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

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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

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,[24 55] 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[23])

Apparently in contradiction, in 1987, Miletich published the results of screening for protein C deficiency among blood donors,[99] 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[99] 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 [115 116 145]

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,[33] 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 [80] 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[96 138] 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 thombosis 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

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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 [4 102] It is uncommon in young individuals and becomes more frequent with advancing age [102] 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 emboli, bleeding complications of anticoagulant treatment) and, although rare, it may cause death due to pulmonary embolism [26 142]

Classical risk factors for deep vein thrombosis (DVT) include surgery, immobilization, fractures, puerperium, paralysis, prolonged bed rest, and use of oral contraceptives [52 85 86] 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 [2 85 86] 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 methylenetetrahydrofolate reductase (MTHFR) gene [47]

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 [138 139] 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,[99] 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

For APC-resistance and factor V Leiden, the estimates among Caucasians range from 3% to 7%,[118 122 136] whereas it is very uncommon in Asians and Africans [30 113]

Among consecutive patients with objectively confirmed DVT, deficiencies of protein C, protein S, and antithrombin combined are found in about 5% [59 83] In 20% of unselected patients with DVT, APC-resistance is present [84 122]

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

Table 1 Risk Factors for Venous Thrombosis

Acquired	Inherited	Mixed
Age	Antithrombin deficiency	Hyperhomocysteinemia
Previous thrombosis	Protein C deficiency	High factor VIII levels
Immobilization	Protein S deficiency	High fibrinogen levels
Major surgery	Factor V Leiden (FV R506Q)	
Orthopedic surgery	Dysfibrinogenemia	
Malignancy	Factor II 20210A	
Oral contraceptives		
Hormonal replacement therapy		
Antiphospholipid syndrome		
Myeloproliferative disorders		
Polycythemia vera		

Table 2. Prevalence of the Major Thrombophilic Clotting Abnormalities (%)

	Protein C Deficiency	Protein S Deficiency	Antithrombin Deficiency	APC-Resistance	Factor II 20210A
Healthy individuals					
Scotland (n = 9669)[138,139]	0.2*		0.02*†		
USA (n = 5422)[99]	0.4				
USA (n = 704)[118]				6*	
Sweden (n = 130)[136]				7	
Netherlands (n = 474)[108,122]				3*	2.3
Netherlands (n = 100)[108]					1.0
Netherlands (n = 646)					1.2
Consecutive patients with first DVT					
Netherlands (n = 277)[59]	3	2	1		
Netherlands (n = 474)[83,108]	3*	1	1		6.2
Netherlands (n = 471)[122]				20*	
Thrombophilic patients					
Spain (n = 204)[137]	1	1	0.5		
Germany (n = 158)[128]	9	6	5		
Netherlands (n = 113)[21]	8	13	4		
Israel (n = 107)[9]	6	3	7		
USA (n = 25)[54]				52	
Netherlands (n = 28)[108]					17.9

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%.[9,21,128,137] APC-resistance is found in more than half of all cases of hereditary thrombophilia, and is the most important cause of hereditary thrombosis.[12,32,33,54,122]

The polymorphism in the prothrombin gene (20210 G to A) that has recently been described[108] 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%.[108] The polymorphism is associated with increased levels of prothrombin, which suggests that these are the effectors of the thrombotic risk.[108]

PREVALENCE OF ACQUIRED AND MIXED RISK FACTORS

The prevalence of hyperhomocysteinemia and high levels of factor VIII depend on the cutoff values that are used because plasma levels are continuous variables rather than dichotomous molecular abnormalities. Here we will use cutoff values that were also used in the studies that reported hyperhomocysteinemia and high levels of factor VIII as risk factors for venous thrombosis. 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.[39,133] Den Heijer found that the risk of venous thrombosis associated with hyperhomocysteinemia only became apparent when levels exceeded 18 μmol/L.[39] Factor VIII:C levels exceeding 150 IU/dL were observed in 11% of healthy Dutch volunteers.[82]

These measurements of homocysteine and factor VIII were all performed only once,[39,82,133] 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,[80] 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.[88] 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.[88] 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.[18,24,55] 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.[115] DVT of the leg is the most common manifestation, although thrombosis may occur at a variety of other sites.[3,58,110]

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.[3,18] In these families, many thrombotic events occur spontaneously, ie, without any obvious cause.[65]

The relative risk of 6.5 obtained from a population-based study is very similar to this result from family studies.[83] The prevalence of protein C deficiency as found in unselected patients with a first thrombotic event (3%)[59,83] and healthy individuals from the general population (0.2%)[139] 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.[22,130] Whereas the clinical symptoms are similar to those found in protein C deficiency,[43] 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.[156] The mutations in the protein S gene have been reported to be associated with protein S deficiency.[1,107,129]

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.[83] 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.[37,41,63,141] The clinical symptoms of antithrombin deficiency closely resemble those of protein C and protein S deficiency, although superficial thrombophlebitis seems to occur less often.[2] A large number of mutations associated with antithrombin deficiency have been described.[87] 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.[63,141] The 50-fold difference between prevalence among patients with a first event of DVT and prevalence in a healthy population[59,83,138] 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.[83]

APC Resistance

Resistance to APC was first described by Dahlbäck in 1993.[33] The defect is associated with an abnormality in clotting factor V,[34] and this mutation (factor V R506Q, factor V Leiden)[11] appears responsible for the large majority of cases of APC resistance.[12,157] 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.[136] Approximately 25% of the patients with APC resistance had suffered thrombosis before the age of 50 years.[136] This risk is lower than the figures reported for families with protein C deficiency,[3] 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.[88] 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.[83,84]

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.[108] 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.[108]

Table 3. Prothrombin Levels, Prothrombin Genotype, and Risk of Thrombosis

Plasma Prothrombin Level (IU/dL)	Relative Risk (OR)	Prevalence of 20210 A Genotype	
		Patients (%)	Controls (%)
<95	1	0	0
95-104	1.3	2.8	0
104-115	1.4	6.9	1.7
>115	2.2	18.2	9.3

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.[108]

Hyperhomocysteinemia

In two case-control studies, hyperhomocysteinemia has been shown to increase the risk of DVT.[39,133] 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,[44,45] which was most apparent on post-methionine loading homocysteine measurements (the studies by Simioni[133] and den Heijer[39] were based on fasting homocysteine measurements). Among patients with a first episode of venous thrombosis before age 40, Falcon found 19% with hyperhomocysteinemia.[44] 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.[39] Hyperhomocysteinemia has also been shown to increase the risk of recurrent thrombotic events.[38]

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.[72,114,146] Heterozygous carriership of CS deficiency, the abnormality that in the homozygous form causes classic homocystinuria,[100] 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.[42,47,73,79] Surprisingly, homozygous carriership of this variant is not associated with an increased risk of venous thrombosis.[5,78,111,117] 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 homocysteine 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.[78] 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.[39,133]

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.[82] 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.[77,97,102,121,140] 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.[82] These findings[82] 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.[104]

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,[82] the population attributable risk is 35%. This indicates that (assuming a causal relation) 35% of all events of DVT in

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.[71] Since then, numerous reports in the 1960s and 1970s have confirmed that oral contraceptives increase the risk of venous as well as arterial thrombosis.[126,135] 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.[68,125,150] In the 1970s, a large case-control study found a relative risk of 11[16] in users versus nonusers, and a prospective cohort study reported a relative risk of 4.[124] A prospective study in the 1980s found a relative risk of 7.[109] 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,[154] oral contraceptives were associated with a 4.2-fold increased risk; in the Transnational study with a fourfold increased risk[134]; whereas in the Leiden Thrombophilia Study the age-adjusted relative risk was 6.[149] In the latter study, the risk conferred by oral contraceptives with 30 μg ethinylestradiol and 50 μg ethinylestradiol was the same.[13] 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.[50] Similar dose-related results have been reported from Sweden.[17] 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.[76]

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,[26] 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.[35,56,69] When investigated in healthy volunteers, the effects on the hemostatic system of oral contraceptives containing either 30 or 50 μg ethinylestradiol did not differ.[127] 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.[8]

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.[149] 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).[13,70,134,153] Between the various studies, extensive adjustment for possible confounders, eg, age, family history, factor V Leiden carriership, 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.[143,148]

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[75] and 0.7 per 1,000 women,[144] 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[75,144] 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).[10,95,101,151] 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 thrombosis.[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[94]; 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

Table 4. Concepts of Interaction

Risk Factor A	Risk Factor B	Incidence	Risk Difference	Relative Risk
−	−	1/1000	0*	1*
+	−	2/1000	1/1000	2
−	+	3/1000	2/1000	3
+	+	?	?	?

*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.[19] 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.[19] Homozygous protein S deficiency has also been reported, and while extremely rare, it appears as severe as homozygous protein C deficiency.[92] Homozygous antithrombin deficiency is extremely rare and probably incompatible with life: two siblings with homozygous antithrombin deficiency died within 3 weeks after birth.[57] 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.[53,122] 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.[122] The majority of symptomatic 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.[122,149] 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.[25,49] Because of a homozygous deficiency of CS, which catabolizes homocysteine to cystathionine, the levels of homocysteine become so high that homocysteine is excreted in the urine.[100] 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.[114] 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 B_{12} 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 B_{12}, 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.[42,47,79] The variant is present in homozygous form in about 10% of the general population.[78] Several studies,[5,78,111,117] although not all,[6] 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[73,114]; 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).[80] 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.[147] In families with thrombophilia and protein S deficiency, the risk of thrombosis was also higher in those with a combined defect, ie, protein S deficiency and factor V Leiden, than in those with either of the two defects.[7,155]

In all these instances of more than one defect, ie, 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.[93] 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).[112] 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.[31,39] It is unclear if there is a synergistic effect in those with combined hyperhomocysteinemia and factor V Leiden. 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, ie, there was no sign of interaction.[39] The distribution of the abnormalities over a mixture of cases with arterial or venous occlusive disease reported by D'Angelo[31] suggests a more than additive effect when re-

evaluated as a case-case analysis.[74] Within the Physicians' Health Study, the risk for the combined abnormalities also exceeded the risk for factor V Leiden and hyperhomocysteinemia alone.[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,[39] and 21.8 in the American study[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[39] included all patients with thrombosis, of all ages, whereas in the other series the results concerned young patients,[31] or were most striking for idiopathic thrombosis.[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.[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).[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.[149] In a subsequent analysis, this interaction was most striking for oral contraceptives containing a third-generation progestogen.[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.[120,122] In a series of homozygous patients, 80% of the women with thrombosis had been using oral contraceptives.[120]

Table 5. Factor V Leiden and Oral Contraceptives: Single and Combined Effects

Factor V Leiden	Oral Contraceptives	Relative Risk	Incidence per 10,000 Women per Year
–	–	1.0*	0.8
–	+	3.7	3.0
+	–	6.9	5.7
+	+	34.7	28.5

*Reference category.

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%.[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.[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.[15,60,62]

Antiphospholipid Antibodies and Factor V Leiden

Antiphospholipid antibodies are a common cause of thrombosis,[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,[40] the simultaneous presence of both abnormalities in one patient may lead to a severe thrombotic tendency.[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.[14] Similar results have been observed by others.[105,132]

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.[98] 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%.[36] 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.[81] 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, ie, 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, ie, risk differences Under a multiplicative model, the expected effect is defined multiplicatively based on incidence ratios, ie, 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 of 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 absence or presence 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 the ones 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 [90] 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 The second public

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

Rothman 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 [123]

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

1 Alach M, Gandrille S, Emmerich J A review of mutations causing deficiencies of antithrombin, protein C and protein S Thromb Haemost 74 81-89, 1995

2 Allaart CF, Briet E Familial venous thrombophilia, in Bloom AL, Forbes CD, Thomas DP, et al, eds Haemostasis and Thrombosis New York, NY, Churchill-Livingstone, 1994, pp 1349-1360

3 Allaart CF, Poort SR, Rosendaal FR, et al Increased risk of venous thrombosis in carriers of protein C deficiency defect Lancet 341 134-138, 1993

4 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 151 933-938, 1991

5 Ankri A, Chadefaux-Vekemans B, Kara Mostefa A, et al Does the prevalent mutation 677>T in the methylenetetrahydrofolate reductase gene contribute to hyperhomocysteinemia related to vascular disease? Blood 88 169a, 1996 (abstract) (suppl 1)

6 Arruda VR, Von Zuben PM, Chiaparini LC, et al The mutation Ala6766>Val in the methylenetetrahydrofolate reductase gene A risk factor for premature arterial disease and venous thrombosis Blood 88 285a, 1996 (abstr) (suppl 1)

7 Beauchamp NJ, Daly ME, Cooper PC, et al Molecular basis of protein S deficiency in three families also showing independent inheritance of factor V Leiden Blood 88 1700-1707, 1996

8 Beaumont V, Lemoit N, Beaumont JL Oral contraceptives, sex steroid-induced antibodies and vascular thrombosis Results from 1318 cases Eur Heart J 12 1219-1224, 1991

9 Ben Tal O, Zivelin A, Seligsohn U The relative frequency of hereditary thrombotic disorders among 107 patients with thrombophilia in Israel Thromb Haemost 61 50-54, 1989

10 Bergqvist D Frequency of thromboembolic complications, in Bergquist D (ed) Postoperative Thromboembolism Berlin, Springer-Verlag, 1983, pp 12-13

11 Bertina RM, Koeleman RPC, Koster T, et al Mutation in blood coagulation factor V associated with resistance to activated protein C Nature 369 64-67, 1994

12 Bertina RM, Reitsma PH, Rosendaal FR, et al Resistance to activated protein C and factor V Leiden as risk factors for venous thrombosis Thromb Haemost 74 449-453, 1995

13 Bloemenkamp KWM, Rosendaal FR, Helmerhorst FM, et al Enhancement by factor V Leiden mutation of risk of deep-vein thrombosis associated with oral contraceptives containing a third-generation progestagen Lancet 346 1593-1596, 1995

14 Bokarewa MI, Blomback M, Bremme K Phospholipid antibodies and resistance to activated protein C in women with thrombophilia Blood Coagul Fibrinolysis 6 417-422, 1995

15 Bokarewa MI, Bremme K, Blomback M Arg506-Gln mutation in factor V and risk of thrombosis during pregnancy Br J Haematol 92 473-478, 1996

16 Boston Collaborative Drug Surveillance Program Oral contraceptives and venous thromboembolic disease, surgically confirmed gall bladder disease and breast tumours Lancet 1 1399-1404, 1973

17 Bottiger LE, Boman G, Eklund G, et al Oral contraceptives and thromboembolic disease Effects of lowering oestrogen content Lancet 1 1097-1101, 1980

18 Bovill EG, Bauer KA, Dickermann JD, et al The clinical spectrum of heterozygous protein C deficiency in a large New England kindred Blood 73 712-717, 1989

19 Branson HE, Marble R, Katz J, et al Inherited protein C deficiency and coumarin-responsive chronic relapsing purpura fulminans in a newborn Lancet II 1165-1168, 1983

20 Brenner B, Vulfsons SL, Lanii N, et al Coexistence of familial antiphospholipid syndrome and factor V Leiden Impact on thrombotic disease Br J Haematol 94 166-167, 1996

21 Briet E, Engesser L, Brommer EJP, et al Thrombophilia Its causes and a rough estimate of its prevalence Thromb Haemost 58 39, 1987

22 Broekmans AW, Bertina RM, Reinalda-Poot J, et al Hereditary protein S deficiency and venous thromboembolism A study in three Dutch families Thromb Haemost 53 273-277, 1985

23 Broekmans AW, Van der Linden IK, Veltkamp JJ, et al Prevalence of isolated protein C deficiency in patients with venous thrombotic disease and in the population Thromb Haemost 50 350, 1983 (abstr)

24 Broekmans AW, Veltkamp JJ, Bertina RM Congenital protein C deficiency and venous thromboembolism A study of three Dutch families N Engl J Med 309 340-344, 1983

25 Carson NAJ, Neill DW Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland Arch Dis Child 37 505-513, 1962

26 Carter CJ The natural history and epidemiology of venous thrombosis Prog Cardiovasc Dis 36 423-438, 1994

27 Chievitz E, Thiede E Complications and causes of death in polycythemia vera Acta Med Scand 172 513-523, 1962

28 Cohen SH, Ehrlich GE, Kaufman MS, et al Thrombophlebitis following knee surgery J Bone Joint Surg [Am] 55 106-111, 1973

29 Conard J, Horellou MH, Van Dreden P, et al Thrombosis and pregnancy in congenital deficiencies in AT III, protein C or protein S Study of 78 women Thromb Haemost 63 319-320, 1990

30 Cox MJ, Rees DC, Martinson JJ, et al Evidence for a single origin of factor V Leiden Br J Haematol 92 1022-1025, 1996

31 D'Angelo A, Fermo I, D'Angelo SV Thrombophilia, homocystinuria and mutation of the factor V gene N Engl J Med 335 289, 1996

32 Dahlback B Inherited thrombophilia Resistance to activated protein C as a pathogenic factor of venous thromboembolism Blood 85 607-614, 1995

33 Dahlback 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 USA 90 1004-1008, 1993

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

35 Daly E, Vessey MP, Hawkins MM, et al Risk of venous thromboembolism in users of hormone replacement therapy Lancet 348 977-980, 1996

36 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 74 793-794, 1995

37 Demers C, Ginsberg JS, Hirsh J, et al Thrombosis in antithrombin III-deficient persons Report of a large kindred and literature review Ann Intern Med 116 754-761, 1992

38 Den Heijer M, Blom HJ, Gerrits WB, et al Is hyperhomocysteinemia a risk factor for recurrent venous thrombosis? Lancet 345 882-885, 1995

39 Den Heijer M, Koster T, Blom HJ, et al Hyperhomocysteinemia as a risk factor for deep-vein thrombosis N Engl J Med 334 759-762, 1996

40 Dizon-Towson D, Hutchison C, Silver R, et al The factor V Leiden mutation which predisposes to thrombosis is not common in patients with antiphospholipid syndrome Thromb Haemost 74 1029-1031, 1995

41 Egeberg O Inherited antithrombin deficiency causing thrombophilia Thromb Diath Haemorrh 13 516-530, 1965

42 Engbertsen AMT, Franken DG, Boers GHJ, et al Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia Am J Hum Genet 56 142-150, 1995

43 Engesser L, Broekmans AW, Briet E, et al Hereditary protein S deficiency Clinical manifestations Ann Intern Med 106 677-682, 1987

44 Falcon CR, Cattaneo M, Panzeri D, et al High prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis Arterioscler Thromb 14 1080-1083, 1994

45 Fermo I, D'Angelo SV, Paroni R, et al Prevalence of moderate hyperhomocysteinemia in patients with early-onset venous and arterial occlusive disease Ann Intern Med 123 747-753, 1995

46 Friederich PW, Sanson BJ, Simioni P, et al Frequency of pregnancy-related venous thromboembolism in anticoagulant factor-deficient women Implications for prophylaxis Ann Intern Med 125 955-960, 1996

47 Frosst P, Blom HJ, Milos R, et al A candidate genetic risk factor for vascular disease A common mutation in methylenetetrahydrofolate reductase Nature Genetics 10 111-113, 1995

48 Geerts WH, Code KI, Jay RM, et al A prospective study of venous thromboembolism after major trauma N Engl J Med 331 1601-1606, 1994

49 Gerritsen T, Vaughn JG, Weisman HA The identification of homocysteine in urine Biochem Biophys Res Commun 9 493, 1962

50 Gerstman BB, Piper JM, Tomita DK, et al Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease Am J Epidemiol 133 32-37, 1991

51 Goldberg RJ, Seneff M, Gore JM, et al Occult malignant neoplasms in patients with deep venous thrombosis Arch Intern Med 147 251-253, 1987

52 Goldhaber SZ Epidemiology of pulmonary embolism and deep vein thrombosis, in Bloom AL, Forbes CD, Thomas DP, et al (eds) Haemostasis and Thrombosis New York, NY, Churchill-Livingstone, 1994, pp 1327-1333

53 Greengard JS, Eichinger S, Griffin JH, et al Variability of thrombosis among homozygous siblings with resistance to activated protein C due to an Arg to Gln mutation in the gene for factor V N Engl J Med 331 1559-1562, 1994

54 Griffin JH, Evatt B, Wideman C, et al Anticoagulant protein C pathway defective in a majority of thrombophilic patients Blood 82 1989-1993, 1993

55 Griffin JH, Evatt B, Zimmerman TS, et al Deficiency of protein C in congenital thrombotic disease J Clin Invest 68 1370-1373, 1981

56 Grodstein F, Stampfer MJ, Goldhaber SZ, et al Prospective study of exogenous hormones and risk of pulmonary embolism in women Lancet 348 983-987, 1996

57 Hakten M, Deniz U, Ozbag G, et al Two cases of homozygous antithrombin III deficiency in a family with congenital deficiency of ATIII, in Senzinger H, Vinazzer H, (eds) Thrombosis and haemorrhagic disorders Wurzberg, Schmitt und Meyer GmbH, 1989, pp 177-181

58 Harle JR, Aillaud MF, Quinsat D, et al Cerebral thrombophlebitis disclosing functional protein C deficiency Ann Med Intern 140 233-234, 1989

59 Heijboer H, Brandjes DPM, Buller HR, et al Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis N Engl J Med 323 1512-1516, 1990

60 Hellgren M, Svensson PJ, Dahlback B Resistance to activated protein C as a basis for venous thromboembolism associated with pregnancy and oral contraceptives Am J Obstetr Gynecol 173 210-215, 1995

61 Hellgren M, Tengborn L, Abildgaard U Pregnancy in women with congenital antithrombin III deficiency Experience of treatment with heparin and antithrombin Gynecol Obstetr Invest 14 127-141, 1982

62 Hirsch DR, Mikkola KM, Marks PW, et al Pulmonary embolism and deep venous thrombosis during pregnancy or oral contraceptive use Prevalence of factor V Leiden Am Heart J 131 1145-1148, 1996

63 Hirsh J, Piovella F, Pini M Congenital antithrombin III deficiency Incidence and clinical features Am J Med 87 34-38, 1989 (suppl)

64 Hjelmstedt A, Bergvall U Incidence of thrombosis in patients with tibial fractures Acta Chir Scand 134 209-218, 1968

65 Horellou MH, Conard J, Bertina RM, et al Congenital

protein C deficiency and thrombotic disease in nine French families Br Med J 289 1285-1287, 1984

66 Hughes GR Thrombosis, abortion, cerebral disease and lupus anticoagulant Br Med J 287 1088-1089, 1983

67 Hull RD, Raskob GE Prophylaxis of venous thromboembolic disease following hip and knee surgery J Bone Joint Surg [Am] 68 146-150, 1986

68 Inman WHW, Vessey MP Investigation of death from pulmonary, coronary, and cerebral thrombosis and embolism in women of child-bearing age Br Med J 1997 193-199, 1968

69 Jick H, Derby LE, Wald Myers M, et al Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens Lancet 348 981-983, 1996

70 Jick H, Jick SS, Gurewich V, et al Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components Lancet 346 1589-1593, 1995

71 Jordan WM Pulmonary embolism Lancet ii 1146-1147, 1961

72 Kang SS, Wong PWK, Norusis M Homocysteinemia due to folate deficiency Metabolism 36 458 462, 1987

73 Kang SS, Zhou J, Wong PWK, et al Intermediate homocysteinemia A thermolabile variant of methyenetetrahydrofolate reductase Am J Hum Genet 48 536 545, 1988

74 Khoury MJ, Flanders WD Non-traditional epidemiologic approaches in the analysis of gene-environment interaction Case-control studies with no controls! Am J Epidemiol 144 207-213, 1996

75 Kierkegaard A Incidence and diagnosis of deep vein thrombosis associated with pregnancy Acta Obstetr Gynecol Scand 62 239-243, 1983

76 Kierkegaard A Deep vein thrombosis and the oestrogen component in oral contraceptives An epidemiological analysis Contraception 31 29-41, 1985

77 Kingsbury KJ Relation of ABO blood-groups to atherosclerosis Lancet i 199-203, 1971

78 Kluijtmans LAJ, Den Heijer M, Reitsma PH, et al Thermolabile methylenetetrahydrofolate reductase and factor V Leiden in the risk of deep vein thrombosis 1997 (submitted)

79 Kluijtmans LAJ, Van den Heuvel LPWJ, Boers GHJ, et al Molecular genetic analysis in mild hyperhomocysteinemia A common mutation in the methelenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease Am J Hum Genet 58 35 41, 1996

80 Koeleman BP, Reitsma PH, Allaart CF, et al Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families Blood 84 1031 1035, 1994

81 Koster T Deep-vein thrombosis A population-based case-control study Leiden Thrombophilia Study Thesis, Leiden, Rijksuniversiteit Leiden, 1995

82 Koster T, Blann AD, Briet E, et al Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis Lancet 345 152-155, 1995

83 Koster T, Rosendaal FR, Briet 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 85 2756 2761, 1995

84 Koster T, Rosendaal FR, De Ronde H, et al Venous thrombosis due to a poor anticoagulant response to activated protein C Leiden Thrombophilia Study Lancet 342 1503-1506, 1993

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

87 Lane DA, Olds RJ, Thein SL Antithrombin III Summary of first database update Nucleic Acids Res 22 3556-3559, 1994

88 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 88 4205-4208, 1997

89 Love PE, Santoro SE Antiphospholipid antibodies Anticardiolipin antibodies and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders Ann Intern Med 112 682-698, 1990

90 MacMahon B, Trichopoulos D Epidemiology Principles and Methods Boston, MA, Little, Brown and Co, 1996, pp 282-283

91 MacMahon B, Trichopoulos D Epidemiology Principles and Methods Boston, MA, Little, Brown and Co, 1996, pp 213-217

92 Mahasandana C, Suvatte V, Chuansumrit A, et al Homozygous protein S deficiency in an infant with purpura fulminans J Pediatr 117 750 753, 1990

93 Mandel H, Brenner B, Berant M, et al Coexistence of hereditary homocystinuria and factor V leiden—Effect on thrombosis N Engl J Med 334 763-768, 1996

94 Maurer BJ, Wray R, Shillingford JP Frequency of venous thrombosis after myocardial infarction Lancet ii 1385-1387, 1971

95 Mayo M, Halil T, Browse NL The incidence of deep vein thrombosis after prostatectomy Br J Urol 43 738-742, 1971

96 McColl M, Tait RC, Walker ID, et al 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 7 689-694, 1996

97 Medalie JH, Levene C, Papier C, et al Blood groups, myocardial infarction and angina pectoris among 10,000 adult males N Engl J Med 285 1348-1353, 1981

98 Melnyk A, Theriault R, Andreeff M, et al Factor V leiden (G1691A) and the risk of thrombosis in patients with solid tumors A prospective case-control study Blood 88 176a, 1996 (abstr) (suppl)

99 Miletich J, Sherman L, Broze G Absence of thrombosis in subjects with heterozygous protein C deficiency N Engl J Med 317 991-996, 1987

100 Mudd SH, Skovby F, Levy HL, et al The natural history of homocystinuria due to cystathionine beta-synthase deficiency Am J Hum Genet 37 1-31, 1985

101 Nicolaides AN, Field ES, Kakkar VV, et al Prostatectomy and deep-vein thrombosis Br J Surg 59 487-488, 1972

102 Nordstrom M, Lindblad B, Bergqvist D, et al A prospective study of the incidence of deep-vein thrombosis within a defined urban population J Intern Med 232 155-160, 1992

103 Nurmohamed MT, Rosendaal FR, Buller HR, et al Low molecular weight heparin versus standard heparin in general and orthopedic surgery A meta-analysis Lancet 340 152-156, 1992

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)

105 Ortel TL, Klemp KF, Moore KD, et al Phenotypic heterogeneity in patients with antiphospholipid antibodies and factor V Leiden Blood 88 176a, 1996 (abstr) (suppl 1)

106 Pabinger I, Schneider B, and the GTH study group Thrombotic risk of women with hereditary antithrombin III-, protein C and protein S-deficiency taking oral contraceptive medication Thromb Haemost 71 548-552, 1994

107 Ploos van Amstel JK, Huisman MV, Reitsma PH, et al Partial protein S gene deletion in a family with hereditary thrombophilia Blood 73 479-483, 1989

108 Poort SR, Rosendaal FR, Reitsma PH, et al 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 88 3698-3703, 1996

109 Porter JB Oral contraceptives and nonfatal vascular disease—Recent experience Obstetr Gynecol 59 299 302, 1982

110 Prat F, Ouzan D, Trecziak N, et al Portal and mesenteric thrombosis revealing constitutional protein C deficiency Gut 30 416, 1989

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

112 Quéré I, Lamarti H Thrombophilia, homocystinuria and mutation of the factor V gene N Engl J Med 335 289, 1996

113 Rees DC, Cox M, Clegg JB World distribution of factor V Leiden Lancet 346 1133-1134, 1995

114 Rees MM, Rodgers GM Homocysteinemia Association of a metabolic disorder with vascular disease and thrombosis Thrombosis Res 71 337-359, 1993

115 Reitsma PH, Bernardi F, Doig RG, et al Protein C deficiency A database of mutations, 1995 update On behalf of the Subcommittee on Plasma Coagulation Inhibitors of the Scientific and Standardization Committee of the ISTH Thromb Haemost 73 876-889, 1995

116 Reitsma PH, Poort SR, Bertina RM Genetic abnormalities in the protein C genes of homozygous and compound heterozygotes for protein C deficiency Thromb Haemost 65 808, 1991

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

118 Ridker PM, Hennekens CH, Lindpainter K, et al Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men N Engl J Med 332 912 917, 1995

119 Ridker PM, Hennekens CH, Selhub J, et al Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risks of future venous thromboembolism Circulation 1997 (in press)

120 Rintelen C, Mannhalter C, Ireland H, et al Oral contraceptives enhance the risk of clinical manifestation of venous thrombosis at a young age in females homozygous for factor V Leiden Br J Haematol 93 487-490, 1996

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

122 Rosendaal FR, Koster T, Vandenbroucke JP, et al High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance) Blood 85 1504 1508, 1995

123 Rothman KJ Modern Epidemiology Boston, MA, Little, Brown, and Co, 1986, pp 313-326

124 Royal College of General Practioners' Oral Contraception Study Oral contraceptives, venous thrombosis, and varicose veins J R Coll Gen Pract 28 393-399, 1978

125 Sartwell PE, Masi AT, Arthes FG, et al Thromboembolism and oral contraceptives An epidemiological case control study Am J Epidemiol 90 365 380, 1969

126 Sartwell PE, Stolley P Oral contraceptives and cardiovascular disease Epidemiol Rev 4 95 109, 1982

127 Scarabin PY, Plu-Bureau G, Zitoun D, et al Changes in haemostatic variables induced by oral contraceptives containing 50 micrograms or 30 micrograms oestrogen Absence of dose-dependent effect on PAI-1 activity Thromb Haemost 74 928-932, 1995

128 Scharrer I, Hach Wunderle V, Heyland H, et al Incidence of defective t-PA release in 158 unrelated young patients with venous thrombosis in comparison to PC-, PS-, AT III-, fibrinogen- and plasminogen deficiency Thromb Haemost 58 72, 1987

129 Schmidel DK, Nelson RM, Broxson EHJ, et al A 5 3-kb deletion including exon XIII of the protein S alpha gene occurs in two protein S-deficient families Blood 77 551-559, 1991

130 Schwarz HP, Fischer M, Hopmeier P, et al Plasma protein S deficiency in familial thrombotic disease Blood 64 1297-1300, 1984

131 Siemiatycki J, Thomas DC Biological models and statistical interactions An example from multistage carcinogenesis Int J Epidemiol 10 383-387, 1981

132 Simantov R, Lo SK, Salmon JE, et al Factor V Leiden increases the risk of thrombosis in patients with antiphospholipid antibodies Thromb Res 84 361-365, 1996

133 Simioni P, Prandoni P, Burlina A, et al Hyperhomocysteinemia and deep-vein thrombosis A case control study Thromb Haemost 76 883-886, 1996

134 Spitzer WO, Lewis MA, Heinemann LA, et al Third generation oral contraceptives and risk of venous thromboembolic disorders An international case-control study Transnational Research group on Oral Contraceptives and the Health of Young Women Br Med J 312 83-88, 1996

135 Stadel BV Oral contraceptives and cardiovascular disease (first of two parts) N Engl J Med 305 612-618, 1981

136 Svensson PJ, Dahlback B Resistance to activated protein C as a basis for venous thrombosis N Engl J Med 330 517-522, 1994

137 Tabernero MD, Tomas JF, Alberca I, et al Incidence and clinical characteristics of hereditary disorders associated with venous thrombosis Am J Hematol 36 249-254, 1991

138 Tait RC, Walker ID, Perry DJ, et al Prevalence of antithrombin deficiency in the healthy population Br J Haematol 87 106-112, 1994

139 Tait RC, Walker ID, Reitsma PH, et al Prevalence of protein C deficiency in the healthy population Thromb Haemost 73 87 93, 1995

140 Talbot S, Ryrie D, Wakley EJ, et al ABO blood groups and venous thromboembolic disease Lancet i 1257-1259, 1970

141 Thaler E, Lechner K Antithrombin III deficiency and thromboembolism Clin Haematol 10 369 390, 1981

142 Thomas DP Pathogenesis of venous thrombosis, in

Bloom AL, Forbes CD, Thomas DP, et al (eds) Haemostasis and Thrombosis New York, NY, Churchill-Livingstone, 1994, pp 1335-1347

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

144 Treffers PE, Huidekoper BL, Weenink GH, et al Epidemiological observations of thrombo embolic disease during pregnancy and in the puerperium, in 56,022 women Int J Gynaecol Obstetr 21 327 331, 1983

145 Tsuda S, Reitsma P, Miletich J Molecular defects causing heterozygous protein C deficiency in three asymptomatic kindreds Thromb Haemost 65 647, 1991 (abstr)

146 Ubbink JB, Vermaak WJ, Van der Merwe A, et al Vitamin B12, vitamin B6, and folate nutritional status in men with hyperhomocysteinemia Am J Clin Nutr 57 47-53, 1993

147 Van Boven HH, Reitsma PH, Rosendaal FR, et al Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency Thromb Haemost 75 417-421, 1996

148 Vandenbroucke JP, Bloemenkamp KWM, Helmerhorst FM, et al Mortality from venous thromboembolism and myocardial infarction in young women in The Netherlands Lancet 348 401-402, 1996

149 Vandenbroucke JP, Koster T, Briet E, et al Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation Lancet 344 1453-1457, 1994

150 Vessey MP, Doll R Investigation of relation between use of oral contraceptives and thromboembolic disease Br Med J 1 199-205, 1968

151 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 Obstetr Gynaecol Br Commonw 81 311 316 1974

152 Warlow C, Ogston D, Douglas AS Deep venous thrombosis of the legs after stroke Br Med J 1 1178 1181, 1976

153 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 346 1582 1588, 1995

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

155 Zoller B, Berntsdotter A, Garcia de Frutos P, et al Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S Blood 85 3518-3523, 1995

156 Zoller B, Garcia de Frutos P, Dahlback B Evaluation of the relationship between protein S and C4b-binding protein isoforms in hereditary protein S deficiency demonstrating type I and type III deficiencies to be phenotypic variants of the same genetic disease Blood 85 3524 3531, 1995

157 Zoller B, Svensson PJ, He X et al Identification of the same factor v gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C J Clin Invest 94 2521 2524, 1994