Risk Factors for Venous Thrombosis: Prevalence, Risk, and Interaction

Frits R Rosendaal

Annually, 1 in 1,000 individuals is affected by venous thrombosis. Risk factors that are known to increase the risk of thrombosis may be either genetic or acquired, or have a combined origin. Many of these risk factors are very frequent, among which several have been recently identified, such as resistance to activated protein C by factor V Leiden, hyperhomocysteinemia, high levels of factor VIII, as well as the classical acquired risk factors, such as surgery and malignancies. When the prevalence of risk factors is high, it becomes likely that in some individuals two or more risk factors will be present simultaneously. The question "What happens to the risk in these circumstances?" is one involving interaction, also known as effect modification or synergy. In this article we review the prevalence and risk estimates for the various genetic and acquired risk factors for venous thrombosis, discuss the concept of interaction, and give an overview of the evidence for interaction of these risk factors.

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One of the most tantalizing problems in thrombosis research in the last decade has recently appeared to be an issue of interaction. Since 1981, many families with hereditary protein C deficiency have been reported, which led to the conclusion that a heterozygous defect of protein C brought about a high risk of thrombosis. From these family studies it also seemed clear that the abnormality was uncommon (an estimate was 1/16,000 heterozygous individuals in the population).

Apparently in contradiction, in 1987, Miletich published the results of screening for protein C deficiency among blood donors, which yielded two surprising results: first, the prevalence of the defect was estimated at between 0.4% to 1.3%, and second, among these individuals and their deficient relatives, thrombosis appeared to be uncommon. Instead of a rare and severe disorder, protein C deficiency appeared to be a common and mild abnormality. It was later shown that the types of mutations leading to protein C deficiency in these blood donor families were not different from those found in protein C-deficient thrombophilia families.

In retrospect, however, it became clear that although in the classical thrombophilic families the thrombosis cosegregated with the defect, the disease was also frequently found in the family members who had normal protein C levels, they experienced thrombosis far more often than was to be expected from general population data. After resistance to activated protein C (APC) had been described as the most common thrombogenic clotting defect, it was shown that among the same families with protein C deficiency of previous reports, many also carried the factor V Leiden abnormality. Family members with neither defect had a low incidence of thrombosis, as in the general population, members with both defects had the highest risk, and those with one defect had an intermediate risk. This not only showed the synergistic effect of combined defects, but also explained the apparent discrepancy between the family studies and the blood donor studies. Families with thrombophilia are recognized because of the interaction of several defects that are present simultaneously in those families.

Recently, a very similar observation has been reported for antithrombin deficiency from the West of Scotland Blood Donor Study among blood donors with the deficiency and their deficient relatives, the frequency of thrombotic events was much lower than has been reported previously for thrombophilic families with antithrombin deficiency.

The study of interactions between risk factors has become relevant because it will help explain the differences in risks between individuals, and it has become possible because many risk factors for thrombosis are now known. Interaction is an issue whenever two or more risk factors are present simultaneously, the chances of such an occurrence depend on the prevalence of the individual risk factors.
factors In this article we discuss the established risk factors for venous thrombosis with regard to prevalence and risks, and subsequently, we review the available evidence on the interaction of risk factors.

VENOUS THROMBOSIS. GENERAL INTRODUCTION

Venous thrombosis has an overall frequency of about 1 in 1,000 individuals per year. It is uncommon in young individuals and becomes more frequent with advancing age. Its most frequent manifestation is thrombosis of the deep veins of the leg, which may have serious morbidity (post-thrombotic syndrome, respiratory insufficiency due to pulmonary embolism, bleeding complications of anticoagulant treatment) and, although rare, it may cause death due to pulmonary embolism.

Classical risk factors for deep vein thrombosis (DVT) include surgery, immobilization, fractures, puerperium, paralysis, prolonged bed rest, and use of oral contraceptives. In some instances, thrombosis appears to be hereditary occurring in families, and often causing thrombosis among individuals in these families at a young age and without apparent cause. This tendency to develop thrombosis has been called thrombophilia, which, if a genetic explanation is likely, is called hereditary thrombophilia. A list of important risk factors for venous thrombosis is given in Table 1. For most of these risk factors it is known whether they are acquired or genetic, however, for several, this is not known or a combined origin has been demonstrated. For instance, hyperhomocysteinemia may be the result of low vitamin intake or of defects in the enzymes in methionine metabolism, such as cystathionine synthase (CS) deficiency or a recently described genetic variant in the methylene-tetrahydrofolate reductase (MTHFR) gene.

PREVALENCE OF GENETIC ABNORMALITIES CAUSING THROMBOSIS

Estimates of the prevalence of deficiencies of protein C, protein S, antithrombin, and of resistance to APC have been derived from three sources: healthy individuals, unselected patients with venous thrombosis, and selected patients with venous thrombophilia. The results of several studies classified in this way are listed in Table 2.

For deficiencies of protein C and antithrombin, the prevalence has been investigated in a study of almost 10,000 blood donors. This led to prevalence estimates of 1 in 500 for protein C deficiency and 1 in 5,000 for type I antithrombin deficiency. The findings for protein C deficiency are similar to those of Miletich among more than 5,000 blood donors, who reported a prevalence of 1 in 250 individuals. For protein S deficiency, there are no studies of sufficient size among healthy individuals to reach an estimate of its prevalence.

Among consecutive patients with objectively confirmed DVT, deficiencies of protein C, protein S, and antithrombin combined are found in about 5%. APC-resistance is present in 20% of unselected patients with DVT, whereas it is very uncommon in Asians and Africans.

Among selected patients with venous thrombosis, higher prevalences of abnormalities can be found. The results depend on the selection criteria used, these are usually thrombosis at a young age, recurrent thrombotic events, thrombotic events that appeared to occur spontaneously, or thrombosis in

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<tr>
<th>Table 1 Risk Factors for Venous Thrombosis</th>
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<tr>
<td><strong>Acquired</strong></td>
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<td>Previous thrombosis</td>
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<td>Oral contraceptives</td>
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<td>Hormonal replacement therapy</td>
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<td>Myeloproliferative disorders</td>
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<td>Polycythemia vera</td>
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Table 2. Prevalence of the Major Thrombophilic Clotting Abnormalities (%)

<table>
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<tr>
<th>Abnormality</th>
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<th>Consecutive patients with first DVT</th>
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<tr>
<td>Protein C deficiency</td>
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<td>Protein S deficiency</td>
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<td>Antithrombin deficiency</td>
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Abbreviation: APC, activated protein C.
*DNA confirmed.
†Type I antithrombin deficiency.

patients with a positive family history. The reported prevalences among selected patient groups for deficiencies of protein C, protein S, and antithrombin are mostly between 5% and 10%. APC-resistance is found in more than half of all cases of hereditary thrombophilia, and is the most important cause of hereditary thrombosis.

The polymorphism in the prothrombin gene (20210 G to A) that has recently been described was found in 6% of unselected patients with thrombosis and in 18% of patients with familial thrombophilia. In a group of nearly 500 healthy individuals, the variant was present in 2%. The polymorphism is associated with increased levels of prothrombin, which suggests that these are the effectors of the thrombotic risk.

As with genetic abnormalities, the prevalences of high levels may differ between populations.

Homocysteine levels exceeding 18.5 μmol/L were found in 5% of the Dutch population and 10% of an Italian group of healthy individuals. Den Heijer found that the risk of venous thrombosis associated with hyperhomocysteinemia only became apparent when levels exceeded 18 μmol/L. Factor VIII:C levels exceeding 150 IU/dL were observed in 11% of healthy Dutch volunteers.

These measurements of homocysteine and factor VIII were all performed only once, so, although they represent the prevalence in the general population at a given time, it is unclear how many individuals have constantly increased levels and how many have only temporarily increased levels.

The prevalence of the acquired risk factors, such as surgery, immobilization, malignancy, pregnancy, puerperium, oral contraceptive use, and hormonal replacement therapy (HRT) all heavily depend on age; some, notably use of oral contraceptives and HRT, vary widely between societies.

RISK OF VENOUS THROMBOSIS

Two approaches have been used to assess the risk of venous thrombosis for individuals with
genetic clotting abnormalities: first, studies in family members of probands with one of these abnormalities; and second, population-based studies. These two types of studies may yield different information. Family studies are based on selected families in which the hereditability of the abnormality has been demonstrated. Typically, in these studies, the occurrence of thrombosis is compared between the family members with and without the clotting factor abnormality while the proband is excluded from the analysis. Because hereditability is a prerequisite in studies of this design, these studies are efficient in qualitatively answering questions concerning the risks associated with specific genotypes.

In studies that are based in the population, quantitative risk estimates can be obtained. In case-control studies, patients with thrombosis are compared to healthy individuals with regard to the prevalence of clotting factor abnormalities. The odds ratios that result from these studies are estimates of the relative risks, which indicate how much higher the risk of thrombosis is in the presence of a particular risk factor than in the absence of that factor. Because only individuals are included and not families, no direct conclusions about hereditability can follow from these studies. However, when unselected patients are included in a population-based study, and compared to appropriately chosen controls, the results on a particular risk factor for thrombosis apply, as an average risk, to all individuals with that risk factor in the population. This is not the case for family studies because these include families that were recognized and referred because of a conspicuously high frequency of thrombosis. So, strictly speaking, the results from the family studies only apply to families detected in a similar way, for they are conducted in a selected high-risk stratum of the population. Many of these selected families with thrombophilia have more than one thrombophilic abnormality, and therefore the results from family studies cannot be extrapolated to unselected individuals, nor vice versa. An individual found to have a thrombogenic abnormality in a study of healthy individuals from the general population will most likely be a carrier of only that one abnormality; if he is recognized as proband in a family with thrombophilia, he may well be a carrier of two or more defects; if he is an unselected patient with thrombosis, he may be either.

The dominant effect of selection on the apparent severity of disease can also be shown by looking at the age of onset. In families selected because of familial thrombophilia, the first thrombosis occurred around the age of 30 years for individuals with protein C deficiency and factor V Leiden alike. In unselected patients with thrombosis and these same abnormalities, the age at which thrombosis occurred was 45 years, again without any difference according to the type of defect. This implies that if patient groups are compared that have been selected with slightly different criteria, differences in clinical severity may be observed that are the result of the selection criteria and not of true differences in severity.

Protein C Deficiency

Many families with hereditary protein C deficiency have been reported since 1981. Heterozygous protein C deficiency increases the risk of thrombosis without any apparent difference according to type of deficiency (type I, low plasma level; and type II, functional defect) or to the underlying mutation. DVT of the leg is the most common manifestation, although thrombosis may occur at a variety of other sites.

Family studies have shown that family members who are protein C-deficient have an 8- to 10-fold increased risk of venous thrombosis; by the age of 40 years, about half of them will have experienced at least one thrombotic event. In these families, many thrombotic events occur spontaneously, ie, without any obvious cause.

The relative risk of 6.5 obtained from a population-based study is very similar to this result from family studies. The prevalence of protein C deficiency as found in unselected patients with a first thrombotic event (3%) and healthy individuals from the general population (0.2%) is in accordance with a relative risk of this magnitude.

Protein S Deficiency

Families with protein S deficiency and venous thrombosis have been reported since 1984. Whereas the clinical symptoms are similar to those found in protein C deficiency, it is not clear whether the different types of protein S deficiency that have been described (type I, low plasma concentrations of total and free protein S; type II, functional defect; type III, low free protein S) lead to similar risks of thrombosis. Type I and type III
protein S deficiency have recently been reported to be phenotypic variations of the same genotype. The mutations in the protein S gene have been reported to be associated with protein S deficiency.

The prevalence of protein S deficiency in the general population is unknown and estimates of incidence rates in families are lacking, so the risk of thrombosis associated with protein S deficiency has not been quantitated. In a population-based case-control study, no relation between protein S deficiency and thrombosis could be established. Although the numerous reports on protein S–deficient kindreds support an increased risk of venous thrombosis, the evidence is much less solid than for protein C deficiency, and the risks are not known quantitatively.

### Antithrombin Deficiency

Since 1965 numerous families with antithrombin deficiency have been reported. The clinical symptoms of antithrombin deficiency closely resemble those of protein C and protein S deficiency, although superficial thrombophlebitis seems to occur less often. A large number of mutations associated with antithrombin deficiency have been described. Type I (low levels in plasma) and type II (functional defect) are both associated with thrombophilia; type IIc, however, only causes a severe form of thrombophilia in the homozygous individual.

Antithrombin deficiency appears to be more severe than deficiencies of protein C and protein S. Thrombosis may occur at a young age, even earlier than 16 years, and about half the patients suffer a first thrombotic event before age 25. The 50-fold difference between prevalence among patients with a first event of DVT and prevalence in a healthy population supports a higher thrombotic risk in antithrombin deficiency than in protein C deficiency. In a direct comparison in a population-based study, however, such a difference was not found.

### APC Resistance

Resistance to APC was first described by Dahlbäck in 1993. The defect is associated with an abnormality in clotting factor V, and this mutation (factor V R506Q, factor V Leiden) appears responsible for the large majority of cases of APC resistance. APC resistance is far more common than the other forms of hereditary thrombophilia (Table 2).

In a family study, the risk of thrombosis was clearly higher in family members who were APC-resistant than in those who were not. Approximately 25% of the patients with APC resistance had suffered thrombosis before the age of 50 years. This risk is lower than the figures reported for families with protein C deficiency, which may indicate a lower thrombotic risk associated with APC resistance than with protein C deficiency. The discrepancy may also be the result of selection bias (in the protein C–deficient families). Because APC resistance is common, families with APC resistance may not have been as heavily selected on the severity of thrombophilia as families with protein C deficiency in previous studies. The relative risk associated with APC resistance observed in a population-based case-control study did not differ from that found for protein C deficiency (relative risk of 7 for APC resistance, and 6.5 for protein C deficiency), which suggests that the two abnormalities do not differ in severity.

### Factor II 20210 G → A

The prevalence of this variant was 6.2% in consecutive patients with thrombosis and 2.3% in healthy control subjects, which yielded a relative risk of 2.8 for carriers of the variant versus noncarriers. This implies that this is a relatively frequent risk factor, which confers less of a risk than deficiencies of protein C, protein S, antithrombin, or factor V Leiden. The allele frequency was determined among 474 healthy subjects, and it has a considerable statistical uncertainty (95%-confidence interval of the prevalence of carriers 1.0% to 3.6%). In a group of 100 healthy volunteers, 1% carried the A-allele, and in a second large sample of more than 600 healthy men from Leiden, 1.2% carriers were observed (Doggen CJ, personal communication, March 1997). If the prevalence in the population is around 1% rather than the 2% observed in the Leiden Thrombophilia Study, the relative risk might be higher (for a prevalence of 1%, it would be 6, ie, similar to that of the other hereditary defects leading to thrombophilia). The polymorphism is closely related to factor II levels, which in turn are associated with the risk of thrombosis (Table 3). A factor II level exceeding 115 IU/dL increases the risk of DVT twofold.
Hyperhomocysteinemia

In two case-control studies, hyperhomocysteinemia has been shown to increase the risk of DVT. In both studies, a 2.5-fold increased risk was found for levels exceeding 18.5 μmol/L, and a three- to fourfold increased risk for levels exceeding 20 μmol/L. Among patients with juvenile thrombosis, a high prevalence of hyperhomocysteinemia has been reported, which was most apparent on post-methionine loading homocysteine measurements (the studies by Simioni and den Heijer were based on fasting homocysteine measurements). Among patients with a first episode of venous thrombosis before age 40, Falcon found 19% with hyperhomocysteinemia. From this study we can infer a more than 10-fold increased relative risk because among healthy young individuals the abnormality is rare. This higher relative risk in the younger age groups could not be confirmed in the Dutch study. Hyperhomocysteinemia has also been shown to increase the risk of recurrent thrombotic events.

Hyperhomocysteinemia may be the result of several underlying abnormalities, genetic as well as environmental. Of the latter, low vitamin intake (notably vitamins B6, B12, and folic acid) are the most common. Heterozygous carriage of CS deficiency, the abnormality that in the homozygous form causes classic homocystinuria, is an infrequent genetic cause. Far more common is the recently described variant of the MTHFR gene that leads to a thermolabile variant of this enzyme and to mildly increased levels of homocysteine. Surprisingly, homozygous carriage of this variant is not associated with an increased risk of venous thrombosis. It is difficult to reconcile these findings with those of an increased risk of venous thrombosis caused by hyperhomocysteinemia. It may be that the homocysteine levels with this variant are not sufficiently increased to cause thrombosis, or that hyperhomocysteinemia itself will not lead to thrombosis unless, for instance, folate levels are normal. Finally, because genotypes are invariant whereas homocystine levels can be affected by other risk factors, it cannot be ruled out that hyperhomocysteinemia is a marker of either disease or risk factor status rather than a cause of disease. Nevertheless, hyperhomocysteinemia is important in the etiology of thrombosis because no less than 5% in the Dutch and 10% in the Italian control group of healthy individuals had levels over 18.5 μmol/L, which were associated with an increased risk.

High Levels of Factor VIII

Factor VIII levels exceeding 150 IU/dL are associated with a sixfold increased risk as compared to levels below 100 IU/dL. Because blood group and von Willebrand factor (vWF) are strong determinants of the factor VIII concentration, these are also risk factors for DVT: individuals with non-O blood groups have a twofold increased risk compared to subjects with other ABO blood groups, and persons with vWF levels exceeding 150 IU/dL have a threefold increased risk compared to those with levels less than 100 IU/dL. The effect of blood group has been known since the 1970s, and, interestingly, has also been observed for arterial vascular disease. However, blood group and vWF levels are only risk factors because they affect the factor VIII level, which implies that the risk will not be increased in an individual with non-O blood group or high vWF levels if the factor VIII concentration is normal. These findings were based on measurements after the thrombotic event, which includes the possibility of post-hoc increases in factor VIII:C. Recently, O’Donnell has shown that among patients with thrombosis, the high factor VIII levels are likely to be based on increased factor VIII synthesis, and are not associated with signs of acute phase reaction.

High factor VIII levels are very frequent and the relative risk is high, which implies that high factor VIII levels are among the most important causes of thrombosis. For factor VIII levels exceeding 150 IU/dL, which are found in 11% of the population and which increase the risk 6-fold, the population attributable risk is 35%. This indicates that (assuming a causal relation) 35% of all events of DVT in

| Table 3. Prothrombin Levels, Prothrombin Genotype, and Risk of Thrombosis |
|-----------------------------|-----------------------------|-----------------------------|
| Plasma Prothrombin Level (IU/dL) | Relative Risk (OR) | Prevalence of 20210 A Genotype |
| <95 | 1 | 0 |
| 95-104 | 1.3 | 2.8 |
| 104-115 | 1.4 | 6.9 |
| >115 | 2.2 | 18.2 |

Abbreviation: OR, odds ratio. Results are given for 424 patients and 474 controls for whom DNA was available and who were not on oral anticoagulant treatment.
the population can be attributed to high factor VIII levels (for protein C deficiency, this is only 3%).

**Oral Contraceptives and Other Hormonal Steroids**

The thrombogenicity of the pill has been known since 1961 when Jordan reported pulmonary embolism in a nurse who had just started oral contraception. Since then, numerous reports in the 1960s and 1970s have confirmed that oral contraceptives increase the risk of venous as well as arterial thrombosis. The early oral contraceptives contained a high level of estrogen (100 μg and more), which over the decades has been decreased to reduce the risk of thrombosis. The evidence that this has in fact led to a reduction of the risk of venous thrombosis is scarce. The early case-control studies in the 1960s found relative risks for idiopathic DVT ranging from 4 to 8. In the 1970s, a large case-control study found a relative risk of 11 in users versus nonusers, and a prospective cohort study reported a relative risk of 4. A prospective study in the 1980s found a relative risk of 7. In the most recent studies in the 1990s, the risks reported are not substantially different from the early reports. In the International World Health Organization (WHO) study, oral contraceptives were associated with a 4.2-fold increased risk; in the Transnational study with a fourfold increased risk; whereas in the Leiden Thrombophilia Study the age-adjusted relative risk was 6. In the latter study, the risk conferred by oral contraceptives with 30 μg ethinylestradiol and 50 μg ethinylestradiol was the same. In another report, however, oral contraceptives containing 50 μg ethinylestradiol or more appeared to confer a higher risk than those containing less than 50 μg. Similar dose-related results have been reported from Sweden. It has been argued that the finding of a decreasing rate of thrombosis with lower estrogen dose was biased by differences in diagnostic methods for DVT and age differences between women using different formulations.

Even if the risk of venous thrombosis has decreased with lower estrogen content, it is clear that this has not been a dramatic decrease and that the risk of venous thrombosis is still present with the current low-dose formulations. There are no data concerning the newest oral contraceptives containing less than 30 μg ethinylestradiol, therefore, claims regarding their superior safety are unfounded. Possibly the most convincing evidence that it is unjustified to assume an ever-decreasing risk of thrombosis with decreasing amounts of estrogen has come from the reports on hormonal replacement therapy. These contain an amount of estrogen that is equivalent to about 5 μg of estradiol, ie, a very low dose compared to oral contraceptives, and still were found to increase the risk of venous thromboembolism 2.1- to 3.6-fold. When investigated in healthy volunteers, the effects on the hemostatic system of oral contraceptives containing either 30 or 50 μg ethinylestradiol did not differ. The thrombogenicity of oral contraceptives is not necessarily partly or completely mediated by the hemostatic system and alternative hypotheses, eg, an immunologic response to estrogens, have been proposed for which a dose-related risk is not even plausible.

Because oral contraceptives are used by young women among whom the incidence of thrombosis is low, the absolute risk brought about by use of oral contraceptives remains low: from approximately 1 per 10,000 women per year to 4 per 10,000 women per year. On the other hand, because oral contraceptives are widely used, they are the most important cause of thrombosis in young women.

Recently, several studies have shown that it is not just the estrogen component in oral contraceptives that is responsible for the risk of venous thrombosis: preparations containing a so-called third-generation progestogen (desogestrel, gestodene) lead to a twofold higher risk of thrombosis than products containing a second-generation progestogen (mostly levonorgestrel). Between the various studies, extensive adjustment for possible confounders, eg, age, family history, factor V Leiden carrierhip, duration of use, previous pregnancy, and obesity, did not alter the findings. It has subsequently been shown that mortality from venous thromboembolism has increased among young women in the United Kingdom and the Netherlands since the mid-1980s, since oral contraceptives with third-generation progestogens have been increasingly in use.

**Pregnancy and Puerperium**

The estimates of the incidence of thrombosis in pregnancy and puerperium vary widely. In two large series, thrombosis in pregnancy was found in 0.13 per 1,000 women and 0.7 per 1,000 women, which translates into incidence rates of 0.17 to 0.93
per 1,000 (pregnant) women-years. These incidence rates are lower than the overall incidence rates for venous thrombosis; this is, however, the result of pregnant women being younger than the general population. Among women aged less than 30, Nordström found an incidence of 0.075 per 1,000 women-years, which is clearly lower than the rates reported in pregnancy.\(^{102}\) In the Leiden Thrombophilia Study, pregnancy was associated with a fourfold increased risk of venous thrombosis.\(^{81}\)

The thrombotic risk is greater in puerperium than in pregnancy. Per 1,000 birth-giving women, it is estimated that 2.3 to 6.1\(^{75}\cdot 144\) will experience thrombosis postpartum. This indicates that a pregnant women has a three to five times higher chance of developing thrombosis shortly after than during pregnancy; and also, because the postpartum period is much shorter than the pregnancy, that the “thrombogenicity” of the postpartum period is much higher than that of pregnancy (20- to 30-fold higher incidence rate).

**Surgery and Trauma**

The risk of thrombosis is greatly increased during surgery, mostly during orthopedic surgery and neurosurgery. In hip and knee surgery, as well as major hip and knee trauma, the risk of thrombosis reaches 30% to 50%.\(^{28,64,67,103}\) The risk is also high, up to 30%, in abdominal surgery, gynecologic surgery, and urologic surgery (especially open prostatectomy).\(^{105,101,151}\) The risk of thrombosis is increased in all forms of major injury,\(^{48}\) with risk estimates of 54% in patients with major head injury, 62% of patients with spinal injury, 61% of patients with pelvic fractures, 80% of patients with femoral fractures, and 77% of patients with tibial fractures.\(^{48}\)

**Malignant and Other Diseases**

Patients with malignancies have an increased incidence of venous thrombosis. In a population-based study in urban Sweden, which included 366 patients with a first or recurrent DVT, 19% of the patients had a malignancy known at the time of the diagnosis of thrombosis, and an additional 5% were diagnosed with malignancy in the year after the thrombosis diagnosis.\(^{102}\) These figures obviously exceed the expected prevalence of malignancies in a control group of individuals without thrombosis. An approximation of the relative risk of thrombosis brought about by malignancies was reached by following patients with and without DVT after (positive and negative) testing for DVT after.\(^{51}\) The relative risk was several-fold increased and very high in those aged younger than 50 (relative risk, 19). The risk of thrombosis is particularly high in (mucin-producing) adenocarcinomas, eg, gastric carcinoma and pancreatic carcinoma.

Other medical conditions predisposing to thrombosis are those associated with immobilization, particularly paralysis\(^{152};\) cardiac disease\(^{84};\) myeloproliferative disorders\(^{27};\) and antiphospholipid antibodies (lupus anticoagulant or anticardiolipin antibodies).\(^{66,89}\)

THE CONCEPT OF INTERACTION

Interaction, also known as effect modification or synergism, is present when the risk in the presence of two risk factors exceeds the sum of the separate effects of the two factors.\(^{123}\) Stated differently, effect modification is present when a certain risk factor has a different effect in the presence of another factor than in the absence of that factor. So, under an interactive effect, more people with a combination of risk factors develop the disease than would be expected based on the disease incidences for the risk factors when present separately.\(^{91}\) This does not necessarily imply any knowledge of the mechanism of action of the risk factors, or of their combination, nor does the presence of interaction indicate common mechanisms.\(^{131}\)

Table 4 shows a hypothetical example. When the background incidence is 1 per 1,000 (per unit of time), A adds one patient to this background risk. When only B is present, the number of diseased individuals increases from one to three, ie, B adds two. The expected number of patients for the combination of A and B is therefore one (background), plus one (A), plus two (B), which is 4 per 1,000. The expected incidence of 4 per 1,000 when there is no interaction is associated with a risk difference of 3 per 1,000 (compared to the category without A and B) and a relative risk of 4.

Here we will use this model for interaction and

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<th>Risk Factor A</th>
<th>Risk Factor B</th>
<th>Incidence</th>
<th>Risk Difference</th>
<th>Relative Risk</th>
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<tr>
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</tr>
<tr>
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<td>-</td>
<td>2/1000</td>
<td>1/1000</td>
<td>2</td>
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<td>+</td>
<td>3/1000</td>
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<td>3</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>?</td>
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</tbody>
</table>

*Reference category.
define departures from additivity as an indication for interaction. Several additional arguments for this definition and a technical discussion are given in the Appendix.

GENE-GENE INTERACTION

Homozygous Defects

Double defects in the same gene are a first example of so-called gene-gene interaction. Patients homozygous for protein C deficiency have been reported. Because the allele frequency of protein C deficiency is low, the patient with a double defect is rare and may often be the result of consanguinity. In these patients protein C activity in plasma is absent or very low, and the thrombotic tendency is usually very high, with severe thrombosis (purpura fulminans) developing shortly after birth. Homozygous protein S deficiency has also been reported, and while extremely rare, it appears as severe as homozygous protein C deficiency. Homozygous antithrombin deficiency is extremely rare and probably incompatible with life: two siblings with homozygous antithrombin deficiency died within 3 weeks after birth. Antithrombin type II heparin-binding site deficiency only leads to increased thrombotic risk in the homozygous form.

Because of its high allele frequency, homozygous carriers of the factor V Leiden are more common. The homozygous abnormality appears much less severe than homozygous protein C deficiency and several of the homozygous patients have remained thrombosis-free well into adult life. Still, the risk of homozygous carriership is 10-fold greater than that of heterozygous carriership, and 90-fold greater compared to individuals without the mutation. Also, these individuals experience thrombosis at a younger age than those with heterozygous factor V Leiden. The majority of asymptomatic factor V Leiden homozygous patients are women; therefore, it seems likely that the estimate of a 90-fold greater risk is partly the result of interaction with sex, and specifically the use of oral contraceptives. This may indicate a less extreme increased risk in men who are homozygous carriers of factor V Leiden or in women who do not use oral contraceptives.

Very high levels of homocysteine are found in homocystinuria, first described in the 1960s. Because of a homozygous deficiency of CS, which catalyzes homocysteine to cystathionine, the levels of homocysteine become so high that homocysteine is excreted in the urine. This classical form of homocystinuria is a severe inborn error of metabolism, which is associated with mental retardation, skeletal abnormalities, ectopia lentis, and arterial vascular disease as well as venous thrombosis. The prevalence is 1 in 335,000 live births. With regard to venous thrombosis—and the same holds true for arterial disease—the risk appears much higher in homocystinuria than in hyperhomocysteinemia, although not as devastatingly high as in homozygous protein C deficiency.

Because homocysteine may be metabolized via two pathways, of which the vitamin B6-dependent transsulfuration by CS to cysteine is one, and the vitamin B12 and folic acid-dependent remethylation to methionine by methionine synthase is the other, hyperhomocysteinemia may be the result of defects in either of the pathways—or in the vitamins that are involved as coenzymes. Whereas homocystinuria is the result of a homozygous defect in the transsulfuration of homocysteine, heterozygous CS deficiency is rarely the cause of hyperhomocysteinemia: more often mildly increased homocysteine levels are the result of poor remethylation due to either low intake of folic acid and vitamin B12, or to genetic defects in this pathway. A recently described variant of the enzyme MTHFR is a very common abnormality leading to increased levels of homocysteine due to inadequate remethylation. The variant is present in homozygous form in about 10% of the general population. Several studies, although not all, report that carriership of this variant in the homozygous form, although associated with increased homocysteine levels, does not affect the risk of venous thrombosis. The variant leads to a reduction of 50% of the normal activity of MTHFR, when the enzyme activity is absent, as in homozygous MTHFR deficiency, a severe homocystinuria is the result. This form accounts for about 10% of homocystinuria, and thus is even more rare than homozygous CS deficiency.

Combined Genetic Defects

Combinations of deficiencies of protein C, protein S, and antithrombin have been reported, but are extremely rare due to the low allelic frequency of each of these defects (and also because consanguinity will not lead to an increased frequency of these combinations as it will for the homozygous form of
each of the individual deficiencies). Factor V Leiden is common, however, and combinations with deficiencies of protein C, protein S, and antithrombin have been described. Although analyzed in a variety of ways, the reports on combined defects all indicate a higher risk for the combined defect than for the single defect, which, however, is not so high as the risk for homozygous protein C or protein S deficiency. In thrombophilic families in which protein C deficiency and factor V Leiden are both present, a history of thrombosis was present in 31% of individuals with protein C deficiency only, in 13% of individuals with factor V Leiden only, and in 73% of individuals with both defects (analysis among sibships in which both defects were segregating). In families with thrombophilia with antithrombin deficiency, the risk of a combination of this defect with factor V Leiden was even higher: whereas 57% of individuals with only antithrombin deficiency had a history of venous thrombosis, and 20% of those with factor V Leiden only, 11 of the 12 carriers of both defects (92%) had suffered venous thrombosis. In families with thrombophilia with antithrombin deficiency, the risk of thrombosis was also higher in those with a combined defect, i.e., protein S deficiency and factor V Leiden, than in those with either of the two defects.

In all these instances of more than one defect, i.e., the homozygous abnormalities, and the combination of several defects in the natural coagulation inhibition mechanisms, the available data do not allow an evaluation of interaction with the concept outlined above. Because the results are reported as age-of-onset, or prevalence of a history of thrombosis, and more importantly, because the families were often selected on the presence of one of the defects, it is not possible to examine if the incidence of thrombosis in the presence of two defects exceeds the sum of the incidences of each of the two defects separately. In fact, little more can be concluded than that the risk for combined defects in the natural clotting inhibitors is higher than for single defects. For homozygous protein C or protein S deficiency the risk becomes nearly absolute. All studies on combinations of two different defects in the natural clotting inhibition pathways (mostly combinations of factor V Leiden with a deficiency of either antithrombin, protein C, or protein S) report very high risks of ever experiencing thrombosis. It should be indicated, however, that all these studies were performed in highly selected families, and it cannot be ruled out that these families may have other yet unknown thrombogenic defects.

**GENE-ENVIRONMENT INTERACTION**

Interaction is most readily observed, and also most relevant, for risk factors with a high prevalence and therefore has a high probability of being present simultaneously in one individual. Risk factors that have such a high prevalence are surgery, immobilization, pregnancy and puerperium, use of sex steroids (oral contraceptives, HRT) for the acquired risk factors, and factor V Leiden, hyperhomocysteinemia, and high levels of factor VIII for the endogenous risk factors. Several of the possible interactions between these factors have been studied.

**Hyperhomocysteinemia and Factor V Leiden**

It is unclear if hyperhomocysteinemia and factor V Leiden have synergistic effects. Mandel, in a study of 45 individuals from seven families with (homozygous) homocystinuria, found venous thrombosis to be associated with hyperhomocysteinemia only in individuals who were also carriers of factor V Leiden. This observation suggests a strong interaction, in which factor V Leiden is a prerequisite for the thrombogenic effect of hyperhomocysteinemia. The interaction is not absolute, however, because other homozygous CS deficient homocystinuric patients have been reported to suffer venous thrombosis while free of factor V Leiden (or deficiencies of protein C, protein S, and antithrombin). For mild hyperhomocysteinemia, there can be little doubt that it is associated with an increased risk of thrombosis also in the majority of individuals free of factor V Leiden (or deficiencies of protein C, protein S, and antithrombin). In the Leiden Thrombophilia Study, isolated hyperhomocysteinemia and isolated factor V Leiden each increased the risk of thrombosis, indicating that neither factor is required for the other factor to have an effect; the risk when both factors were present did not exceed these separate risks, i.e., there was no sign of interaction. The distribution of the abnormalities over a mixture of cases with arterial or venous occlusive disease reported by D'Angelo suggests a more than additive effect when re-
evaluated as a case-case analysis.\textsuperscript{74} Within the Physicians' Health Study, the risk for the combined abnormalities also exceeded the risk for factor V Leiden and hyperhomocysteinemia alone.\textsuperscript{119} It is difficult to reach a conclusion from these widely varying results: a relative risk of only 2.0 for the combined defect (as compared to individuals with neither factor V Leiden nor hyperhomocysteinemia) in the Dutch study,\textsuperscript{39} and 21.8 in the American study\textsuperscript{119} (both using a 95th percentile cutoff point for hyperhomocysteinemia). One explanation may be the wide statistical uncertainty resulting from the small number of individuals with the combined defect in these studies. A clue for a biological explanation may be that the Leiden Thrombophilia Study\textsuperscript{39} included all patients with thrombosis, of all ages, whereas in the other series the results concerned young patients,\textsuperscript{31} or were most striking for idiopathic thrombosis.\textsuperscript{119}

**Oral Contraceptives and Thrombophilic Defects**

Oral contraceptives lead to a high risk of thrombosis in deficiencies of natural anticoagulant proteins, especially in antithrombin deficiency, with an estimated incidence of 27% per year.\textsuperscript{106} For factor V Leiden, the interaction with oral contraceptives has been shown in a population-based case-control study, ie, in consecutive patients with thrombosis (Table 5).\textsuperscript{149}

As Table 5 shows, the risk of thrombosis in users of oral contraceptives who are carriers of the factor V Leiden mutation exceeds the sum of the separate effects of these two risk factors.\textsuperscript{149} In a subsequent analysis, this interaction was most striking for oral contraceptives containing a third-generation progesterogen.\textsuperscript{13}

Among homozygous carriers of the factor V Leiden mutation there is a preponderance of women among symptomatic patients, the majority of whom have used oral contraceptives.\textsuperscript{120,122} In a series of homozygous patients, 80% of the women with thrombosis had been using oral contraceptives.\textsuperscript{120}

**Pregnancy, Puerperium, and Thrombophilic Defects**

In women from families with familial thrombophilia due to deficiencies of protein C, protein S, or antithrombin, or due to factor V Leiden, the risk of thrombosis in pregnancy and puerperium is extremely high, with estimates ranging from 10% to more than 40%.\textsuperscript{29,36,61} The risk is highest in antithrombin deficiency, whereas for protein S deficiency the risk during pregnancy does not appear to be increased. These risk estimates indicate a synergistic effect but may largely be restricted to women from selected families with thrombophilia. In a recent study of female relatives of probands with thrombophilia, in which the proband was excluded from the analysis, the overall risk of pregnancy-associated thrombosis (pregnancy and puerperium) was 4.1% in women with antithrombin, protein C, or protein S deficiency.\textsuperscript{46} Although substantially less than the previous estimates, this is still much higher than the risk in nondeficient women.

Deficiencies of protein C, protein S, and antithrombin are rare, and therefore even a highly increased risk of thrombosis in pregnancy will only concern a fraction of all pregnancies. This is different for the much more common factor V Leiden. Among women with thrombosis during pregnancy, 20% to 60% proved to be APC-resistant in subsequent investigations.\textsuperscript{15,60,62}

**Antiphospholipid Antibodies and Factor V Leiden**

Antiphospholipid antibodies are a common cause of thrombosis,\textsuperscript{14} and therefore a combination of this abnormality with factor V Leiden carriership may be encountered. Although it has been shown that factor V Leiden carriership is not a prerequisite for the occurrence of thrombosis in the antiphospholipid syndrome,\textsuperscript{40} the simultaneous presence of both abnormalities in one patient may lead to a severe thrombotic tendency.\textsuperscript{20} In a series of 78 women with a history of thrombosis, the combination of anticardiolipin antibodies or lupus anticoagulant and resistance to APC was found in 22%, which suggests a strong synergistic effect.\textsuperscript{14} Similar results have been observed by others.\textsuperscript{105,132}

<p>| Table 5. Factor V Leiden and Oral Contraceptives: Single and Combined Effects |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Factor V Leiden</th>
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</table>

*Reference category.
**Factor V Leiden and Malignancies**

Because malignancies are often the underlying disease in thrombosis, it is important to know if the thrombotic risk is modified in the presence of APC resistance. In one article, it is reported that among patients with malignancies, carriers of the factor V Leiden mutation had a fivefold increased risk. Given the high risk brought about by malignancies, this indicates a synergistic effect.

**Surgery and Thrombophilic Defects**

The overall frequency of thrombotic complications in surgery in individuals with deficiencies of protein C, protein S, or antithrombin has been estimated at about 20%. It is unclear whether surgery in factor V Leiden carriers has a different thrombotic risk from surgery in other individuals. From the Leiden Thrombophilia Study, no interaction of more than an additive nature was apparent. It may well be that surgery is such a strong risk factor for venous thrombosis, that the simultaneous presence of other risk factors loses its importance. It should be noted, however, that the issue is confounded by the use of anticoagulant prophylaxis for many surgical interventions.

**CONCLUSION**

When a risk factor is established as such, it can be said that it increases the risk by a certain amount, or that it increases the probability of disease in those exposed to that factor as compared to those not exposed to it. The prior probabilities of disease are higher in those with the risk factor than in those without it. Some individuals who were exposed, however, will not experience thrombosis, and some who were not exposed will. So, in retrospect, each individual either increases his prior probability to unity, or decreases it to zero. It may be argued that the differences between these real events, and the prior probabilities, are all the result of interaction: among those with a specific overall or average risk of disease, there are subgroups with a much higher risk, and subgroups with a much lower risk. The factors that discriminate between these subgroups are the factors that lead to interaction.

Future research will be aimed at further elucidating interactive effects because this will help to pinpoint an individual's risk of thrombosis, and give insights into the biological mechanisms underlying thrombosis.

**APPENDIX**

The risk brought about by a factor can be expressed in absolute and relative terms, i.e., as the difference of the disease frequencies in the presence or absence of the risk factor (incidence rate difference, or risk difference) or as the ratio of these two frequencies (incidence rate ratio or relative risk). This has led to the concepts of additivity and multiplicativity. Under an additive model, interaction is said to be present when the combined effect of two factors exceeds the sum of the separate effects in terms of incidence rates, i.e., relative risks. Under a multiplicative model, the expected effect is defined multiplicatively based on incidence ratios, i.e., relative risks, and interaction is considered present when the combined effect exceeds the product of the two relative risks. This is shown in a hypothetical example in Table 4.

In an additive model, the expected incidence of disease for the combined presence of risk factors A and B in Table 4 is 4/1,000, the sum of the disease incidences of the separate effects. The risk difference, always in reference to those with neither of the risk factors, is 3/1,000. In this way, the effect of factor A is to add one case of disease in the absence of factor B (from 1/1,000 to 2/1,000) as well as in the presence of factor B (from 3/1,000 to 4/1,000). If the combined effect differs from this expected incidence of 4/1,000, interaction is considered to be present.

In a multiplicative model, the expected effect (of no interaction) is derived from the relative risks. Factor A doubles the risk in the absence of factor B (from 1/1,000 to 2/1,000), and is therefore also expected to double the risk in the presence of factor B (from 3/1,000 to 6/1,000). Therefore, under a multiplicative model, interaction is considered to be present when the combined effect exceeds, or more generally differs from, the expected frequency of 6/1,000.

It is obvious from this example that when risk differences are constant, as in an additive model, relative risks will not be constant in the example of Table 4. When the combined effect of A and B has an incidence of 4/1,000 (and thus an overall relative risk of the combination of 4), A will have a relative risk of 2 in the absence of B, and 1.25 in the presence of B. Conversely, when relative risks are constant, as assumed under a multiplicative model, risk differences will not be constant when factor A doubles the risk regardless of the presence or absence of factor B (and therefore factor B triples the risk regardless of the presence or absence of factor A), factor A will add 1/1,000 in the absence of factor B, and 3/1,000 in the presence of factor B.

Many techniques that are used to estimate the effect of risk factors, especially those used for multivariate analysis such as calculation of the Mantel-Haenszel odds ratio or the use of logistic regression, assume multiplicativity of risk factors. There are several reasons, however, to prefer a definition of departure from additivity as a sign of interaction. MacMahon and Trichopoulos state that comparisons of effects in additive models are intuitively more appealing, and more relevant for public health policies. The first argument is that, whereas it may be computationally convenient in regression models to assume multiplicativity, there is no biologically plausible reason why risk factors should multiply one another.
health argument is that when we say that the effect of a risk factor is constant when its relative risk is constant, we will call very different effects the same. For instance, if a factor doubles the risk of thrombosis for each age class, this may be considered negligible in the very young among whom thrombosis is extremely rare, but may lead to a large number of excess cases of thrombosis in older individuals, among whom thrombosis is prevalent.

Rosman even takes the view that the additive model, being the parsimonious one, will have the closest association with the underlying disease mechanism, and asserts that departures from additivity indicate interaction on the basic cellular or biochemical level.[27]

A final argument to use departures from additivity as the yard stick for interaction is that it conforms to the view of the individual patient “What is my risk of developing disease?” Obviously, the patient will be interested in the absolute risk of disease, and not the relative risk, and so will his or her doctor.

REFERENCES


34. Dahlback B, Hildebrand B. Inherited resistance to activated protein C is corrected by anticoagulant cofactor activity found to be a property of factor V. Proc Natl Acad Sci USA 91:1396-1400, 1994


71 Jordan WM Pulmonary embolism. Lancet i 1146-1147, 1961
77 Kingsbury KJ Relation of ABO blood-groups to atherosclerosis. Lancet i 199-203, 1971
85 Lane DA, Mannucci PM, Bauer KA, et al Inherited thrombophilia Part 1 Thromb Haemost 76 651-662, 1996
86 Lane DA, Mannucci PM, Bauer KA, et al Inherited thrombophilia Part 2 Thromb Haemost 76 824-834, 1996
104 O'Donnell J, Tuddenham EGD, Manning R, et al High prevalence of elevated FVIII levels in patients referred for
thrombophilia screening is due to increased synthesis independent of the acute phase reaction. Blood 88 470a, 1996 (abstr) (suppl 1)


111 Quéré I, Dupuy E, Cludefous-Veckermans B, et al Homocysteine and deep vein thrombosis C677T MTHFR genetic thermolabile variant and folate acid modulation of plasma homocysteine levels. Haemostasis 26 188, 1996 (abstr) (suppl 3)


117 Riddell AF, Pasi KJ, Perry DJ. Thermolabile methylene-tetrahydrofolate reductase (TL-MTHFR) and venous thromboembolic disease. Haemostasis 26 189, 1996 (abstr) (suppl 3)


121 Rosendaal FR. Factor VIII and coronary heart disease. Eur J Epidemiol 8 71-75, 1992 (suppl 2)


142 Thomas DP. Pathogenesis of venous thrombosis, in

143 Thomas SH Mortality from venous thromboembolism and myocardial infarction in young adults in England and Wales Lancet 348 402, 1996


154 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 346 1575-1582, 1995

