Thrombophilia screening: a matter of debate

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A B S T R A C T

In the last ten years, several risk factors that increase the risk of venous thrombosis have been discovered. Venous thrombosis is a multicausal disease in which several risk factors, both genetic and acquired, have to occur simultaneously to cause thrombosis. This means that most individuals with single thrombophilia are asymptomatic. Although testing thrombosis patients and their relatives for thrombophilia factors seems important for tailoring the duration of (prophylactic) anticoagulant therapy or estimating the risk of recurrence of thrombosis, current data do not support screening for thrombophilia. The risk of recurrences or the duration of anticoagulant therapy are generally not altered by thrombophilia. Future research should focus on identifying clusters of thrombosis risk factors to better estimate the individual risk of thromboembolic events.

I N T R O D U C T I O N

Before 1993, an inherited risk factor was detectable in only 10% of symptomatic patients with venous thrombosis. In the last ten years, the knowledge of risk factors for venous thrombosis has increased significantly. With the discovery of several inherited coagulation abnormalities associated with an increased tendency for venous thrombosis, such as factor V Leiden and the prothrombin 20210A mutation, many patients with a first episode of venous thrombosis have a detectable disorder.

Rudolph Virchow stated that the development of thrombosis was the result of changes in blood composition (hypercoagulability), reduced blood flow, or changes in the vessel wall.[1] Disturbance of this balance favours fibrin formation and may ultimately lead to the formation of occlusive thrombi. Examples of this pathophysiological phenomenon are trauma, immobilisation, pregnancy, surgery, malignancy and infection. These are acquired risk factors for venous thrombosis that may cause tissue damage, stasis of the blood or changes in blood composition. Both family studies and case-control studies led to important discoveries of heritable causes of thrombosis. The Leiden Thrombophilia Study (LETS), a population-based large case-control study, assessed the importance of various risk factors for thrombosis, which in most cases had been identified by family studies.[2] *Table 1* summarises the main results of the LETS. The thrombophilia factors can roughly be divided in two groups: deficiencies in the anticoagulant proteins antithrombin, protein C, and protein S are loss of function mutations and are rare in the general population. The prothrombotic abnormalities have a gain of function through subtle changes in the regulation of the gene activity. Factor V Leiden is relatively resistant to inactivation by activated protein C (APC) and the prothrombin mutation leads to increased prothrombin levels. High levels of procoagulant factors, such as factor VIII, IX and XI, lead to prolonged formation of fibrin as a result of excessive generation of thrombin. Finally, high thrombin-activatable fibrinolysis inhibitor (TAFI) levels result in prolonged down-regulation of fibrinolysis. Since no mutations have been found that elevate these coagulation factors, we do not know whether a gain or loss of function is responsible.

Table 1

Results from the Leiden Thrombophilia Study

RISK FACTOR	PREVALENCE IN PATIENTS (%)	PREVALENCE IN CONTROLS (%)	OR	95% CI
ANTICOAGULANT PROTEINS				
Protein C <0.67 U/ml	4.6	0.8	3.8	1.7-7.0
Protein S <0.67 U/ml	1.1	1.3	0.8	0.2-3.0
Antithrombin <0.80 U/ml	1.1	0.2	5.0	0.7-34
PROTHROMBOTIC MUTATIONS				
Factor V Leiden mutation	19	3	7.9	4.4-14
Prothrombin 20210A mutation	6.2	2.3	2.8	1.4-5.6
ELEVATED LEVELS OF PROCOAGULANT FACTORS				
Factor VIII >150 IU/dl	25	11	6.2	3.4-11
Factor IX >129 U/dl	20	10	2.5	1.6-3.9
Factor XI >120.8%	19	10	2.2	1.5-3.2
FIBRINOLYTIC FACTORS				
TAFI >122 U/dl	17	10	1.7	1.1-2.5
Protein C inhibitor >125.5%	13	10	1.4	0.9-2.0
OTHER LABORATORY ABNORMALITIES				
Homocysteine >18.5 μmol/l	10	5	2.5	1.2-5.2
APC resistance for wild-type factor V <0.92	36	16	4.4	2.9-6.6

INTERACTION, REGULATION AND CLUSTERING OF RISK FACTORS

Interaction

Venous thrombosis like many other diseases is multicausal. The discovery of common risk factors was a prerequisite for the study of interaction and made it clear that risk factors for thrombosis result from genetic differences or differences brought about by the environment or even behaviour. Plasma levels of proteins can, for instance, be determined by polymorphisms in the functional allele and by age or hormones. A good example of this complicated regulation is factor VIII. ABO blood group is an important genetic determinant of plasma factor VIII levels.[3] Von Willebrand factor is the carrier protein of factor VIII in plasma and also determines the factor VIII level.[4] If both blood group and von Willebrand factor are taken into account, a clear familial clustering remains, suggesting a third set of genes that regulate factor VIII levels.[5] Apart from the genetic causes, factor VIII is also influenced by environmental factors such as acute phase reactions and age. It is clear that not only is thrombosis a multicausal disease, but that the level of coagulation factors also reflects a mixture of genetic and environmental determinants.[6,7] The mean age at first thrombosis for patients from thrombophilic families is much younger than for consecutive patients with thrombosis.[8] This phenomenon is probably due to interaction of several genetic defects. In thrombophilic families, the risk of thrombosis in combination with protein C deficiency and factor V Leiden was much higher than for relatives with only protein C deficiency.[8] This gene-gene interaction results in variation within and between families. Homozygous disease is another example of this interaction. More commonly, a gene-environment interaction is present in patients with thrombosis. The synergistic effect of factor V Leiden and oral contraceptive use was described in 1994.[9] The annual absolute risk of women who were taking oral contraceptives and were carriers of factor V Leiden was 28.5 per 10,000 people, whereas this risk was 5.7 per 10,000 women per year for those with factor V Leiden without contraceptives and 3.0 per 10,000 per year for women with contraceptives without factor V Leiden.[9] An example of environment-environment interaction is oral contraceptive use and age.[9] This all shows that the nature of thrombosis is complex. The model of multicausal disease is not always sufficient to explain why the clustering of these different risk factors is sufficient to cause thrombosis in one patient but not in the other. Refinement of this model by including the dynamic influence of age is more useful for an individual risk estimate.[6] In this way we can better incorporate interaction of different risk factors. *Figure 1* shows the hypothetical situation of a patient who is followed through life.[6] This person has a certain basic thrombosis potential, which is formed by genetic factors (in this case factor V Leiden). Through life, several events lead to an increased thrombosis potential.
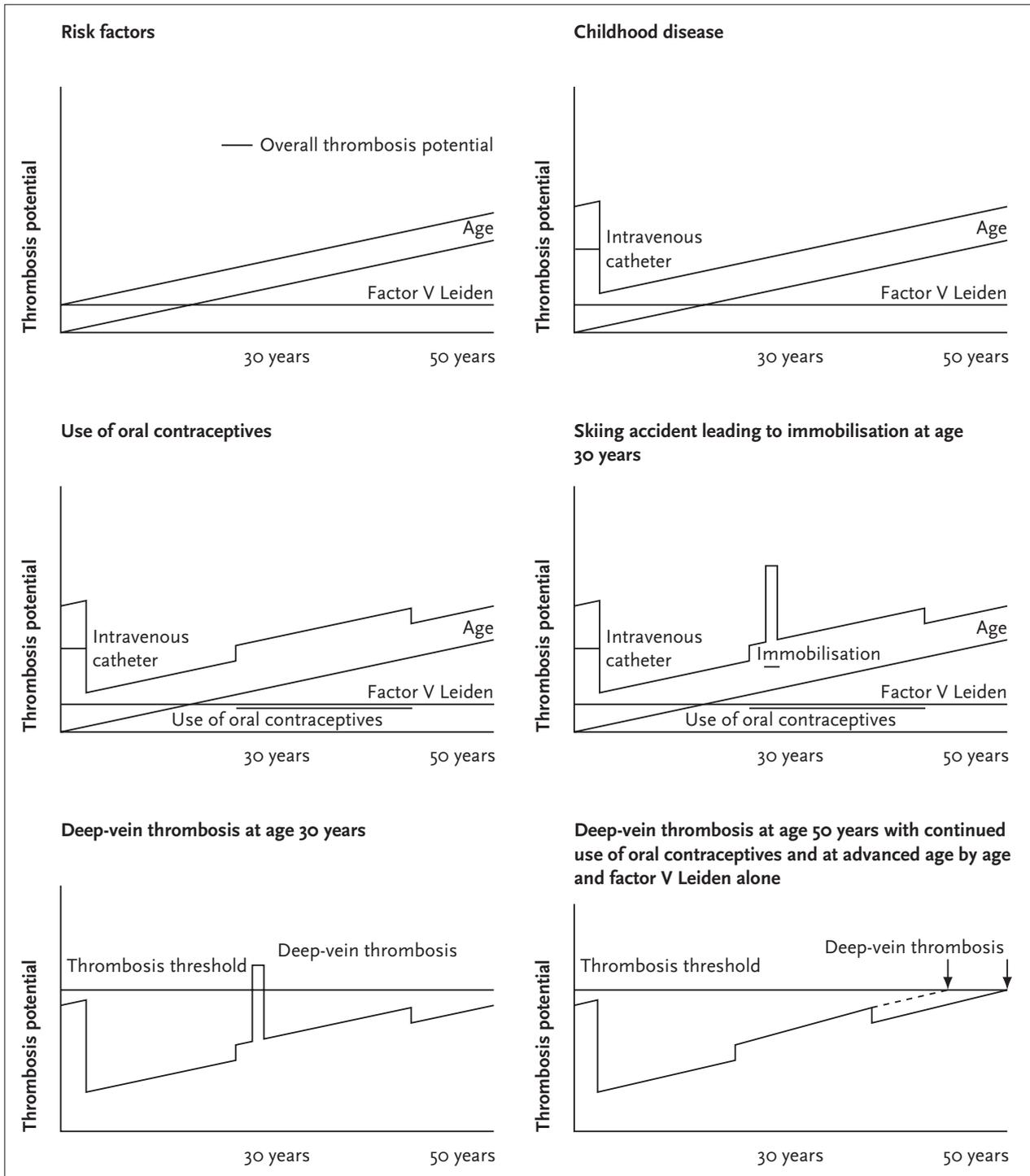
Figure 1

Models of thrombosis risk.[6] In each panel, the figure shows the thrombosis potential of each risk factor during an individual's life and the resultant thrombosis potential

At the age of 30 years, the combination of several risk factors and the thrombosis potential exceeds the thrombosis threshold and leads to clinical disease. Since increasing age itself is a risk factor for thrombosis, the threshold will be reached easier at later age and less risk factors will be needed to cause thrombosis.

Clustering and regulation

Since several procoagulant risk factors for thrombosis are closely related in the haemostatic system, a common genetic determinant of these coagulation factor levels could regulate these levels additionally to environmental determinants. A significant genetic component of coagula-

tion factors has been found in the Spanish population,[10] the United Kingdom[11] and the USA.[12] Interestingly, six families with a thrombotic tendency were reported in which high levels of coagulation factors XI, IX and VIII aggregated.[13] The inheritance pattern seemed to be dominant autosomal.[13] To date, the genetic basis of high levels is unknown. It is, however, possible that regulatory genes outside the genes of the coagulation factors regulate the protein levels. These levels would then cluster in an individual due to pleiotropic effects.

The evaluation between a potential risk factor and the occurrence of thrombosis is becoming more difficult, since adjustment is needed for more and more already known thrombotic risk factors. To better estimate the role of possible confounders and clustering of these factors, *a priori* knowledge of the interrelations of procoagulant and anticoagulant factors is important. With the data from the LETS, factor analysis was conducted using principal-component analysis with varimax rotation.[14] The number of variables is reduced by constructing relatively independent summary factors (the so-called principal components), which explain most of the variation in the data. In large studies where several risk factors seem to cluster, it is important to find the smallest number of principal components that still reflects the original data and variance. The newly formed principal loadings can be compared with the original variables by factor loadings, comparable with Pearson's correlation coefficients. When all the measured coagulation factors of the LETS were analysed, three relatively separate cluster patterns were found (*figure 2*). There was a clustering of the vitamin K dependent factors II, VII, IX and X, together with coagulation factors XI and XII. The second cluster consisted of factors V, VIII, IX, and fibrinogen. The third 'cluster' was made up of only one clotting factor, namely factor XIII subunit levels. These results show that interrelations exist between

different coagulation factors in the haemostatic system. Therefore, common shared genetic mechanisms may be responsible for the clustering of these coagulation factors. Transcription factors, such as hepatocyte nuclear factor-4, may contribute to the first clustering pattern.[15-17] Factors V and VIII share a great part of homology and post-translational modifications and could explain the second clustering.[18] By using factor analysis, a better overall estimation of the overall risk associated with coagulant factors may become possible. The described method facilitates the interpretation of epidemiological studies and hopefully the determination of the thrombosis risk for individual patients. Family studies might be helpful in unravelling the genetic basis of these findings.

CONSEQUENCES OF THROMBOPHILIA

Nowadays, a dozen different thrombophilia factors for thrombosis have been elucidated. However, venous thrombosis is a multicausal disease in which several risk factors, both genetic and acquired, have to occur simultaneously to cause thrombosis.[6,7] The interaction between these risk factors is dynamic rather than static, with age as an important contributor. In this complex situation, what is the contribution of inherited thrombophilia? And, now that we know so many thrombophilia factors, what is the consequence of thrombophilia? We will address this question by reviewing the influence of thrombophilia on the intensity and duration of anticoagulant therapy after a thromboembolic event, the risk of recurrence of venous thrombosis and the type of thrombosis. Thrombophilia could further be of importance for asymptomatic individuals.

Treatment of patients with thrombophilia

The intensity of anticoagulant treatment of patients with thrombosis who have a thrombophilia factor usually seems identical to patients without inherited defects, although this subject has never been thoroughly investigated.[19] Even in patients with deficiencies of antithrombin, protein C or protein S the therapeutic approach of thrombosis is generally the same. The optimal intensity of the international normalised ratio (INR) is 2.0 to 3.5, and this regimen is sufficient for preventing recurrences during therapy.[20] Recently it was shown that also in subjects with the antiphospholipid syndrome, moderate intensity anticoagulant therapy is adequate.[21] The optimal duration of anticoagulant therapy is uncertain, but does not seem to be influenced by the common thrombophilia factors. The goal of therapy is mainly to prevent recurrences. Since factor V Leiden and the prothrombin mutation are common in patients with thrombosis, several studies have analysed the risk of recurrent thrombosis in association with these prothrombotic defects. Neither of these mutations seem
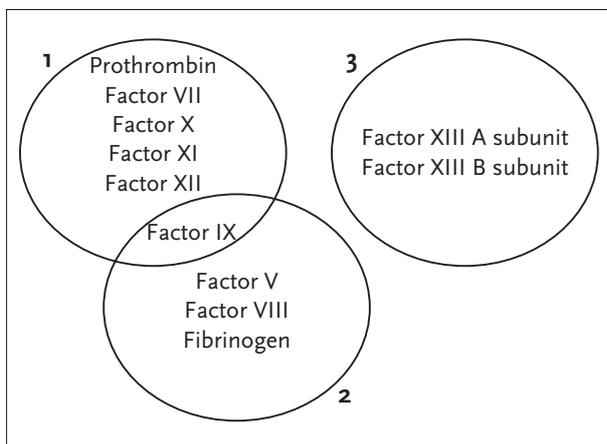

Figure 2
Factor loading pattern of procoagulant factors and fibrinogen in 466 healthy individuals[14]

to increase the risk of recurrences, although the data are not in complete agreement.[22-27] High levels of factor VIII and homocysteine seem to be associated with recurrences,[28,29] but these results have to be confirmed in other studies. Recurrent venous thrombosis might be more common in patients with a deficiency of antithrombin, protein C or protein S, but these results are based on retrospective data.[30] Given the low prevalence of these defects, it will be difficult to accurately determine the risk of recurrent thrombosis. From the other known prothrombotic defects, the effect on recurrent thrombosis is unknown. The combination of defects or homozygous factor V Leiden is probably associated with an increased risk of recurrence, although the information on patients studied so far is low.[31-36] So, apart from the antiphospholipid syndrome,[37] combined or homozygous defects, and possibly antithrombin deficiency, the impact of thrombophilia on the optimal duration of therapy to prevent recurrent thrombosis is probably small.[38]

Clinical manifestations of thrombophilia

Thrombosis in patients with thrombophilia usually manifests as deep vein thrombosis or pulmonary embolism. In patients with thrombophilia, thrombosis can also occur at unusual sites, such as the cerebral, visceral and axillary veins (*table 2*). Superficial thrombophlebitis is more common in protein C or protein S deficiency. In rare cases coumarin skin necrosis can occur.[39] Recurrence of thrombosis, a family history of thrombosis and first episode of thrombosis at young age are more common in patients with thrombophilia. In unselected thrombosis patients with a prothrombotic defect, such as factor V Leiden or prothrombin mutation, the difference with thrombosis patients without a defect is less clear.[8]

Table 2
Clinical manifestations of thrombophilia

Venous thrombosis at unusual site: mesenteric, pelvic, cerebral sinuses, portal, axillary
Family history of venous thromboembolism
Onset of thrombosis at young age
Recurrent episodes of venous thromboembolism
Warfarin induced skin necrosis
Recurrent foetal loss
Thrombophlebitis
Neonata purpura fulminans

Thrombophilia in asymptomatic patients

In women with the factor V Leiden or prothrombin mutation, oral contraceptive use, hormone replacement therapy and pregnancy further increase the risk of thrombosis, but the absolute risk seems to be low. Middeldorp *et al.*

prospectively followed asymptomatic carriers of the factor V Leiden mutation.[40] In 470 individuals, the annual incidence of venous thrombosis was 0.58%, which does not justify routine screening of family members. Also in risk situations, such as pregnancy or oral contraceptive use, the rate of thrombosis was low.[40] In pregnant asymptomatic women heterozygous for factor V Leiden or the prothrombin mutation, absolute risk of thrombosis is less than 3%,[41,42] whereas a deficiency of antithrombin, protein C or protein S leads to a risk of 4.1%.[43] Taken together, the risk of thrombosis in asymptomatic carriers of thrombophilia defects seems low and does not justify screening. The optimal strategy of thrombosis prophylaxis of asymptomatic carriers is probably not different from patients without heritable thrombophilia, but this subject remains controversial as long as there are no trials comparing prolonged prophylaxis with standard prophylaxis in high-risk situations or prophylaxis *vs* placebo during pregnancy.[44]

IMPLICATIONS OF THROMBOPHILIA SCREENING

Testing for thrombophilia is subject to an intense pro-con debate.[45,46] Clinicians who perform thrombophilia screening usually argue that a better understanding of the pathogenesis of thrombosis is important for both the treating physician and for the patient. Family members of the proband with a prothrombotic defect can also be screened, in order to tailor prophylactic treatment during high-risk situations.[47] Others argue against screening since screening is not cost-effective and leads to anxiety among asymptomatic carriers or false reassurance in those without the defect.[46] Apart from the discussion whether screening should be performed, it is important how to interpret the results of studies for thrombophilia. What are the implications for an individual patient, for the family members, the treating physician, researcher or even the society?

Influence of patient selection on the association of thrombophilia and thrombosis

The strength of an association between an inherited coagulation defect and venous thrombosis can be influenced by the type of study and the selection of thrombosis patients and controls.[7] In cohort (follow-up) studies, quantitative estimates (i.e. absolute risks) can be obtained. In case-control studies one can estimate relative risks (as an odds ratio) by comparing thrombosis patients with healthy individuals. This figure indicates how much higher the thrombosis risk is in the presence of a certain risk factor than in the absence of that factor. In unselected cases from population-based studies, relative risks can be applied to all individuals with that particular risk factor, provided cases are well selected. Population-based case-

control studies can be used to calculate the attributable risk, i.e., the proportion of all thrombotic events that would have been prevented by removing the risk factor. Family studies often consist of subjects that were selected because of a conspicuously high frequency of thrombosis. In these studies, the occurrence of thrombosis is compared between family members with and without the risk factor. These studies are ideal for studying the type of inheritance of a certain risk factor and to qualitatively estimate the thrombosis risks. These thrombophilia families usually have more than one thrombophilic defect and results cannot be extrapolated to the general population. The influence of selection is well reflected in the age of onset of thrombosis that clearly differs between individuals from thrombophilia families and unselected thrombosis patients.[48] Finally, other aspects such as an objective diagnosis of thrombosis and prospective *vs* retrospective studies also influence the estimates of risk.

Importance of a risk factor for thrombosis

With so many new risk factors emerging, the question is what impact they have in daily clinical practice. In other words, how can the results from research be translated into practical clinical guidelines? First of all, we must make sure that the new risk factor is independent and clinically relevant. This requires full adjustment for potential confounders, such as age, sex, body mass index, and other coagulation factors. This does not apply for genetic risk factors, since these are by definition unconfounded.

It is important to appreciate and interpret the differences between absolute and relative risks. The relative risks that have been calculated from case-control studies are mainly important to the researcher, whereas absolute risk estimates (the probability to develop thrombosis and the possibility to lower this probability) is relevant to the individual patient and his physician.[49] Population-attributable risk estimates are also important for the population and can influence decision-making. Asymptomatic females with deficiencies of antithrombin, protein C, or protein S have an eightfold increased relative risk of thrombosis during pregnancy.[30] The absolute risk is 4.1% (7 in 169 pregnancies). So, these deficiencies have a high magnitude of risk, but because of the low prevalence, account for only a small percentage of the overall thrombosis risk. Likewise, oral contraceptives and hormone replacement therapy both increase the risk of the thrombosis approximately fourfold. Since the baseline risk of thrombotic disease is nearly tenfold higher in the older HRT users, their basic risk of thrombosis is greater than in women who are on oral contraceptives.[49] Hypertension is a moderate risk factor for congestive heart failure, but accounts for nearly half of the cases of heart failure in a population.[50] So, in general, common risk factors usually have moderate relative risks, but are important at population level when its prevalence

is high. Strong risk factors are generally less important for healthy individuals.

If we consider thrombophilia screening for risk factors, the measurement must be reliable (low coefficient of variation) and reflect a true representation of the risk factor. For prothrombotic mutations, such as factor V Leiden and prothrombin mutation, this is generally not a problem. Most coagulation factors, such as factor VIII, however, have a large intra-individual and inter-assay variation, as reflected in the decision to use the factor VIII measurements of three different plasma samples to calculate the probability of carriership of haemophilia A. Further, there is the important question how we should interpret the result of a measurement in terms of risk of a first thrombotic event and risk of recurrences. Most risk factors have wide ranges of values with large overlap between individuals with and without thrombosis. These risk factors typically increase with increasing levels, without a clear threshold. So, artificial cut-off values which were used in clinical research are being used now for decision-making in individual patients. It is unknown whether these cut-off values are practical and reliable. We do not know the sensitivity or specificity of most risk factors for predicting future occurrences of thrombosis. Factor VIII levels can easily rise above the cut-off value of 150 IU/dl due to acute phase reactions, such as a thrombotic event. This transient rise may cause a mislabelling of a person with venous thrombosis who normally has a low factor VIII level.[51] Since factor VIII levels may be associated with the risk of recurrences,[28] a treating physician might decide to prolong anticoagulant therapy on the basis of a single measurement. This shows that the results from research cannot simply be extrapolated to patient care and can even lead to wrong decisions (*primum non nocere*).

IMPLICATIONS OF THROMBOPHILIA SCREENING FOR THE INDIVIDUAL PATIENT

As already stated, relative risk has no value in the clinic, and only knowledge of the absolute risk of developing thrombosis may have relevance for the individual patient, and then still only if this leads to the possibility of prevention. This would imply that for each patient at risk of a first episode of thrombosis or for a recurrent event, an individualised risk profile should be available with age, sex, current risk factors and the possibility of future risk factors, such as trauma, surgery and pregnancy, while for each factor its strength should be known, as well as its interaction with the other factors. This scenario is still far away. It is not even feasible to readily identify patients with thrombophilia unless all thrombosis patients are screened, since half of the first thrombotic events in patients with

thrombophilia are not idiopathic and occur in high-risk situations. Practical recommendations have been suggested to guide screening strategies in patients with thrombosis, in which patients are divided in 'strongly' and 'weakly' thrombophilic.[19] The 'strongly' thrombophilic patients include patients with age at onset <50 years, patients with recurrent thrombosis, or first-degree family members with a thrombotic event before 50 years of age. All other patients are 'weakly' thrombophilic and should be screened for the common defects such as factor V Leiden and prothrombin mutation, while the former group should also be tested for the more rare defects such as deficiencies of protein C, protein S and antithrombin. This strategy optimises the likelihood of finding a prothrombotic abnormality, but does not necessarily benefit the patient. With the current knowledge it is questionable whether the presence of a risk factor leads to any difference in clinical management, and therefore screening does not seem helpful. The most compelling question is whether, based on laboratory tests, we can predict the risk of recurrence and, while the various studies are not in complete agreement, it may well be that the risk of recurrence is not increased in the presence of prothrombotic defects. In that case it makes more sense to base clinical strategy on clinical history, i.e., the severity of the event or the age of the patient, than on laboratory tests. The next question concerns asymptomatic relatives: is it useful to screen asymptomatic individuals from a family with hereditary thrombophilia, for instance women who intend to become pregnant or want to start oral contraceptives? Again, the literature offers little assistance, except that in most cases the risk of thrombosis appears to be low. Women from families with a strong history of thrombosis may consider not using oral contraceptives.

CONCLUSION

The last decade revealed several new risk factors that contribute to a better understanding of the pathogenesis of venous thrombosis. Well-designed large population-based case-control studies were a prerequisite for establishing new risk factors, such as factor V Leiden, prothrombin 20210A mutation, procoagulant factors, as factor VIII, IX, and XI, and antifibrinolytic factors, such as TAFI. Since many individuals with a thrombophilic factor are asymptomatic, a single defect is seldom sufficient to cause thrombosis. Thrombosis is thus a multicausal disease, in which genetic and environmental factors interact dynamically. The common risk factors with a high prevalence in the general population make a major contribution to the overall risk of thrombosis. These risk factors are likely to occur simultaneously in an individual with thrombosis. When these clusters of risk factors can be identified, preventive measures can be installed, mainly for those individuals

with a genetic predisposition. This can only be assessed adequately through sufficient knowledge of important risk factors for thrombosis, their effect and interaction with other genetic and environmental factors, and the beneficial effect of intervention. Until that time, screening for thrombophilia will remain a matter of debate.

REFERENCES

1. Virchow R. Phlogose und Thrombose im gefäßsystem; Gesammelte Abhandlungen zur Wissenschaftlichen Medizin. Frankfurt: Staatsdruckerei, 1856.

2. Meer FJ van der, Koster T, Vandenbroucke JP, Briët E, Rosendaal FR. The Leiden Thrombophilia Study (LETS). Thromb Haemost 1997;78:631-5.

3. Jeremic M, Weisert O, Gedde-Dahl TW. Factor VIII (AHG) levels in 1016 regular blood donors. The effects of age, sex, and ABO blood groups. Scand J Clin Lab Invest 1976;36:461-6.

4. Wise RJ, Dorner AJ, Krane M, Pittman DD, Kaufman RJ. The role of von Willebrand factor multimerisation and propeptide cleavage in the binding and stabilisation of factor VIII. J Biol Chem 1991;266:21948-55.

5. Kamphuisen PW, Houwing-Duistermaat JJ, Houwelingen HC van, Eikenboom JCJ, Bertina RM, Rosendaal FR. Familial clustering of factor VIII and von Willebrand factor levels. Thromb Haemost 1998;79:323-7.

6. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet 1999;353:1167-73.

7. Rosendaal FR. Risk factors for venous thrombosis: prevalence, risk, and interaction. Semin Hematol 1997;34:171-87.

8. Lensen RP, Rosendaal FR, Koster T, et al. Apparent different thrombotic tendency in patients with factor V Leiden and protein C deficiency due to selection of patients. Blood 1996;88:4205-8.

9. Vandenbroucke JP, Koster T, Briët E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. Lancet 1994;344:1453-7.

10. Souto JC, Almasy L, Borrell M, et al. Genetic determinants of hemostasis phenotypes in Spanish families. Circulation 2000;101:1546-51.

11. Lange M de, Snieder H, Ariens RA, Spector TD, Grant PJ. The genetics of haemostasis: a twin study. Lancet 2001;357:101-5.

12. Rosendaal FR, Bovill EG. Heritability of clotting factors and the revival of the prothrombotic state. Lancet 2002;359:638-9.

13. Lavigne G, Mercie E, Queré I, Dauzat M, Gris JC. Thrombophilic families with inheritably associated high levels of coagulation factors VIII, IX and XI. J Thromb Haemost 2003;1:2134-9.

14. Hylckama Vlieg A van, Callas PW, Cushman M, Bertina RM, Rosendaal FR. Inter-relation of coagulation factors and d-dimer levels in healthy individuals. J Thromb Haemost 2003;1:516-22.

15. Erdmann D, Heim J. Orphan nuclear receptor HNF-4 binds to the human coagulation factor VII promoter. J Biol Chem 1995;270:22988-96.

16. Reijnen MJ, Sladek FM, Bertina RM, Reitsma PH. Disruption of a binding site for hepatocyte nuclear factor 4 results in hemophilia B Leyden. Proc Natl Acad Sci USA 1992;89:6300-3.

17. Hung HL, High KA. Liver-enriched transcription factor HNF-4 and ubiquitous factor NF-Y are critical for expression of blood coagulation factor X. J Biol Chem 1996;271:2323-31.

18. Kaufman RJ. Post-translational modifications required for coagulation factor secretion and function. Thromb Haemost 1998;79:1068-79.

19. Bauer KA. The thrombophilias: well-defined risk factors with uncertain therapeutic implications. Ann Intern Med 2001;135:367-73.

20. Kearon C, Ginsberg JS, Kovacs MJ, et al. Comparison of low-intensity warfarin therapy with conventional-intensity warfarin therapy for long-term prevention of recurrent venous thromboembolism. N Engl J Med 2003;349:631-9.

21. Crowther MA, Ginsberg JS, Julian J, et al. A comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. N Engl J Med 2003;349:1133-8.

22. Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. Circulation 1995;92:2800-2.

23. Simioni P, Prandoni P, Lensing AW, et al. The risk of recurrent venous thromboembolism in patients with an Arg506—>Gln mutation in the gene for factor V (factor V Leiden). N Engl J Med 1997;336:399-403.

24. Kearon C, Gent M, Hirsh J, et al. A comparison of three months of anti-coagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999;340:901-7.

25. Eichinger S, Minar E, Hirschl M, Bialonczyk C, Stain M, Mannhalter C. The risk of early recurrent venous thromboembolism after oral anti-coagulant therapy in patients with the G20210A transition in the prothrombin gene. Thromb Haemost 1999;81:14-7.

26. Eichinger S, Pabinger I, Stumpflen A, Hirschl M, Bialonczyk C, Schneider B, The risk of recurrent venous thromboembolism in patients with and without factor V Leiden. Thromb Haemost 1997;77:624-8.

27. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999;81:684-9.

28. Kyrle PA, Minar E, Hirschl M, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med 2000;343:457-62.

29. Heijer M den, Blom HJ, Gerrits WB, et al. Is hyperhomocysteinaemia a risk factor for recurrent venous thrombosis? Lancet 1995;345:882-5.

30. Belt AG van den, Sanson BJ, Simioni P, et al. Recurrence of venous thromboembolism in patients with familial thrombophilia. Arch Intern Med 1997;157:2227-32.

31. De Stefano V, Martinelli I, Mannucci PM, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 1999;341:801-6.

32. Emmerich J, Rosendaal FR, Cattaneo M, et al. Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism-pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism. Thromb Haemost 2001;86:809-16.

33. Koeleman BP, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. Blood 1994;84:1031-5.

34. Boven HH van, Reitsma PH, Rosendaal FR, et al. Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency. Thromb Haemost 1996;75:417-21.

35. Zoller B, Berntsdotter A, Garcia de Frutos P, Dahlback B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. Blood 1995;85:3518-23.

36. Meinardi JR, Middeldorp S, Kam PJ de, et al. The incidence of recurrent venous thromboembolism in carriers of factor V Leiden is related to concomitant thrombophilic disorders. Br J Haematol 2002;116:625-31.

37. Schulman S, Svenungsson E, Granqvist S. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. Am J Med 1998;104:332-8.

38. Bauer KA. Management of thrombophilia. J Thromb Haemost 2003;1:1429-34.

39. Makris M, Rosendaal FR, Preston FE. Familial thrombophilia: genetic risk factors and management. J Intern Med Suppl 1997;740:9-15.

40. Middeldorp S, Meinardi JR, Koopman MM, et al. A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. Ann Intern Med 2001;135:322-7.

41. Lindqvist PG, Svensson PJ, Marsaal K, Grennert L, Luterkort M, Dahlback B. Activated protein C resistance (FV:Q506) and pregnancy. Thromb Haemost 1999;81:532-7.

42. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM. The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 2000;111:1223-9.

43. Ginsberg JS, Greer I, Hirsh J. Use of antithrombotic agents during pregnancy. Chest 2001;119(suppl):122-31.

44. Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

45. Martinelli I. Pros and cons of thrombophilia testing: pros. J Thromb Haemost 2003;1:410-1.

46. Machin SJ. Pros and cons of thrombophilia testing: cons. J Thromb Haemost 2003;1:412-3.

47. Martinelli I, Mannucci PM, De Stefano V, et al. Different risks of throm-bosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. Blood 1998;92:2353-8.

48. Koeleman BP, Reitsma PH, Bertina RM. Familial thrombophilia: a complex genetic disorder. Semin Hematol 1997;34:256-64.

49. Rosendaal FR, Hylckama Vlieg A van, Tanis BC, Helmerhorst FM. Estrogens, progestogens and thrombosis. J Thromb Haemost 2003;1:1371-80.

50. Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. JAMA 1996;275:1557-62.

51. Kamphuisen PW, Wolde M ten, Jacobs EM, Ullmann EF, Büller HR. Screening of high factor VIII levels is not recommended in patients with recently diagnosed pulmonary embolism. J Thromb Haemost 2003;1:2239-40.