Syst Rev. 2013;1:CD000400.
13. Schmidt M, Christiansen CF, Horvath-Puho E, Glynn RJ, Rothman KJ, Sorensen HT. Non-steroidal anti-inflammatory drug use and risk of venous thromboembolism. J Thromb Haemost. 2011 Jul;9(7):1326-33.
14. Huerta C, Johansson S, Wallander MA, Garcia Rodriguez LA. Risk factors and short-term mortality of venous thromboembolism diagnosed in the primary care setting in the United Kingdom. Arch Intern Med. 2007 May 14;167(9):935-43.
15. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR. Cardiovascular risk factors and venous thromboembolism incidence: the longitudinal investigation of thromboembolism etiology. Arch Intern Med. 2002 May 27;162(10):1182-9.

16. Lacut K, van der Maaten J, Le Gal G, Cornily G, Mottier D, Oger E. Antiplatelet drugs and risk of venous thromboembolism: results from the EDITH case-control study. Haematologica. 2008 Jul;93(7):1117-8.
17. Biere-Rafi S, Di Nisio M, Gerdes V, Porreca E, Souverein P, Boer A, et al. Non-steroidal anti-inflammatory drugs and risk of pulmonary embolism. Pharmacoepidemiol Drug Saf. 2011 Jun;20(6):635-42.
18. Sundstrom A, Seaman H, Kieler H, Alfredsson L. The risk of venous thromboembolism associated with the use of tranexamic acid and other drugs used to treat menorrhagia: a case-control study using the General Practice Research Database. BJOG. 2009 Jan;116(1):91-7.
19. Trigg DE, Wood MG, Kouides PA, Kadir RA. Hormonal influences on hemostasis in women. Semin Thromb Hemost. 2011 Feb;37(1):77-86.
20. Strom BL. Pharmacoepidemiology 4th edition. Wiley.
21. Blomgren KB, Sundstrom A, Steineck G, Genell S, Sjostedt S, Wiholm BE. A Swedish case-control network for studies of drug-induced morbidity--acute pancreatitis. Eur J Clin Pharmacol. 2002 Jul;58(4):275-83.
22. Rothman KJ. Epidemiology An Introduction. Oxford University Press, Inc. 2002.
23. Hennekens B. Epidemiology in medicine. Lippincott Williams and Wilkins. 1987;1st edition.
24. Szumilas M. Explaining odds ratios. J Can Acad Child Adolesc Psychiatry. 2010 Aug;19(3):227-9.
25. Cornfield J. A method of estimating comparative rates from clinical data; applications to cancer of the lung, breast, and cervix. J Natl Cancer Inst. 1951 Jun;11(6):1269-75.
26. Anderson FA, Jr., Wheeler HB, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B, et al. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. Arch Intern Med. 1991 May;151(5):933-8.
27. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ, 3rd. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med. 1998 Mar 23;158(6):585-93.
28. Lippi G, Franchini M. Pathogenesis of venous thromboembolism: when the cup runneth over. Semin Thromb Hemost. 2008 Nov;34(8):747-61.
29. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost. 2007 Apr;5(4):692-9.
30. Girard P, Sanchez O, Leroyer C, Musset D, Meyer G, Stern JB, et al. Deep venous thrombosis in patients with acute pulmonary embolism: prevalence, risk factors, and clinical significance. Chest. 2005 Sep;128(3):1593-600.
31. Nordstrom M, Lindblad B, Bergqvist D, Kjellstrom T. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J Intern Med. 1992 Aug;232(2):155-60.
32. Lindblad B, Bergqvist D, Nordstrom M, Kjellstrom T, Bjorgell O, Nylander G, et al. [A survey in Malmo. The frequency of venous thromboembolism has not changed during the last 30 years]. Lakartidningen. 1992 Sep 9;89(37):2941-2, 7.
33. Oger E. Incidence of venous thromboembolism: a community-based study in Western France. EPI-GETBP Study Group. Groupe d'Etude de la Thrombose de Bretagne Occidentale. Thromb Haemost. 2000 May;83(5):657-60.
34. Danilenko-Dixon DR, Heit JA, Silverstein MD, Yawn BP, Petterson TM, Lohse CM, et al. Risk factors for deep vein thrombosis and pulmonary embolism during

pregnancy or post partum: a population-based, case-control study. Am J Obstet Gynecol. 2001 Jan;184(2):104-10.
35. Tormene D, Ferri V, Carraro S, Simioni P. Gender and the risk of venous thromboembolism. Semin Thromb Hemost. 2011 Apr;37(3):193-8.
36. White RH. The epidemiology of venous thromboembolism. Circulation. 2003 Jun 17;107(23 Suppl 1):I4-8.
37. Carter CJ. The pathophysiology of venous thrombosis. Prog Cardiovasc Dis. 1994 May-Jun;36(6):439-46.
38. Virchow. Phlogose und Thrombose im Gefa¨ßsystem. Gesammelte Abhandlungen zur Wissenschaftlichen Medizin Frankfurt, Germany: Staatsdruckerei;. 1856; .
39. Mammen EF. Pathogenesis of venous thrombosis. Chest. 1992 Dec;102(6 Suppl):640S-4S.
40. Laporte S, Mismetti P, Decousus H, Uresandi F, Otero R, Lobo JL, et al. Clinical predictors for fatal pulmonary embolism in 15,520 patients with venous thromboembolism: findings from the Registro Informatizado de la Enfermedad TromboEmbolica venosa (RIETE) Registry. Circulation. 2008 Apr 1;117(13):1711-6.
41. Turpie AG, Chin BS, Lip GY. Venous thromboembolism: pathophysiology, clinical features, and prevention. BMJ. 2002 Oct 19;325(7369):887-90.
42. Ageno W, Agnelli G, Imberti D, Moia M, Palareti G, Pistelli R, et al. Factors associated with the timing of diagnosis of venous thromboembolism: results from the MASTER registry. Thromb Res. 2008;121(6):751-6.
43. Goldhaber SZ. Risk factors for venous thromboembolism. J Am Coll Cardiol. 2010 Jun 29;56(1):1-7.
44. Rosendaal FR. Venous thrombosis: prevalence and interaction of risk factors. Haemostasis. 1999 Dec;29 Suppl S1:1-9.
45. Wells PS, Anderson DR, Rodger M, Forgie M, Kearon C, Dreyer J, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. N Engl J Med. 2003 Sep 25;349(13):1227-35.
46. Wells PS, Hirsh J, Anderson DR, Lensing AW, Foster G, Kearon C, et al. Accuracy of clinical assessment of deep-vein thrombosis. Lancet. 1995 May 27;345(8961):1326-30.
47. Schoepf UJ, Costello P. CT angiography for diagnosis of pulmonary embolism: state of the art. Radiology. 2004 Feb;230(2):329-37.
48. Hogg K, Wells PS, Gandara E. The diagnosis of venous thromboembolism. Semin Thromb Hemost. 2012 Oct;38(7):691-701.
49. Kearon C, Akl EA, Comerota AJ, Prandoni P, Bounameaux H, Goldhaber SZ, et al. Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012 Feb;141(2 Suppl):e419S-94S.
50. Patel A, Schwarz EB. Cancer and contraception. Release date May 2012. SFP Guideline #20121. Contraception. 2012 Sep;86(3):191-8.
51. Heit JA, Kobbervig CE, James AH, Petterson TM, Bailey KR, Melton LJ, 3rd. Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. Ann Intern Med. 2005 Nov 15;143(10):697-706.
52. Jacobsen AF, Skjeldestad FE, Sandset PM. Incidence and risk patterns of venous thromboembolism in pregnancy and puerperium--a register-based case-control study. Am J Obstet Gynecol. 2008 Feb;198(2):233 e1-7.

53. Macklon NS, Greer IA, Bowman AW. An ultrasound study of gestational and postural changes in the deep venous system of the leg in pregnancy. Br J Obstet Gynaecol. 1997 Feb;104(2):191-7.
54. Pabinger I, Grafenhofer H. Thrombosis during pregnancy: risk factors, diagnosis and treatment. Pathophysiol Haemost Thromb. 2002 Sep-Dec;32(5-6):322-4.
55. James AH, Jamison MG, Brancazio LR, Myers ER. Venous thromboembolism during pregnancy and the postpartum period: incidence, risk factors, and mortality. Am J Obstet Gynecol. 2006 May;194(5):1311-5.
56. Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW. Haemostasis in normal pregnancy. Thromb Haemost. 1984 Oct 31;52(2):176-82.
57. Clark P, Brennand J, Conkie JA, McCall F, Greer IA, Walker ID. Activated protein C sensitivity, protein C, protein S and coagulation in normal pregnancy. Thromb Haemost. 1998 Jun;79(6):1166-70.
58. Bergrem A, Dahm AE, Jacobsen AF, Mowinckel MC, Sandvik L, Sandset PM. Resistance to activated protein C is a risk factor for pregnancy-related venous thrombosis in the absence of the F5 rs6025 (factor V Leiden) polymorphism. Br J Haematol. 2011 Jul;154(2):241-7.
59. Cordts PR, Gawley TS. Anatomic and physiologic changes in lower extremity venous hemodynamics associated with pregnancy. J Vasc Surg. 1996 Nov;24(5):763-7.
60. Horsted F, West J, Grainge MJ. Risk of venous thromboembolism in patients with cancer: a systematic review and meta-analysis. PLoS Med. 2012;9(7):e1001275.
61. Cardiovascular disease and steroid hormone contraception. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser. 1998;877:i-vii, 1-89.
62. Manzoli L, De Vito C, Marzuillo C, Boccia A, Villari P. Oral contraceptives and venous thromboembolism: a systematic review and meta-analysis. Drug Saf. 2012 Mar 1;35(3):191-205.
63. Gerstman BB, Piper JM, Tomita DK, Ferguson WJ, Stadel BV, Lundin FE. Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease. Am J Epidemiol. 1991 Jan;133(1):32-7.
64. Vessey M, Mant D, Smith A, Yeates D. Oral contraceptives and venous thromboembolism: findings in a large prospective study. Br Med J (Clin Res Ed). 1986 Feb 22;292(6519):526.
65. Lidegaard O, Lokkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ. 2009;339:b2890.
66. van Hylckama Vlieg A, Helmerhorst FM, Vandenbroucke JP, Doggen CJ, Rosendaal FR. The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study. BMJ. 2009;339:b2921.
67. Jick H, Jick SS, Gurewich V, Myers MW, Vasilakis C. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components. Lancet. 1995 Dec 16;346(8990):1589-93.
68. Spitzer WO, Lewis MA, Heinemann LA, Thorogood M, MacRae KD. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. Transnational Research Group on Oral Contraceptives and the Health of Young Women. BMJ. 1996 Jan 13;312(7023):83-8.

69. Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet. 1995 Dec 16;346(8990):1582-8.
70. Lidegaard O, Milsom I, Geirsson RT, Skjeldestad FE. Hormonal contraception and venous thromboembolism. Acta Obstet Gynecol Scand. 2012 Jul;91(7):769-78.
71. Jacobsen AF, Sandset PM. Venous thromboembolism associated with pregnancy and hormonal therapy. Best Pract Res Clin Haematol. 2012 Sep;25(3):319-32.
72. Child TJ, MacKenzie IZ, Rees M. Terminations of pregnancy, not unplanned deliveries, increased as result of pill scare. BMJ. 1996 Oct 19;313(7063):1005.
73. Szarewski A, Mansour D. The 'pill scare': the responses of authorities, doctors and patients using oral contraception. Hum Reprod Update. 1999 Nov-Dec;5(6):627-32.
74. Ramsay S. UK "pill scare" led to abortion increase. Lancet. 1996 Apr 20;347(9008):1109.
75. Martinez F, Ramirez I, Perez-Campos E, Latorre K, Lete I. Venous and pulmonary thromboembolism and combined hormonal contraceptives. Systematic review and meta-analysis. Eur J Contracept Reprod Health Care. 2012 Feb;17(1):7-29.
76. Odlind V, Milsom I, Persson I, Victor A. Can changes in sex hormone binding globulin predict the risk of venous thromboembolism with combined oral contraceptive pills? Acta Obstet Gynecol Scand. 2002 Jun;81(6):482-90.
77. Tchaikovski SN, Rosing J. Mechanisms of estrogen-induced venous thromboembolism. Thromb Res. 2010 Jul;126(1):5-11.
78. Tchaikovski S, Tans G, Rosing J. Venous thrombosis and oral contraceptives: current status. Womens Health (Lond Engl). 2006 Sep;2(5):761-72.
79. van Rooijen M, Silveira A, Thomassen S, Hansson LO, Rosing J, Hamsten A, et al. Rapid activation of haemostasis after hormonal emergency contraception. Thromb Haemost. 2007 Jan;97(1):15-20.
80. Mantha S, Karp R, Raghavan V, Terrin N, Bauer KA, Zwicker JI. Assessing the risk of venous thromboembolic events in women taking progestin-only contraception: a meta-analysis. BMJ. 2012;345:e4944.
81. Winkler UH. Effects of progestins on cardiovascular diseases: the haemostatic system. Hum Reprod Update. 1999 May-Jun;5(3):200-4.
82. Schindler AE. Differential effects of progestins on hemostasis. Maturitas. 2003 Dec 10;46 Suppl 1:S31-7.
83. Blanco-Molina MA, Lozano M, Cano A, Cristobal I, Pallardo LP, Lete I. Progestin-only contraception and venous thromboembolism. Thromb Res. 2012 May;129(5):e257-62.
84. WHO EG. Medical eligibility criteria for contraceptive use
2009.
85. Canonico M, Scarabin PY. Hormone therapy and risk of venous thromboembolism among postmenopausal women. Climacteric. 2009;12 Suppl 1:76-80.
86. Canonico M, Fournier A, Carcaillon L, Olie V, Plu-Bureau G, Oger E, et al. Postmenopausal hormone therapy and risk of idiopathic venous thromboembolism: results from the E3N cohort study. Arterioscler Thromb Vasc Biol. 2010 Feb;30(2):340-5.
87. Canonico M, Oger E, Plu-Bureau G, Conard J, Meyer G, Levesque H, et al. Hormone therapy and venous thromboembolism among postmenopausal women:

impact of the route of estrogen administration and progestogens: the ESTHER study. Circulation. 2007 Feb 20;115(7):840-5.
88. Olie V, Canonico M, Scarabin PY. Postmenopausal hormone therapy and venous thromboembolism. Thromb Res. 2011 Feb;127 Suppl 3:S26-9.
89. Meilahn EN, Kuller LH, Matthews KA, Kiss JE. Hemostatic factors according to menopausal status and use of hormone replacement therapy. Ann Epidemiol. 1992 Jul;2(4):445-55.
90. Lowe GD, Upton MN, Rumley A, McConnachie A, O'Reilly DS, Watt GC. Different effects of oral and transdermal hormone replacement therapies on factor IX, APC resistance, t-PA, PAI and C-reactive protein--a cross-sectional population survey. Thromb Haemost. 2001 Aug;86(2):550-6.
91. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med. 2000 Nov 23;343(21):1520-8, 2 p following 8.
92. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ. 2006 Jun 3;332(7553):1302-8.
93. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. JAMA. 2006 Oct 4;296(13):1633-44.
94. Lijfering WM, Flinterman LE, Vandenbroucke JP, Rosendaal FR, Cannegieter SC. Relationship between venous and arterial thrombosis: a review of the literature from a causal perspective. Semin Thromb Hemost. 2011 Nov;37(8):885-96.
95. Pottier P, Hardouin JB, Lejeune S, Jolliet P, Gillet B, Planchon B. Immobilization and the risk of venous thromboembolism. A meta-analysis on epidemiological studies. Thromb Res. 2009 Sep;124(4):468-76.
96. Chandra D, Parisini E, Mozaffarian D. Meta-analysis: travel and risk for venous thromboembolism. Ann Intern Med. 2009 Aug 4;151(3):180-90.
97. Shorr AF, Kwong LM, Sarnes M, Happe L, Farrelly E, Mody-Patel N. Venous thromboembolism after orthopedic surgery: implications of the choice for prophylaxis. Thromb Res. 2007;121(1):17-24.
98. Wong P, Baglin T. Epidemiology, risk factors and sequelae of venous thromboembolism. Phlebology. 2012;27 Suppl 2:2-11.
99. Geerts WH, Bergqvist D, Pineo GF, Heit JA, Samama CM, Lassen MR, et al. Prevention of venous thromboembolism: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008 Jun;133(6 Suppl):381S-453S.
100. Enga KF, Braekkan SK, Hansen-Krone IJ, le Cessie S, Rosendaal FR, Hansen JB. Cigarette smoking and the risk of venous thromboembolism: the Tromso Study. J Thromb Haemost. 2012 Oct;10(10):2068-74.
101. Severinsen MT, Overvad K, Johnsen SP, Dethlefsen C, Madsen PH, Tjonneland A, et al. Genetic susceptibility, smoking, obesity and risk of venous thromboembolism. Br J Haematol. 2010 Apr;149(2):273-9.
102. Pomp ER, Rosendaal FR, Doggen CJ. Smoking increases the risk of venous thrombosis and acts synergistically with oral contraceptive use. Am J Hematol. 2008 Feb;83(2):97-102.

103. Pomp ER, le Cessie S, Rosendaal FR, Doggen CJ. Risk of venous thrombosis: obesity and its joint effect with oral contraceptive use and prothrombotic mutations. Br J Haematol. 2007 Oct;139(2):289-96.
104. Allman-Farinelli MA. Obesity and venous thrombosis: a review. Semin Thromb Hemost. 2011 Nov;37(8):903-7.
105. Rosito GA, D'Agostino RB, Massaro J, Lipinska I, Mittleman MA, Sutherland P, et al. Association between obesity and a prothrombotic state: the Framingham Offspring Study. Thromb Haemost. 2004 Apr;91(4):683-9.
106. Bowles LK, Cooper JA, Howarth DJ, Miller GJ, MacCallum PK. Associations of haemostatic variables with body mass index: a community-based study. Blood Coagul Fibrinolysis. 2003 Sep;14(6):569-73.
107. Middeldorp S, van Hylckama Vlieg A. Does thrombophilia testing help in the clinical management of patients? Br J Haematol. 2008 Nov;143(3):321-35.
108. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994 May 5;369(6475):64-7.
109. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet. 1995 Oct 28;346(8983):1133-4.
110. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood. 1995 Mar 15;85(6):1504-8.
111. Lindqvist PG, Dahlback B. Carriership of Factor V Leiden and evolutionary selection advantage. Curr Med Chem. 2008;15(15):1541-4.
112. Emmerich J, Rosendaal FR, Cattaneo M, Margaglione M, De Stefano V, Cumming T, et al. Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism--pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism. Thromb Haemost. 2001 Sep;86(3):809-16.
113. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996 Nov 15;88(10):3698-703.
114. Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism. A meta-analysis involving approximately 120,000 cases and 180,000 controls. Thromb Haemost. 2009 Aug;102(2):360-70.
115. Bereczky Z, Muszbek L. Factor XIII and venous thromboembolism. Semin Thromb Hemost. 2011 Apr;37(3):305-14.
116. Tsantes AE, Nikolopoulos GK, Bagos PG, Rapti E, Mantzios G, Kapsimali V, et al. Association between the plasminogen activator inhibitor-1 4G/5G polymorphism and venous thrombosis. A meta-analysis. Thromb Haemost. 2007 Jun;97(6):907-13.
117. Tsantes AE, Nikolopoulos GK, Bagos PG, Bonovas S, Kopterides P, Vaiopoulos G. The effect of the plasminogen activator inhibitor-1 4G/5G polymorphism on the thrombotic risk. Thromb Res. 2008;122(6):736-42.
118. Bauduer F, Lacombe D. Factor V Leiden, prothrombin 20210A, methylenetetrahydrofolate reductase 677T, and population genetics. Mol Genet Metab. 2005 Sep-Oct;86(1-2):91-9.
119. Ducros V, Barro C, Yver J, Pernod G, Polack B, Carpentier P, et al. Should plasma homocysteine be used as a biomarker of venous thromboembolism? A case-control study. Clin Appl Thromb Hemost. 2009 Oct;15(5):517-22.

120. Feng D, Lindpaintner K, Larson MG, Rao VS, O'Donnell CJ, Lipinska I, et al. Increased platelet aggregability associated with platelet GPIIIa PlA2 polymorphism: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol. 1999 Apr;19(4):1142-7.
121. Li H, Forstermann U. Nitric oxide in the pathogenesis of vascular disease. J Pathol. 2000 Feb;190(3):244-54.
122. Ronaghi M, Uhlen M, Nyren P. A sequencing method based on real-time pyrophosphate. Science. 1998 Jul 17;281(5375):363, 5.
123. Norell SE, Boethius G, Persson I. Oral contraceptive use: interview data versus pharmacy records. Int J Epidemiol. 1998 Dec;27(6):1033-7.
124. de Graaf MA, Jager KJ, Zoccali C, Dekker FW. Matching, an appealing method to avoid confounding? Nephron Clin Pract. 2011;118(4):c315-8.
125. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Epidemiology. 2007 Nov;18(6):805-35.
126. Guentert TW, Banken L, Hilton S, Holford NH. Moclobemide: relationships between dose, drug concentration in plasma, and occurrence of adverse events. J Clin Psychopharmacol. 1995 Aug;15(4 Suppl 2):84S-94S.
127. Tran C, Knowles SR, Liu BA, Shear NH. Gender differences in adverse drug reactions. J Clin Pharmacol. 1998 Nov;38(11):1003-9.
128. Harris RZ, Benet LZ, Schwartz JB. Gender effects in pharmacokinetics and pharmacodynamics. Drugs. 1995 Aug;50(2):222-39.
129. Saxon L, Hiltunen AJ, Hjemdahl P, Borg S. Gender-related differences in response to placebo in benzodiazepine withdrawal: a single-blind pilot study. Psychopharmacology (Berl). 2001 Jan 1;153(2):231-7.
130. Strohle A. Increased response to a putative panicogenic nocebo administration in female patients with panic disorder. J Psychiatr Res. 2000 Nov-Dec;34(6):439-42.