Ham-Wasserman Lecture

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Venous Thrombosis: The Role of Genes, Environment, and Behavior

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Over the last decade we have witnessed an avalanche of newly identified risk factors for venous thrombosis. This has advanced our knowledge of its etiology, because more determinants have been described and because the underlying concepts have received a new and broader understanding.

Venous thrombosis is a common multicausal disease occurring as the result of interacting genetic, environmental and behavioral risk factors. Some of these have been known since medieval times, such as the increased risk of thrombosis during immobilization in pregnancy and after childbirth (although retained milk of the breast-feeding mother was seen as the primary cause for the latter). Pregnancy and puerperium still cause thrombosis, as do exogenous hormones in oral contraceptives and hormonal replacement therapy. Furthermore, the immobilization in the puerperium of the old days translates directly to situations of immobilization in current times, such as prolonged travel in airplanes or excessive electronic gaming.

While pedigrees with abundant thrombosis were observed in the early 1900s, the first cause of heritable thrombophilia (antithrombin deficiency) was discovered in 1965, with the subsequent identification of deficiencies of protein C and protein S in the early 1980s. These were uncommon and strong risk factors, whereas the more recently discovered genetic variants are common and weak, and cause disease only in the presence of other factors.

Thrombosis

Thrombosis may occur in arteries and in veins. The obstructive clot formation that defines thrombosis is the end product of an imbalance of procoagulant, anticoagulant and fibrinolytic factors. Arterial thrombosis is seen predominantly as myocardial infarction and ischemic stroke, and more rarely in other locations. It is almost invariably superimposed on vessel wall disease, i.e., atherosclerosis. Although its symptoms are acute due to the blocking of the vital blood flow to an organ, arterial thrombosis could be seen as a chronic disorder related to a slowly increasing severity of atherosclerosis. Venous thrombosis contrasts with this, since the development of the clot is a relatively sudden phenomenon that does not follow a build-up of disease but often occurs in reaction to an acute and short-lasting risk. However, impeded venous blood flow from an organ or body part is much less endangering to vital function, so symptoms may sometimes be mild with an unclear time of onset, and it may even go undiagnosed. So, while arterial thrombosis is a chronic disease with acute symptoms, venous thrombosis is an acute disorder with chronic symptoms.

Due to the association of venous thrombosis to acute, transient risk factors, insight into its etiology preceded that of arterial disease. Some of the acquired causes of venous thrombosis, particularly pregnancy and the puerperium, have been known for centuries. Peripartum venous thrombosis was called 'milk leg' because of the sometimes shiny-white appearance of the thrombosed leg. Early physicians thought it was related to milk production, with unexpelled milk accumulating in the leg. In the late 1700s this led to the public-health advice that breast-feeding was recommended to prevent milk leg.[1]

The modern era of understanding the etiology of thrombosis began with the pathologist Virchow, who in the mid-1800s postulated three major causes of thrombosis: changes in the vessel wall, changes in the blood flow, and changes in the blood composition.[2] This broad classification is still valid. However, these classes of causes do not have the same role in arterial and venous thrombosis; therefore, arterial and venous thrombosis share some causes, but also each have causes that are vastly different. Arterial thrombosis is

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dominated by atherosclerosis (vessel wall changes) and its risk factors include hypertension, hyperlipidemia, smoking, and diabetes mellitus. Recent analyses including very large numbers of patients show that these risk factors are still the most important, with one of them present in approximately 90% of patients with myocardial infarction.[3-5] Stasis does not play a role in the etiology of the arterial thrombosis because of the high blood pressure and flow through the arteries. Hypercoagulability does affect the risk of arterial thrombosis, as witnessed, for example, by the low rate of myocardial infarction in hemophilia patients and the increased rate in patients with non-O blood groups, which are associated with increased levels of von Willebrand factor and factor VIII.[6-8] However, for most thrombophilic defects, their role in arterial disease is much less pronounced than in venous thrombosis. Some have even disputed the role altogether in view of the overriding effects of atherosclerosis. This is in contrast to venous thrombosis, where stasis and immobilization are equally important risk factors as prothrombotic abnormalities. However, atherogenic factors (vessel wall) such as smoking, hypertension or hyperlipidemia do not appear to affect the risk of venous thrombosis. **Table 1** lists the risk factors for venous thrombosis.

Venous Thrombosis

The incidence of venous thrombosis is 1-3 individuals per 1000 per year.[9-12] The most common forms of venous thrombosis are deep vein thrombosis of the leg and pulmonary embolism, although it also occurs in other veins (upper extremities, liver, cerebral sinus, retina, mesenteric), but rarely. Chronic sequelae can be severely debilitating due to a disabling postthrombotic syndrome, which occurs in up to 20% of patients.[13] The case-fatality rate of deep-vein thrombosis, mainly due to fatal pulmonary embolism, ranges from 1% in young patients to 10% in older patients, and is highest in those with underlying malignancies.[10,12]

Venous thrombosis occurs about equally often in men and women, with possibly higher rates among women in the younger age groups, and among men in the older age groups (**Figure 1**).[11,12] The incidence of venous thrombosis is strongly age-dependent: it is extremely uncommon (1 in 100,000 per year) in childhood, and rises to nearly 1% per year in old age.[11,12,14]

Risk Factors for Venous Thrombosis

The causes of venous thrombosis can be divided into those that are characterized by stasis and those that are reflected in abnormalities in the blood plasma (hypercoagulability). Another common classification separates acquired and genetic risk factors; acquired (environmental) risk factors are often related to stasis (but not exclusively, for example, oral contraceptives) and genetic risk factors to hypercoagulability.

Some environmental risk factors for venous thrombosis have been known for centuries; they include bed rest, surgery, trauma, plaster casts, pregnancy, puerperium, lupus anticoagulants, cancer and female hormones.[15,16] Acquired risk factors still play a major role in the burden of

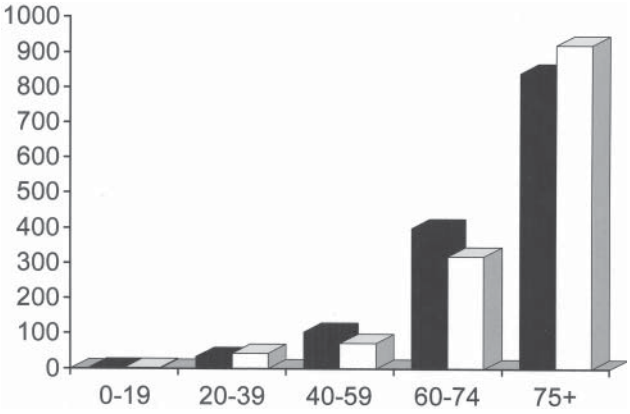

Figure 1. Incidence of first venous thrombosis (deep-vein thrombosis and pulmonary embolism, by age and sex (data from the district of Brest, France[11]). Rates are shown per 100,000 per year; men = filled bars, women = open bars

Table 1. Risk factors for venous thrombosis.

Acquired	Inherited	Mixed/Unknown
Bed rest	Antithrombin deficiency	High levels of factor VIII
Plaster cast	Protein C deficiency	High levels of factor IX
Trauma	Protein S deficiency	High levels of factor XI
Major surgery	Factor V Leiden (FVL)	High levels of fibrinogen
Orthopedic surgery	Prothrombin 20210A	High levels of TAFI
Malignancy	Dysfibrinogenemia	Low levels of TFPI
Oral contraceptives	Factor XIII 34val	APC-resistance in the absence of FVL
Hormonal replacement therapy		
Antiphospholipid syndrome		Hyperhomocysteinemia
Myeloproliferative disorders		High levels of PCI (PAI-3)
Polycythemia vera		
Central venous catheters		
Age		
obesity		

Abbreviations: TAFI, thrombin activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; PCI, protein C inhibitor; PAI-3, plasminogen activator inhibitor-3; APC, activated protein C

venous thrombosis, even though their impact has lessened because of the implementation of prophylactic antithrombotic strategies (**Table 2**).

A familial tendency to venous thrombosis was first described in the early 1900s, when pedigrees with a large number of individuals with venous thrombotic events suggested a heritable hypercoagulability. Egeberg described the first family with an identified hereditary tendency to thrombosis (thrombophilia), caused by antithrombin (AT) deficiency (previously known as antithrombin III), in 1965.[17] Subsequently, in the 1980s, protein C deficiency and protein S deficiency were recognized as causes underlying familial thrombophilia.[18,19]

Over the last 10 years, several new defects have been identified that increase the risk of venous thrombosis. Generally, these abnormalities tend to confer less increased thrombotic risk than do the deficiencies of antithrombin, protein C and protein S. However, although more mild, they are also far more common and therefore responsible for a larger proportion of all venous thrombotic events.

Table 2. Thrombosis risk in acquired risk situations—the Leiden Thrombophilia Study.

Risk Factor	Patients (*n* = 474) *n* (%)	Controls (*N* = 474) *n* (%)	OR	CI95
Surgery	85 (18)	17 (3.6)	5.9	3.4–10.1
Hospitalization	59 (12)	6 (1.3)	11.1	4.7–25.9
Bed rest (at home)	17 (3.6)	2 (0.4)	8.9	2.0–38.2
Pregnancy	8 (5.0)	2 (1.3)	4.2	0.9–19.9
Puerperium	13 (8.2)	1 (0.6)	14.1	1.8–109
Oral contraceptives	109 (70)	65 (38)	3.8	2.4–6.0

Time window for surgery, hospitalization (without surgery) and bed rest (not in the hospital, > 13 days) was 1 year preceding the index date (i.e., date of thrombosis diagnosis in patients, similar date in controls), for puerperium it was delivery 30 days or less prior to the index date, and for pregnancy and oral contraceptives it was at the index date.

Data on pregnancy, puerperium and oral contraceptive use refer to women in reproductive age.

Environmental Causes of Thrombosis

Age

One of the strongest risk factors for thrombosis is age, with a steep gradient of risk, in which the incidence is a thousand-fold higher in the very old than in the very young.[11,14] Why age is such a strong determinant of venous thrombosis is unclear. Several explanations seem obvious, all of which probably contribute to some extent. These are decreased mobility, an increased frequency of risk-enhancing diseases, decreased muscular tone, and acquisition of other risk factors as well as aging of the veins themselves, and particularly of the valves in the veins, that are crucial for good venous flow.

Surgery and trauma

Surgical interventions carry a very high risk of thrombosis, which, dependent on the type of surgery, may occur in over 50% of the patients in the absence of antithrombotic prophylaxis. The highest risks are conferred by orthopedic surgery and neurosurgery. In hip and knee surgery, the risk of thrombosis reaches 30% to 50%.[20,21] Risks that are nearly as high have been reported for abdominal surgery (up to 30%), gynecologic surgery and urologic surgery (in particular open prostatectomy).[22-24] Generally, the larger the intervention, the greater the risk, but in orthopedic surgery even minor interventions, such as arthroscopy, considerably affect the risk of venous thrombosis.

The incidence of venous thrombosis is also very high in patients with multiple trauma, and thrombosis occurs in 50%-60% of patients with head trauma, spinal injury, pelvic fractures, femoral fractures and tibial fractures.[25-27] Such high rates of symptomatic thrombosis are no longer observed due to anticoagulant prophylaxis that is routinely prescribed after major and orthopedic surgery. Still, even with anticoagulant treatment, high-risk surgery such as total hip or knee replacement will lead to symptomatic venous thrombosis in 1% to 3% of the patients.[28] Therefore, surgery remains a major cause of venous thrombosis. In the Leiden region, where extended anticoagulant prophylaxis is routinely prescribed for most surgical interventions, we observed that 18% of patients with thrombosis had had a surgical intervention, which increased the risk of venous thrombosis 6-fold (**Table 2**).[29] In a more recent analysis of over 4000 patients with a first venous thrombosis and a similar number of controls, we still noted a 4-fold increased risk of symptomatic thrombosis following orthopedic and major non-orthopedic surgery.[30]

Immobilization

As stasis is the major cause of thrombosis, the risk is increased during all circumstances that are associated with immobilization of the extremities, such as paralysis, bed rest, plaster casts and prolonged travel.[31-33] The common denominator is that immobilization interferes with the function of the calf musculature in pumping the blood upstream though the veins. Immobilization in a sitting position confers a higher risk than other positions. This was shown in London in World War II, where a 6-fold increased risk of pulmonary embolism was observed shortly after air raids, during which people sough shelter in the underground where they waited in deck-chairs. The risk went down after the chairs had been replaced by bunks.[34] More recently, it was reported that during massive traffic jams due to public transport strikes in Paris, several cases of deep-vein thrombosis occurred.[35] An even more contemporary example of thrombosis due to immobilization occurred in a young man who regularly spent 12 hours per day behind a computer screen, which was coined 'eThrombosis.'[36]

Long-distance travel

Although the first cases of venous thrombosis after air travel were reported in 1954,[33] the death of a young passenger from pulmonary embolism shortly after a flight from Australia to the United Kingdom has rekindled the interest in this association. Over the years, many case reports have been published, and thrombosis following long-haul air travel became known as the 'economy class syndrome,' without, however, controlled studies demonstrating a truly increased risk.[37,38] A very elegant study was published in 1986, in which data on sudden deaths occurring over several years at Heathrow airport in London had been collected and categorized as to where the death took place: in the arrival hall or in the departure hall.[39] There were far more deaths from pulmonary embolism in the arrival than in the departure hall, yielding a relative risk of around six.[39] In a similar design, severe pulmonary emboli (fatal and non-fatal) occurring in passengers shortly after their arrival at Charles de Gaulle airport in Paris were categorized according to the distance that had been travelled, and related to the distances travelled by all passengers arriving at this airport.[40] There was a clear association of risk of pulmonary embolism with the duration of the flight, with a 50-fold difference in risk between flights of less than 2500 km, and those over 10,000 km.[40] While these reports make it very plausible that air travel increases the risk of thrombosis, they give little information about the magnitude of the risk, since they dealt only with severe events occurring shortly after arrival. Controlled studies in which consecutive patients with venous thrombosis were included have yielded conflicting results, with some showing no risk increase at all, perhaps reflecting that the number of travellers in these studies was small.[41-44] In one of the studies it was found that recent air travel doubled the risk of thrombosis, which was, however, over 14-fold increased for individuals with thrombophilia or women who used oral contraceptives.[44] This was confirmed in the much larger 'MEGA-study,' in which we analyzed data from 1851 patients with a first deep-vein thrombosis and 1851 matched controls (SC Cannegieter et al, manuscript submitted). Travel was associated with a 3-fold increased risk of thrombosis, without much difference for the various modes of travel. The risk was particularly increased for individuals with factor V Leiden, those who were obese, and users of oral contraceptives. This analysis is part of the 'WRIGHT' project (WHO Research Into Global Hazards of Travel). This initiative combines several studies of the risks, mechanisms and prevention of travel-related thrombosis, in which the role of airplane cabin conditions on coagulation activation will also be studied. There are some data to suggest that mild hypoxic hypobaria, as can be found in an airplane at high altitude, leads to activation of the coagulation system.[45] However, a study that was conducted as part of the WRIGHT project, in which a large number of volunteers were exposed to a test condition of hypoxic hypobaria and a control condition of normoxic normobaria in hypobaric chambers in the United Kingdom, found no evidence for a broad shift towards coagulation activation.[46] In another study that was also part of the WRIGHT project, volunteers were first exposed to a real flight of 8 hours and then to 8 hours of immobilization in a cinema at ground level. Blood samples were taken before and after each exposure as well as during a day of normal daily activities.[47] The study population consisted mainly of women, many of whom had factor V Leiden, used oral contraceptives, or both. In this study there were several women, mainly those with one or two risk factors, who showed pronounced coagulation activation, as evidenced by increases in thrombin-antithrombin complexes (TAT), prothrombin fragment 1+2 (F1+2) and D-Dimers.[47] In a similar study in France, no clotting activation was observed in healthy male volunteers who were flown to Réunion.[48] So it seems that the conditions of air travel, e.g., hypoxic hypobaria, may lead to a hypercoagulant response in a minority of individuals, mainly those with other risk factors.

Several studies aimed at detecting asymptomatic clots after air travel have found them in several percent of travellers, with reduced rates of clots in those who used elastic stockings or antithrombotic drugs.[49-52] The relevance of asymptomatic clots is unclear, although in one study, with particularly long flights, several cases of pulmonary embolism were observed.[53] Asymptomatic clots also appear to occur often in daily life. This was demonstrated in a German study where extensive ultrasound scanning was performed in nearly 1000 individuals who had just flown for over 8 hours and in over 1000 controls who had not travelled.[54] Asymptomatic thrombosis was found in 2.8% of the travellers, but also in 1% of the controls.[54]

Cancer

Trousseau was the first, in 1865, to observe that cancer and venous thrombosis are associated.[55] Recurrent thrombophlebitis at various and changing locations (saltans et migrans) suggests occult cancer, especially of the pancreas. It is not entirely clear why cancer causes thrombosis, and several factors are likely to be involved. It is very likely that the tumor itself increases the risk of thrombosis by causing a procoagulant state through release of humoral factors. In addition, large tumors may cause thrombosis due to mechanical effects and venous obstruction.[56,57] Furthermore, there may be less mobility due to the illness, as well as thrombogenic effects of treatment.[58,59] Central venous catheters, which are often used to administer chemotherapeutics, are the most important cause of thrombosis of the arm,[60] and over 10% of patients with a central venous catheter develop symptomatic venous thrombosis of the upper extremity.[61]

It is a well-known observation among clinicians that adenocarcinomas confer a higher risk of thrombosis than other types of solid tumors. However, until recently there were surprisingly few data to support this notion. We constructed a historical cohort of 537 patients with a first diag-

nosis of lung carcinoma, including 133 patients with an adenocarcinoma and 258 patients with squamous cell cancer.[62] Thrombotic risk in lung cancer patients was 20-fold higher than in the general population (CI95 14.6–27.4). In the group of patients with adenocarcinoma the risk of venous thrombosis, after controlling for sex, age and stage of disease, was 3-fold higher than in those with squamous cell cancer, at an annualized rate of nearly 7%.[62]

How many patients diagnosed with venous thrombosis have cancer? In a population-based Swedish study, 19% of patients with thrombosis had a malignancy known at the time of diagnosis, and cancer was diagnosed in the year after the thrombotic event in an additional 5%.[63] In an analysis of the MEGA study, of 3220 unselected patients with deep-vein thrombosis or pulmonary embolism and 2131 controls, 389 of the patients (12.1%) had been diagnosed with cancer before the thrombotic event, and another 35 were diagnosed with cancer in the 6 months after the thrombosis.[64] The presence of a malignancy increased the risk 4.3-fold compared to individuals without cancer (CI95 3.3–5.6). When information on non-participants was included in the analyses, this odds ratio became 6.7 (CI95 5.2–8.6); many of these individuals had died before the examination date for the study.[64] Patients with hematological malignancies had the highest risk of venous thrombosis (OR 28.0; CI95 4.0–199.7), followed by lung cancer and gastrointestinal cancer. The risk of venous thrombosis was highest in the first few months after the diagnosis of malignancy (OR 53.5; CI95 8.6–334.3). The presence of distant metastases led to a further 20-fold increase in risk of thrombosis (OR 19.8; CI95 2.6–149.1). Carriers of the factor V Leiden mutation who also had cancer had a 12-fold increased risk versus individuals without cancer and factor V Leiden (OR 12.1; CI95 1.6–88.1), and similar results were found for the prothrombin 20210A variant.[64]

Oral contraceptives

Oral contraceptives first went on the market in 1959, and the first case of pulmonary embolism was reported in 1961 in a nurse using them for treatment of endometriosis.[65] Shortly after, the first cases of myocardial infarction and ischemic stroke in oral contraceptive users were reported.[66,67] These early case reports have been substantiated in large controlled studies, showing that even the low-dose oral contraceptives that are used today increase the risk of arterial thrombosis and venous thrombosis 2- to 5-fold.[68-75]

Combined monophasic oral contraceptives, consisting of a constant daily dose of a combination of an estrogen and a progestogen, are the most commonly prescribed form of birth control pills. The progestogen is the anti-ovulatory agent. The estrogen content in almost all cases is ethinylestradiol, while the progestogen content has changed over the years, from first generation progestogens, which are no longer used, to second generation progestogens (levonorgestrel), to third generation progestogens (desogestrel, gestodene). Two progestogens are somewhat different: the anti-androgen cyproteronacetate, which is mainly prescribed to women with acne vulgaris, seborrhoea or mild hirsutism, and drospirenone, which is an anti-mineralocorticoid with anti-aldosterone and anti-androgenic effects that inhibits ovulation.[76,77]

Over the years the dose of estrogen in oral contraceptives has been substantially reduced, from 100 µg or more in the earliest preparations, to 30 µg and even lower in the currently used oral contraceptives. While there is good evidence that the reduction from the very high dose to moderate doses of 50 µg and 30 µg ethinylestradiol was accompanied by a reduction in risk of venous thrombosis, this is not clear for further dose reductions, and the latest studies still show a 4-fold increased risk in users versus non-users.[73,78-83]

For women using oral contraceptives the absolute risk is more relevant than the relative risk. Oral contraceptives are used by young women, who have a low baseline risk of thrombosis. Therefore, even a quadrupled risk in oral contraceptive users confers a rate of thrombosis that is small in absolute terms. The absolute risk of venous thrombosis in women of reproductive age is less than 1 per 10,000 per year.[84] In oral contraceptive users, it becomes 2 to 3 per 10,000 per year.[84,85] These risks are acceptably low for many women. Nevertheless, because so many women use oral contraceptives, oral contraceptives remain the most common cause of venous thrombosis in young women. Since thrombotic events may be fatal, and when non-fatal may still be associated with postthrombotic syndrome, the search for the safest oral contraceptive remains an important goal.

The risk of venous thrombosis is highest during the first year of oral contraceptive use, reaching an absolute risk of 12 per 10,000 women per year (for second generation progestogen containing oral contraceptives).[86,87] However, there is no 'duration of use' effect: the effect is immediate and reversible, i.e., returns to baseline shortly after discontinuation.

Both the estrogen and the progestogen content of combined oral contraceptives affect the risk of thrombosis. Starting in 1995, reports were published that users of oral contraceptives containing third generation progestogens (desogestrel and gestodene) had a 2-fold higher risk of venous thrombosis than users of other formulations, which has subsequently been confirmed by several others.[73,88-90] The risk is again highest in the first year, with a risk of over 30 per 10,000 per year.[86]

Estrogens markedly affect the coagulation system, with increased levels of procoagulant factors VII, IX, X, XII and XIII, and reduced concentrations of the anticoagulant factors protein S and antithrombin. The overall effect is a mild prothrombotic state. This overall effect is most clearly seen in studies of global tests of hemostasis, i.e., activated protein C (APC)-resistance and thrombin generation tests.[91-95] Many of the effects on the coagulation system are more pronounced in oral contraceptives containing

desogestrel or gestodene (third generation progestogens) than in those containing levonorgestrel (second generation progestogens), leading to a more pronounced prothrombotic state, as evidenced by global assays.[93-97] In a crossover study in which women were exposed to a combination oral contraceptive with either levonorgestrel or desogestrel, and subsequently to only levonorgestrel or desogestrel, it appeared that the progestogens themselves did not affect the coagulation factor levels. However, the estrogenic effects were less compensated by desogestrel than by levonorgestrel in the combination preparation, leading to a prothrombotic state.[98]

Oral contraceptives that contain cyproteronacetate substantially increase the risk of venous thrombosis, with a higher risk even than for third generation oral contraceptives.[99] There are no clear data on the safety of oral contraceptives containing drospirenone, although cases of venous thrombosis have been reported.[100]

Some women have a higher risk of venous thrombosis when using oral contraceptives than others. These are older women, obese women, and women with prothrombotic abnormalities. Obesity itself mildly affects the risk of thrombosis, with a doubling of the risk for those with a BMI over 30 kg/m^2.[101,102] Overweight (BMI > 25 kg/m^2) and obese (BMI > 30 kg/m^2) women have a 10-fold increased risk of thrombosis when they use oral contraceptives.[102] Oral contraceptives also greatly increase the risk of thrombosis in familial thrombophilia caused by deficiencies of protein C, protein S or antithrombin.[103,104] Heterozygous factor V Leiden carriers or prothrombin 20210A carriers have a 15- to 30-fold increased risk of thrombosis when they use oral contraceptives.[84,105] High levels of several procoagulant factors (FII, FVIII, FIX, FX, FXI) confer a 2- to 3-fold increased risk when levels exceed the 90th percentile of the distribution in the population.[106-110] These risks are increased when oral contraceptives are used, particularly those of high levels of factor II, factor V and factor XI.[111]

Hormone replacement therapy

Postmenopausal hormone replacement therapy has been prescribed for the treatment of symptoms of menopause, to reduce the progression of osteoporosis, and to prevent ischemic heart disease. There is little doubt that estrogens relieve symptoms of menopause, although many symptoms resolve spontaneously without therapy.[112] Prolonged use of estrogens reduces the progression of osteoporosis,[113-115] but it is unclear whether this leads to a reduced incidence of fractures: two observational studies showed clear effects,[116,117] but two recent large randomized trials yielded conflicting results. In the Women's Health Initiative (WHI), the risk of hip fractures was reduced by one-third,[118] whereas in the Heart and Estrogen/progestin Replacement Study (HERS) no effect on fractures was observed.[119] These and one other randomized trial have shown that estrogens have no beneficial effect on the occurrence of arterial disease.[118,120-122]

The effective content of hormonal replacement therapy is the estrogen. Since hormone replacement with only estrogens increases the risk of endometrial cancer, it is now common to combine an estrogen with a progestin, except in women without a uterus. The hormones can be administered either orally or via transdermal patches. The estrogens in most preparations are conjugated estrogens, while the progestin usually is medroxyprogesterone.[82]

Several studies have demonstrated that hormone replacement therapy increases the risk of thrombosis 2- to 4-fold.[123-131] It has been postulated that the absence of a first-pass effect through the liver with transdermal administration might lead to less risk, but an increased risk has been shown for patches, too.[127,129] However, in one recent study, oral administration was associated with an increased risk of venous thrombosis, and transdermal administration was not.[132]

Virtually all studies of oral hormone replacement therapy used a preparation containing equine conjugated estrogens. In a recent case-control study the effects of conjugated estrogens were contrasted to those of esterified estrogens among 586 postmenopausal women with venous thrombosis and 2268 control women.[133] Women who used esterified estrogen had no increase in venous thrombotic risk (OR 0.9; CI95 0.7–1.2) relative to non-users, while women taking conjugated equine estrogen had an elevated risk of venous thrombosis (OR 1.7; CI95 1.2–2.2).[133]

Once again, the risk of venous thrombosis is higher shortly after therapy has started.[123,125,127,129,130] Women with increased risk are those who are older, are overweight or obese, have factor V Leiden or high factor IX levels.[84,134-137] In women with a previous venous thrombosis, the risk of a recurrence becomes very high during use of postmenopausal hormones, as was demonstrated in a randomized trial.[138]

Pregnancy and puerperium

In a large study of over 72,000 deliveries in Scotland, 62 venous thrombotic events occurred, for an incidence of deep vein thrombosis and pulmonary embolism of 0.86 per 1000 deliveries.[139] About one-third of these were postpartum.[139] This risk of around 1 venous thrombotic event per 1000 deliveries is at least 10-fold increased compared to non-pregnant women.[139,140] Thrombophilic abnormalities affect the risk of thrombosis during pregnancy, particularly antithrombin deficiency. In the Scottish series, 12% of the patients had antithrombin deficiency.[139]

Antiphospholipid antibodies

Patients with antiphospholipid antibodies, both those with systemic lupus erythematosus (half of whom have these antibodies) and those with isolated antiphospholipid antibodies, have an increased risk of thrombosis. The clinical presentation and the risk of thrombosis varies widely between patients.[141-143] In the Leiden Thrombophilia Study of consecutive patients with a first deep vein thrombosis, a

lupus anticoagulant was found in 3.1% of patients and 0.9% of controls, yielding a relative risk of 3.6 (CI95 1.2–10.9).[144] The risk of thrombosis was only elevated when anti-beta-2 glycoprotein I antibodies could be detected, in which case the odds ratio became 10.[144]

Genetic Causes of Thrombosis

Deficiencies of natural coagulation inhibitors

Deficiencies of antithrombin, protein C, and its cofactor protein S are found in less than 1% of the population (antithrombin deficiency in only 1 per 5000).[145-148] They increase the risk of thrombosis and therefore are found more often among patients with thrombosis. However, these deficiencies still represent only a few percent of patients with thrombosis.[149-150] Population-based studies to estimate risk are scarce due to the low prevalence of these deficiencies, and most information has been garnered from family studies. However, these results should be interpreted cautiously and not be extrapolated to a patient with one of the same defects who does not come from a 'thrombophilic family,' i.e., a family with abundant thrombosis. It has been shown that carriers of thrombophilic defects who come from thrombophilic families have a more severe phenotype and a younger age-at-onset than individuals with the same defects who do not come from such families.[151] Thrombophilic families harbor more than one genetic defect, including unknown ones that may epistatically interact with the deficiency of a coagulation inhibitor, yielding a higher risk of thrombosis.[152-155]

Deficiencies of protein C, protein S and antithrombin increase the risk of thrombosis about 10-fold in heterozygotes, with the highest risk for antithrombin deficiency.[150,156,157] Homozygous deficiencies are exceedingly rare and lead to a life-threatening thrombotic tendency (purpura fulminans) shortly after birth.[158,159]

Factor V Leiden

First identified in 1994, factor V Leiden (factor V R506Q, G1691A) is the most common genetic prothrombotic defect, with an overall prevalence of carriers among Caucasians of around 5%, although there are regional differences due to founder effects.[160,161] It is found in 20% of all patients with venous thrombosis, and in up to 50% of patients with thrombophilia.[162] Factor V Leiden leads to resistance to APC-resistance as the result of the mutation of one of the cleavage sites where APC inactivates factor V.[160] Because inactivation of the procoagulant mutant factor V occurs less efficiently, 'factor Va persistence' leads to an increased risk of thrombosis.[163,164] In heterozygotes the risk of venous thrombosis is 3- to 8-fold increased, and in homozygotes, 50- to 80-fold.[162,164] Although factor V Leiden is a weaker risk factor than deficiencies of other natural anticoagulants, it is also far more common. This implies that it is responsible for a substantial proportion of all thrombotic events in the population (population attributable risk [PAR] = 20%-25%). Due to the high prevalence, homozygous patients are not exceedingly rare, about 1 per 5000 in the population.

Prothrombin 20210A

Like factor V Leiden, prothrombin 20210A mutation is quite common and is seen only in Caucasians. The mutation is in the 3'-untranslated region of prothrombin at position 20210 (G to A, PT20210A) and is associated with increased levels of prothrombin.[106] It is found in 2% to 3% of Caucasians, again with regional variations in the prevalence.[64,106,165] Among all patients with venous thrombosis it is present in 6%.[106] It increases the risk of thrombosis about 3-fold, which is mediated through elevated prothrombin levels.[106,166-169]

Blood group

The association between ABO blood group and the risk of venous thrombosis has been known since the late 1960s, with a higher risk in those with non-O blood groups than those with blood group O (2- to 4-fold increased risk).[170] These individuals also have higher von Willebrand factor levels and higher factor VIII levels, and in all likelihood this is the mechanism by which blood group is related to thrombotic risk.[107] In the Leiden Thrombophilia Study (LETS) we observed that, with OO-genotypes as a reference group, the risk was almost 2-fold increased for all non-OO genotypes (A1A1, A1A2, A1O1/A1O2, BB/BO1/BO2, A1B/A2B), except for A2O1/A2O2/A2A2.[171] Carriers of non-OO blood group genotypes and factor V Leiden had a 23-fold (CI95 9.1–59.3) increased thrombosis risk compared to subjects with OO genotypes without factor V Leiden (separate effects of non-OO genotype: OR 1.7; CI95 1.3–2.3, and factor V Leiden: OR 4.6; CI 2.0–10.1).[171]

MTHFR 677T

A variant in the gene for methylene hydrofolate reductase (MTHFR), which plays a role in homocysteine metabolism, has been shown to be associated with mildly elevated homocysteine levels.[172,173] The variant (C677T) is common (about 10% of the general population are homozygous carriers), but the elevation of homocysteine levels is so small that little effect on risk can be expected.[174] It has been estimated that if hyperhomocysteinemia is a true cause of thrombosis, homozygous carriers of the MTHFR 677T variant have no more than a 16% increased risk of venous thrombosis. In a recent meta-analysis of 53 studies (n = 8364 cases), the MTHFR 677TT genotype was associated with a 20% (OR 1.20, CI95 1.1-1.3) higher risk of venous thrombosis compared with the 677CC genotype.[175]

Other Plasma Abnormalities Associated with the Risk of Thrombosis

Hyperhomocysteinemia

Mildly elevated levels of homocysteine (over 18 µmol/L) are associated with an increased risk of thrombosis.[176-178] Such levels are found in 5%-10% of the general population and roughly double the risk of venous thrombosis. The mechanism by which hyperhomocysteinemia affects the risk of thrombosis is unknown. Hyperhomocysteinemia is usually the result of acquired causes (low intake of folate, or vitamins B_6 or B_{12}) and only rarely of heterozygous cystathionine beta-synthase (CS) deficiency, the mutation causing homocystinuria in homozygotes.

In the meta-analysis of homocysteine levels, MTHFR genotype and risk of venous thrombosis, it was observed that a 5 µmol/L higher homocysteine level was associated with a 27% (CI95 1%–59%) higher risk of venous thrombosis in prospective studies and a 60% (CI95 10%–134%) higher risk in retrospective studies.[175]

Elevated clotting factor levels

When deficiencies of anticoagulant factors are risk factors for thrombosis, one would expect likewise for high levels of procoagulant factors. Indeed, elevated levels of fibrinogen, prothrombin (factor II), factor VIII, factor IX and factor XI, as well as of thrombin activatable fibrinolysis inhibitor (TAFI) are all associated with an increased risk of thrombosis.[106-109,179,180] The risk of thrombosis is 2- to 3-fold increased in individuals exceeding the 90th percentile of the distribution of clotting factors in the general population. For most of these elevated levels, with the exception of prothrombin and factor VIII, it is unclear whether they are genetically determined or acquired, although a combination seems likely. For high levels of factor VIII, it has been shown that there is familial clustering of high levels, even when the blood group effect has been taken into account.[181-182]

Thrombosis as a Multicausal Disease

Venous thrombosis is a multicausal disease, caused by the simultaneous presence of genetic and acquired risk factors. Examples of the interactive effects of risk factors are the combination of deficiencies of natural coagulation inhibitors (protein C, protein S, antithrombin) and factor V Leiden or prothrombin 20210A;[183-187] the joint effect of genetic thrombophilia due to deficiencies of protein C, of protein S, or of antithrombin, factor V Leiden or prothrombin 20210A, and pregnancy, puerperium, use of oral contraceptives, use of postmenopausal hormones;[84,135,188-190] the combined effects of obesity and exogenous hormones;[102,137] or the effects of factor V Leiden and travel.[44]

The genetic defects that were known to cause venous thrombosis a decade ago were uncommon and strong risk factors, whereas the more recently discovered genetic variants are common and weak, and cause disease only in the presence of other factors. Black and white has become a grayscale, and no longer is thrombophilia a rare disorder: all are at risk for thrombosis, but some are more at risk than others.

References

1. White, C. An inquiry into the nature and cause of that swelling in one or both of the lower extremities which sometimes happen to in-lying women together with the propriety of drawing the breasts of those who do and also who do not give suck. 1784. London, Warrington.
2. Virchow R: Phlogose und Thrombose im Gefäßsystem; Gesammelte Abhandlungen zur Wissenschaftlichen Medizin. Frankfurt, Staatsdruckerei, 1856.
3. Khot UN, Khot MB, Bajzer CT, et al. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA. 2003;290:898-904.
4. Greenland P, Knoll MD, Stamler J, et al. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA. 2003;290:891-897.
5. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364:937-952.
6. Rosendaal FR, Varekamp I, Smit C, et al. Mortality and causes of death in Dutch haemophiliacs, 1973-86. Br J Haematol. 1989;71:71-76.
7. Triemstra M, Rosendaal FR, Smit C, van der Ploeg HM, Briët E. Mortality in patients with hemophilia: changes in a Dutch population from 1986 to 1992 and 1973 to 1986. Ann Intern Med. 1995;123:823-827.
8. Medalie JH, Levene C, Papier C, et al. Blood groups, myocardial infarction and angina pectoris among 10,000 adult males. N Engl J Med. 1971;285:1348-1353.
9. Nordström M, Lindblad B, Bergqvist D, Kjellström T. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J Intern Med. 1992;232:155-160.
10. Anderson FA, Wheeler HB, Goldberg RJ, 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;151:933-938.
11. Oger E. Incidence of venous thromboembolism: a community-based study in western France. Thromb Haemost. 2000;83:657-660.
12. Cushman M, Tsai AW, White RH, et al. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. Am J Med. 2004;117:19-25.
13. Brandjes DP, Büller HR, Heijboer H, et al. Randomised trial of effect of compression stockings in patients with symptomatic proximal-vein thrombosis. Lancet. 1997;349:759-762.
14. Rosendaal FR. Thrombosis in the young: epidemiology and risk factors, a focus on venous thrombosis. Thromb Haemost. 1997;78:1-6.
15. Rosendaal FR. Risk factors for venous thrombosis: prevalence, risk and interaction. Semin Hematol. 1997;34:171-187.
16. Heit JA, O'Fallon WM, Petterson TM, et al. Relative impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-based study. Arch Intern Med. 2002;162:1245-1248.
17. Egeberg O. Inherited antithrombin deficiency causing thrombophilia. Thromb Diath Haemorrh. 1965;13:516-530.
18. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of protein C in congenital thrombotic disease. J Clin Invest. 1981;68:1370-1373.
19. Schwarz HP, Fischer M, Hopmeier P, Batard MA, Griffin JH. Plasma protein S deficiency in familial thrombotic disease. Blood. 1984;64:1297-1300.
20. Cohen SH, Ehrlich GE, Kaufman MS, Cope C. Thrombophlebitis following knee surgery. J Bone Joint Surg. 1973;55:106-111.
21. Hull RD, Raskob GE. Prophylaxis of venous thromboembolic disease following hip and knee surgery. J Bone Joint Surg. 1986;68:146-150.
22. Nicolaides AN, Field ES, Kakkar VV, Yates-Bell AJ, Taylor S, Clarke MB. Prostatectomy and deep-vein thrombosis. Br J

Surg. 1972;59:487-488.
23. Mayo M, Halil T, Browse NL. The incidence of deep vein thrombosis after prostatectomy. Br J Urol. 1971;43:738-742.
24. Walsh JJ, Bonnar J, Wright FW. A study of pulmonary embolism and deep vein thrombosis after major gynaecological surgery using labelled fibrinogen, phlebography and lung scanning. J Obstet Gynaecol Br Commonw. 1974;81:311-316.
25. Geerts WH, Code KI, Jay RM, Chen E, Szalai JP. A prospective study of venous thromboembolism after major trauma. N Engl J Med. 1994;331:1601-1606.
26. Myllynen P, Kammonen M, Rokkanen P, Bostman O, Lalla M, Laasonen E. Deep venous thrombosis and pulmonary embolism in patients with acute spinal cord injury: a comparison with nonparalyzed patients immobilized due to spinal fractures. J Trauma. 1985;25:541-543.
27. Hjelmstedt A, Bergvall U. Incidence of thrombosis in patients with tibial fractures. Acta Chir Scand. 1968;134:209-218.
28. Eikelboom JW, Quinlan DJ, Douketis JD. Extended-duration prophylaxis against venous thromboembolism after total hip or knee replacement: a meta-analysis of the randomised trials. Lancet. 2001;358:9-15.
29. Koster T. Deep-vein thrombosis. A population-based case-control study: Leiden Thrombophilia Study; Thesis. Leiden, Rijksuniversiteit Leiden, 1995.
30. Bannink L, Doggen CJM, Nelissen RGHH, Rosendaal FR. Increased risk of venous thrombosis after orthopedic and general surgery: results of the MEGA study (abstract). J Thromb Haemost. 2005;3 (Suppl1):P1653.
31. Gibbs NM. Venous thrombosis of the lower limbs with particular reference to bed-rest. Br J Surg. 1957;45:209-235.
32. Warlow C, Ogston D, Douglas AS. Deep venous thrombosis of the legs after stroke. Br Med J. 1976;1:1178-1181.
33. Homans J. Thrombosis of the leg veins due to prolonged sitting. N Engl J Med. 1954;250:148-149.
34. Simpson K. Shelter deaths from pulmonary embolism. Lancet. 1940;ii:744.
35. Eschwège V, Robert A. Strikes in French public transport and resistance to activated protein C. Lancet. 1996;347:206.
36. Beasley R, Raymond N, Hill S, Nowitz M, Hughes R. eThrombosis: the 21st century variant of venous thromboembolism associated with immobility. Eur Respir J. 2003;21:374-376.
37. Symington IS, Stack BHR. Pulmonary thromboembolism after travel. Br J Dis Chest. 1977;71:138-140.
38. Cruickshank JM, Gorlin R, Jennett B. Air travel and thrombotic episodes: the economy class syndrome. Lancet. 1988;ii:497-498.
39. Sarvesvaran R. Sudden natural deaths associated with commercial air travel. Med Sci Law. 1986;26:35-38.
40. Lapostolle F, Surget V, Borron SW, et al. Severe pulmonary embolism associated with air travel. N Engl J Med. 2001;345:779-783.
41. Ferrari E, Chevallier T, Chapelier A, Baudouy M. Travel as a risk factor for venous thromboembolic disease: a case-control study. Chest. 1999;115:440-444.
42. Kraaijenhagen RA, Haverkamp D, Koopman MM, Prandoni P, Piovella F, Buller HR. Travel and risk of venous thrombosis. Lancet. 2000;356:1492-1493.
43. Arya R, Barnes JA, Hossain U, Patel RK, Cohen AT. Long-haul flights and deep vein thrombosis: a significant risk only when additional factors are also present. Br J Haematol. 2002;116:653-654.
44. Martinelli I, Taioli E, Battaglioli T, et al. Risk of venous thromboembolism after air travel: interaction with thrombophilia and oral contraceptives. Arch Intern Med. 2003;163:2771-2774.
45. Bendz B, Rostrup M, Sevre K, Andersen TO, Sandset PM. Association between acute hypobaric hypoxia and activation of coagulation in human beings. Lancet. 2000;356:1657-1658.
46. Jones CI, Ford I, Pearse RJ, Chudasama V, et al. Hypobaric hypoxia does not influence markers of coagulation, platelet, endothelial or fibrinolytic activation [abstract]. J Thromb Haemost. 2005;3 (suppl 1):P0474.
47. Schreijer AJM, Cannegieter SC, Meijers JCM, Middeldorp S, Rosendaal FR, Büller HR. Coagulation in aviation: activation of the coagulation system during air travel (the WRIGHT Volunteers Study)(abstract). J Thromb Haemost. 2005;3 (suppl 1):OR289.
48. Boccalon H, Boneu B, Emmerich J, Thalamas C, Ruidavets JB. Long-haul flights do not activate hemostasis in young healthy men. J Thromb Haemost. 2005;3:1539-1541.
49. Scurr JH, Machin SJ, Bailey-King S, Mackie IJ, McDonald S, Smith PD. Frequency and prevention of symptomless deep-vein thrombosis in long-haul flights: a randomised trial. Lancet. 2001;357:1485-1489.
50. Belcaro G, Geroulakos G, Nicolaides AN, Myers KA, Winford M. Venous thromboembolism from air travel: the LONFLIT study. Angiology. 2001;52:369-374.
51. Belcaro G, Cesarone MR, Shah SS, et al. Prevention of edema, flight microangiopathy and venous thrombosis in long flights with elastic stockings. A randomized trial: The LONFLIT 4 Concorde Edema-SSL Study. Angiology. 2002;53:635-645.
52. Cesarone MR, Belcaro G, Nicolaides AN, et al. Venous thrombosis from air travel: the LONFLIT3 study—prevention with aspirin vs low-molecular-weight heparin (LMWH) in high-risk subjects: a randomized trial. Angiology. 2002;53:1-6.
53. Hughes RJ, Hopkins RJ, Hill S, et al. Frequency of venous thromboembolism in low to moderate risk long distance air travellers: the New Zealand Air Traveller's Thrombosis (NZATT) study. Lancet. 2003;362:2039-2044.
54. Schwarz T, Siegert G, Oettler W, et al. Venous thrombosis after long-haul flights. Arch Intern Med. 2003;163:2759-2764.
55. Trousseau A. Phlegmasia alba dolens; Clinique Médicale de l'Hôtel-Dieu de Paris. Paris, J.B. Ballière et fils. 1865;3:652-695.
56. Bick RL. Coagulation abnormalities in malignancy: a review. Sem Thromb Hemost. 1992;18:353-369.
57. Zurborn KH, Duscha H, Gram J, Bruhn HD. Investigations of coagulation system and fibrinolysis in patients with disseminated adenocarcinomas and non-Hodgkin lymphomas. Oncology. 1990;47:376-380.
58. Meier CR, Jick H. Tamoxifen and risk of idiopathic venous thromboembolism. Br J Clin Pharmacol. 1998;45:608-612.
59. Weijl NI, Rutten MF, Zwinderman AH, et al. Thromboembolic events during chemotherapy for germ cell cancer: a cohort study and review of the literature. J Clin Oncol. 2000;18:2169-2178.
60. Blom JW, Doggen CJM, Osanto S, Rosendaal FR. Old and new risk factors for upper extremity deep venous thrombosis. J Thromb Haemost 2005;(in press).
61. van Rooden CJ, Rosendaal FR, Barge RM, et al. Central venous catheter related thrombosis in haematology patients and prediction of risk by screening with Doppler-ultrasound. Br J Haematol 2003;123:507-512.
62. Blom JW, Osanto S, Rosendaal FR. The risk of a venous thrombotic event in lung cancer patients: higher risk for adenocarcinoma than squamous cell carcinoma. J Thromb Haemost. 2004;2:1760-1765.
63. Nordström M, Lindblad B, Anderson H, Bergqvist D, Kjellström T. Deep venous thrombosis and occult malignancy: an epidemiological study. Br Med J. 1994;308:891-894.
64. Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. JAMA. 2005;293:715-722.
65. Jordan WM. Pulmonary embolism. Lancet. 1961;ii:1146-1147.
66. Boyce J, Fawcett JW, Noall EWP. Coronary thrombosis and Conovid. Lancet. 1963;i:111.
67. Lorentz I. Parietal lesions and Enavid. Br Med J. 1962;ii:1191.
68. World Health Organization. Ischaemic stroke and combined oral contraceptives: results of an international, multicentre, case-control study. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet. 1996;348:498-505.
69. World Health Organization. Haemorrhagic stroke, overall stroke risk, and combined oral contraceptives: results of an international, multicentre, case-control study. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet. 1996;348:505-510.
70. World Health Organization. Acute myocardial infarction and combined oral contraceptives: results of an international multicentre case-control study. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet. 1997;349:1202-1209.
71. Kemmeren JM, Tanis BC, van den Bosch MA, et al. Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) study: oral contraceptives and the risk of ischemic

stroke. Stroke. 2002;33:1202-1208.
72. van den Bosch MAAJ, Kemmeren JM, Tanis BC, et al. The RATIO study: oral contraceptives and the risk of peripheral arterial disease in young women. J Thromb Haemost. 2003;1:439-444.
73. Bloemenkamp KWM, Rosendaal FR, Helmerhorst FM, Büller HR, Vandenbroucke JP. Enhancement by factor V Leiden mutation of risk of deep-vein thrombosis associated with oral contraceptives containing a third-generation progestagen. Lancet. 1995;346:1593-1596.
74. World Health Organization. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet. 1995;346:1575-1582.
75. Tanis BC, van den Bosch MA, Kemmeren JM, et al. Oral contraceptives and the risk of myocardial infarction. N Engl J Med. 2001;345:1787-1793.
76. Lachnit-Fixson U. The development and evaluation of an ovulation inhibitor (DIAne) containing an antiandrogen. Acta Obstet Gynecol Scand Suppl. 1979;88:33-42.
77. Muhn P, Fuhrmann U, Fritzemeier KH, Krattenmacher R, Schillinger E. Drospirenone: a novel progestogen with antimineralocorticoid and antiandrogenic activity. Ann N Y Acad Sci. 1995;761:311-335.
78. 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;133:32-37.
79. Thorogood M, Mann J, Murphy M, Vessey M. Risk factors for fatal venous thromboembolism in young women: a case-control study. Int J Epidemiol. 1992;21:48-52.
80. Farmer RDT, Preston TD. The risk of venous thromboembolism associated with low-oestrogen oral contraceptives. J Obst Gynecol. 1995;15:195-200.
81. Vandenbroucke JP, Rosing J, Bloemenkamp KW, et al. Oral contraceptives and the risk of venous thrombosis. N Engl J Med. 2001;344:1527-1535.
82. Rosendaal FR, Helmerhorst FM, Vandenbroucke JP. Female hormones and thrombosis. Arterioscler Thromb Vasc Biol. 2002;22:201-210.
83. Rosendaal FR, van Hylckama Vlieg A, Tanis BC, Helmerhorst FM. Estrogens, progestogens and thrombosis. J Thromb Haemost. 2003;1:1371-1380.
84. Vandenbroucke JP, Koster T, Briët E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. Lancet. 1994;344:1453-1457.
85. 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. Br Med J. 2000;321:1190-1195.
86. Herings RMC, Urquhart J, Leufkens HGM. Venous thromboembolism among new users of different oral contraceptives. Lancet. 1999;354:127-128.
87. Bloemenkamp KWM, Rosendaal FR, Helmerhorst FM, Vandenbroucke JP. Higher risk of venous thrombosis during early use of oral contraceptives in women with inherited clotting defects. Arch Intern Med. 2000;160:49-52.
88. World Health Organization. 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;346:1582-1588.
89. 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;346:1589-1593.
90. Kemmeren JM, Algra A, Grobbee DE. Third generation oral contraceptives and risk of venous thrombosis: meta-analysis. Br Med J. 2001;323:131-134.
91. Henkens CM, Bom VJ, Seinen AJ, van der Meer J. Sensitivity to activated protein C; influence of oral contraceptives and sex. Thromb Haemost. 1995;73:402-404.
92. Olivieri O, Friso S, Manzato F, et al. Resistance to activated protein C in healthy women taking oral contraceptives. Br J Haematol. 1995;91:465-470.
93. Rosing J, Middeldorp S, Curvers J, et al. Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. Lancet. 1999;354:2036-2040.
94. Middeldorp S, Meijers JCM, van den Ende AE, et al. Effects on coagulation of levonorgestrel- and desogestrel-containing low dose oral contraceptives: a cross-over study. Thromb Haemost. 2000;84:4-8.
95. Tans G, Curvers J, Middeldorp S, et al. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. Thromb Haemost. 2000;84:15-21.
96. Rosing J, Tans G, Nicolaes GA, et al. Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. Br J Haematol. 1997;97:233-238.
97. Meijers JCM, Middeldorp S, Tekelenburg W, et al. Increased fibrinolytic activity during use of oral contraceptives is counteracted by an enhanced factor XI-independent down regulation of fibrinolysis: a randomized cross-over study of two low-dose oral contraceptives. Thromb Haemost. 2000;84:9-14.
98. Kemmeren JM, Algra A, Meijers JC, Bouma BN, Grobbee DE. Effects of second and third generation oral contraceptives and their respective progestagens on the coagulation system in the absence or presence of the factor V Leiden mutation. Thromb Haemost. 2002;87:199-205.
99. Vasilakis-Scaramozza C, Jick H. Risk of venous thromboembolism with cyproterone or levonorgestrel contraceptives. Lancet. 2001;358:1427-1429.
100. Sheldon T. Dutch GPs warned against new contraceptive pill. Br Med J. 2002;324:869.
101. 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;162:1182-1189.
102. Abdollahi M, Cushman M, Rosendaal FR. Obesity: risk of venous thrombosis and the interaction with coagulation factors and oral contraceptive use. Thromb Haemost. 2003;89:493-498.
103. Pabinger I, Schneider B; the GTH study group. Thrombotic risk of women with hereditary antithrombin III-, protein C and protein S-deficiency taking oral contraceptive medication. Thromb Haemost. 1994;71:548-552.
104. Martinelli I, Mannucci PM, de Stefano V, et al. Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. Blood. 1998;92:2353-2358.
105. Martinelli I, Taioli E, Bucciarelli P, Akhavan S, Mannucci PM. Interaction between the G20210A mutation of the prothrombin gene and oral contraceptive use in deep vein thrombosis. Arterioscler Thromb Vasc Biol. 1999;19:700-703.
106. 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;88:3698-3703.
107. Koster T, Blann AD, Briët E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet. 1995;345:152-155.
108. van Hylckama Vlieg A, Van der Linden IK, Bertina RM, Rosendaal FR. High levels of factor IX increase the risk of venous thrombosis. Blood. 2000;95:3678-3682.
109. Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med. 2000;342:696-701.
110. de Visser MCH, Poort SR, Vos HL, Rosendaal FR, Bertina RM. Factor X levels, polymorphisms in the promoter region of factor X and the risk of venous thrombosis. Thromb Haemost. 2001;85:1011-1017.
111. van Hylckama Vlieg A, Rosendaal FR. Interaction between oral contraceptive use and coagulation factor levels in deep venous thrombosis. J Thromb Haemost. 2003;1:2186-2190.
112. MacLennan A, Lester S, Moore V. Oral oestrogen replacement therapy versus placebo for hot flushes. Cochrane

Database Syst Rev. 2001;CD002978.
113. Doren M, Samsioe G. Prevention of postmenopausal osteoporosis with oestrogen replacement therapy and associated compounds: update on clinical trials since 1995. Hum Reprod Update. 2000;6:419-426.
114. Villareal DT, Binder EF, Williams DB, Schechtman KB, Yarasheski KE, Kohrt WM. Bone mineral density response to estrogen replacement in frail elderly women: a randomized controlled trial. JAMA. 2001;286:815-820.
115. Watts NB. Therapies to improve bone mineral density and reduce the risk of fracture: clinical trial results. J Reprod Med. 2002;47:82-92.
116. Hutchinson TA, Polansky SM, Feinstein AR. Post-menopausal oestrogens protect against fractures of hip and distal radius. A case-control study. Lancet. 1979;2:705-709.
117. Weiss NS, Ure CL, Ballard JH, Williams AR, Daling JR. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med. 1980;303:1195-1198.
118. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002;288:321-333.
119. Hulley S, Furberg C, Barrett-Connor E, et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). JAMA. 2002;288:58-66.
120. Hulley S, Grady D, Bush T, et al, for the Heart and Estrogen/progestin Replacement Study (HERS) Research Group. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA. 1998;280:605-613.
121. Grady D, Herrington D, Bittner V, et al. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS-II). JAMA. 2002;288:49-57.
122. The ESPRIT team. Oestrogen therapy for prevention of reinfarction in postmenopausal women: a randomised placebo-controlled trial. Lancet. 2002;360:2001-2008.
123. Daly E, Vessey MP, Hawkins MM, Carson JL, Gough P, Marsh S. Risk of venous thromboembolism in users of hormone replacement therapy. Lancet. 1996;348:977-980.
124. Daly E, Vessey MP, Painter R, Hawkins MM. Case-control study of venous thromboembolism risk in users of hormone replacement therapy. Lancet. 1996;348:1027.
125. Jick H, Derby LE, Wald Myers M, Vasilakis C, Newton KM. Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopasusal oestrogens. Lancet. 1996;348:981-983.
126. Grodstein F, Stampfer MJ, Goldhaber SZ, et al. Prospective study of exogenous hormones and risk of pulmonary embolism in women. Lancet. 1996;348:983-987.
127. Perez Gutthann S, Garcia Rodriguez LA, Castellsague J, Duque Oliart A. Hormone replacement therapy and risk of venous thromboembolism: population based case-control study. Br Med J. 1997;314:796-800.
128. Grady D, Furberg C. Venous thromboembolic events associated with hormone replacement therapy. JAMA. 1997;278:477.
129. Varas Lorenzo C, Garcia Rodriguez LA, Cattaruzzi C, et al. Hormone replacement therapy and the risk of hospitalization for venous thromboembolism: a population-based study in southern Europe. Am J Epidemiol. 1998;147:387-390.
130. Hoibraaten E, Abdelnoor M, Sandset PM. Hormone replacement therapy with estradiol and risk of venous thromboembolism—a population-based case-control study. Thromb Haemost. 1999;82:1218-1221.
131. Grady D, Wenger NK, Herrington D, et al. Postmenopausal hormone therapy increases risk for venous thromboembolic disease. Ann Intern Med. 2000;132:689-696.
132. Scarabin PY, Oger E, Plu-Bureau. Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. Lancet. 2003;362:428-432.
133. Smith NL, Heckbert SR, Lemaitre RN, et al. Esterified estrogens and conjugated equine estrogens and the risk of venous thrombosis. JAMA. 2004;292:1581-1587.
134. Lowe G, Woodward M, Vessey M, Rumley A, Gough P, Daly E. Thrombotic variables and risk of idiopathic venous thromboembolism in women aged 45-64 years: relationships to hormone replacement therapy. Thromb Haemost. 2000;83:530-535.
135. Rosendaal FR, Vessey M, Rumley A, et al. Hormonal replacement therapy, prothrombotic mutations and the risk of venous thrombosis. Br J Haematol. 2002;116:851-854.
136. Herrington DM, Vittinghoff E, Howard TD, et al. Factor V Leiden, hormone replacement therapy, and risk of venous thromboembolic events in women with coronary disease. Arterioscler Thromb Vasc Biol. 2002;22:1012-1017.
137. Cushman M, Kuller LH, Prentice R, et al. Estrogen plus progestin and risk of venous thrombosis. JAMA. 2004;292:1573-1580.
138. Hoibraaten E, Qvigstad E, Arnesen H, Larsen S, Wickstrom E, Sandset PM. Increased risk of recurrent venous thromboembolism during hormone replacement therapy—results of the randomized, double-blind, placebo-controlled estrogen in venous thromboembolism trial (EVTET). Thromb Haemost. 2000;84:961-967.
139. McColl MD, Ramsay JE, Tait RC, et al. Risk factors for pregnancy associated venous thromboembolism. Thromb Haemost. 1997;78:1183-1188.
140. Kierkegaard A. Incidence and diagnosis of deep vein thrombosis associated with pregnancy. Acta Obstet Gynecol Scand. 1983;62:239-243.
141. Ginsberg JS, Wells PS, Brill-Edwards P, et al. Antiphospolipid antibodies and venous thromboembolism. Blood. 1995;86:3685-3691.
142. Simioni P, Prandoni P, Zanon E, et al. Deep venous thrombosis and lupus anticoagulant. Thromb Haemost. 1996;76:187-189.
143. Mateo J, Oliver A, Borrell M, Sala N, Fontcuberta J, and the EMET Group. Laboratory evaluation and clinical characteristics of 2,132 consecutive unselected patients with venous thromboembolism—results of the Spanish multicentric study on thrombophilia (EMET-study). Thromb Haemost. 1997;77:444-451.
144. de Groot PhG, Lutters B, Derksen RHWM, Lisman T, Meijers JCM, Rosendaal FR. Lupus anticoagulants and the risk of a first episode of deep venous thrombosis. J Thromb Haemost. 2005; 3: 1993-1997.
145. Tait RC, Walker ID, Reitsma PH, et al. Prevalence of protein C deficiency in the healthy population. Thromb Haemost. 1995;73:87-93.
146. Tait RC, Walker ID, Perry DJ, et al. Prevalence of antithrombin deficiency in the healthy population. Br J Haematol. 1994;87:106-112.
147. McColl M, Tait RC, Walker ID, Perry DJ, McCall F, Conkie JA. Low thrombosis rate seen in blood donors and their relatives with inherited deficiencies of antithrombin and protein C: correlation with type of defect, family history, and absence of the factor V Leiden mutation. Blood Coag Fibrinol. 1996;7:689-694.
148. Rosendaal FR. Risk factors for venous thrombotic disease. Thromb Haemost. 1999;82:610-619.
149. Heijboer H, Brandjes DPM, Büller HR, Sturk A, Ten Cate JW. Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis. N Engl J Med. 1990;323:1512-1516.
150. Koster T, Rosendaal FR, Briët E, et al. Protein C deficiency in a controlled series of unselected outpatients: an infrequent but clear risk factor for venous thrombosis (Leiden Thrombophilia Study). Blood. 1995;85:2756-2761.
151. Lensen RPM, Rosendaal FR, Koster T, et al. Apparent different thrombotic tendency in patients with factor V Leiden and protein C deficiency due to selection of patients. Blood. 1996;88:4205-4208.
152. Hasstedt SJ, Bovill EG, Callas PW, Long GL. An unknown genetic defect increases venous thrombosis risk, through interaction with protein C deficiency. Am J Hum Genet. 1998;63:569-576.
153. Lensen RP, Bertina RM, de Ronde H, Vandenbroucke JP, Rosendaal FR. Venous thrombotic risk in family members of unselected individuals with factor V Leiden. Thromb Haemost. 2000;83:817-821.
154. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol. 2000;110:939-945.
155. Lensen R, Bertina RM, Vandenbroucke JP, Rosendaal FR.

High factor VIII levels contribute to the thrombotic risk in families with factor V Leiden. Br J Haematol. 2001;114:380-386.
156. Demers C, Ginsberg JS, Hirsh J, Henderson P, Blajchman MA. Thrombosis in antithrombin III-deficient persons: report of a large kindred and literature review. Ann Intern Med. 1992;116:754-761.
157. Van Boven HH, Vandenbroucke JP, Briet E, Rosendaal FR. Gene-gene and gene-environment interactions determine risk of thrombosis in families with inherited antithrombin deficiency. Blood. 1999;94:2590-2594.
158. Branson HE, Marble R, Katz J, Griffin JH. Inherited protein C deficiency and coumarin-responsive chronic relapsing purpura fulminans in a newborn. Lancet. 1983;ii:1165-1168.
159. Mahasandana C, Suvatte V, Chuansumrit A, et al. Homozygous protein S deficiency in an infant with purpura fulminans. J Pediatr. 1990;117:750-753.
160. Bertina RM, Koeleman RPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369:64-67.
161. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet. 1995;346:1133-1134.
162. Rosendaal FR, Koster T, Vandenbroucke JP, ReitsmaPH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood. 1995;85:1504-1508.
163. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognised mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci U S A. 1993;90:1004-1008.
164. Koster T, Rosendaal FR, De Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to a poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet. 1993;342:1503-1506.
165. Rosendaal FR, Doggen CJM, Zivelin A, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost. 1998;79:706-708.
166. Bank I, Libourel EJ, Middeldorp S, et al. Prothrombin 20210A mutation: a mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study. Arch Intern Med. 2004;164:1932-1937.
167. Ceelie H, Spaargaren-van Riel CC, Bertina RM, Vos HL. G20210A is a functional mutation in the prothrombin gene; effect on protein levels and 3'-end formation. J Thromb Haemost. 2004;2:119-127.
168. Colucci M, Binetti BM, Tripodi A, Chantarangkul V, Semeraro N. Hyperprothrombinemia associated with prothrombin G20210A mutation inhibits plasma fibrinolysis through a TAFI-mediated mechanism. Blood. 2004;103:2157-2161.
169. von Ahsen N, Oellerich M. The intronic prothrombin 19911A>G polymorphism influences splicing efficiency and modulates effects of the 20210G>A polymorphism on mRNA amount and expression in a stable reporter gene assay system. Blood. 2004;103:586-593.
170. Jick H, Slone D, Westerholm B, et al. Venous thromboembolic disease and ABO blood type. Lancet. 1969;i:539-542.
171. Morelli VM, De Visser MC, Vos HL, Bertina RM, Rosendaal FR. ABO blood group genotypes and the risk of venous thrombosis: effect of factor V Leiden. J Thromb Haemost. 2005;3:183-185.
172. Kang SS, Zhou J, Wong PWK, Kowlisyn J, Strokosch G. Intermediate homocysteinemia: a thermolabile variant of methyenetetrahydrofolate reductase. Am J Hum Genet. 1988;48:536-545.
173. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10:111-113.
174. Kluijtmans LAJ, Den Heijer M, Reitsma PH, Heil SG, Blom HJ, Rosendaal FR. Thermolabile methylenetetrahydrofolate reductase and factor V Leiden in the risk of deep-vein thrombosis. Thromb Haemost. 1998;79:254-258.
175. Den Heijer M, Lewington S, Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost. 2005;3:292-299.
176. Fermo I, D'Angelo SV, Paroni R, Mazzola G, Calori G, D'Angelo A. Prevalence of moderate hyperhomocysteinemia in patients with early-onset venous and arterial occlusive disease. Ann Intern Med. 1995;123:747-753.
177. Den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med. 1996;334:759-762.
178. Simioni P, Prandoni P, Burlina A, et al. Hyperhomocysteinemia and deep-vein thrombosis: a case-control study. Thromb Haemost. 1996;76:883-886.
179. van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood. 2000;95:2855-2859.
180. van Hylckama Vlieg A, Rosendaal FR. High levels of fibrinogen are associated with the risk of deep venous thrombosis mainly in the elderly. J Thromb Haemost. 2003;1:2677-2678.
181. Kamphuisen PW, Houwing-Duistermaat JJ, van Houwelingen JC, et al. Familial clustering of factor VIII and von Willebrand factor levels. Thromb Haemost. 1998;79:323-327.
182. Kamphuisen PW, Lensen R, Houwing-Duistermaat JJ, et al. Heritability of elevated factor VIII antigen levels in factor V Leiden families with thrombophilia. Br J Haematol. 2000;109:519-522.
183. Zöller B, Berntsdotter A, Garcia de Frutos P, Dahlbäck B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. Blood. 1995;85:3518-3523.
184. Koeleman BPC, Reitsma PH, Allaart CF, Bertina RM. APC-resistance as an additional risk factor for thrombosis in protein C deficient families. Blood. 1994;84:1031-1035.
185. Koeleman BPC, Van Rumpt D, Hamulyak K, Reitsma PH, Bertina RM. Factor V Leiden: an additional risk factor for thrombosis in protein S deficient families? Thromb Haemost. 1995;74:580-583.
186. Van Boven HH, Reitsma PH, Rosendaal FR, et al. Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency. Thromb Haemost. 1996;75:417-421.
187. Emmerich J, Rosendaal FR, Cattaneo M, 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;86:809-816.
188. Conard J, Horellou MH, Van Dreden P, Lecompte T, Samama M. Thrombosis and pregnancy in congenital deficiencies in AT III, protein C or protein S: study of 78 women. Thromb Haemost. 1990;63:319-320.
189. De Stefano V, Leone G, Mastrangelo S, et al. Thrombosis during pregnancy and surgery in patients with congenital deficiency of antithrombin III, protein C, protein S. Thromb Haemost. 1995;74:793-794.
190. Hellgren M, Tengborn L, Abildgaard U. Pregnancy in women with congenital antithrombin III deficiency: experience of treatment with heparin and antithrombin. Gynecol Obstet Invest. 1982;14:127-141.