Memoranda / Mémorandums

Inherited thrombophilia: Memorandum from a joint WHO/International Society on Thrombosis and Haemostasis meeting*

Inherited thrombophilias are common disorders with a worldwide distribution, including antithrombin, protein C, and protein S deficiencies as well as resistance to activated protein C. Increased understanding of these disorders suggests that thrombophilia can arise from interaction between defective genes and environmental factors. WHO and the International Society on Thrombosis and Haemostasis (ISTH) discussed the problems of inherited thrombophilia at a joint meeting held in Geneva on 6–8 November 1995. The present article reports on the various possibilities for controlling the disorder and makes a series of recommendations for diagnosis, treatment, and research into the condition.

Introduction

Before 1990 the results of studies to identify isolated hereditary deficiencies or defects were consistent with two principle endogenous anticoagulant pathways (Fig. 1): the antithrombin–heparan sulfate pathway and the protein C/protein S pathway. However, until that time, only three single gene disorders had been identified that were associated with a significant increase in the risk for venous thromboembolism in families with a symptomatic deficient patient: antithrombin, protein C and protein S deficiencies. These deficiencies occurred only in about 15% of families with familial thrombosis and only in a small proportion of all patients with venous thrombosis (I).

The awareness that for 85% of families predisposed to thrombosis no explanation could be found stimulated the application of genetic and epidemiological approaches to the problem. As a result, a major breakthrough in the study of familial thrombosis has been achieved over the past 3 years. First, activated protein C resistance (APC-R) was discovered, and, second, a mutation in the factor V gene (1691 G → A in exon 10, leading to 506Arg → Gln substitution) was identified as the molecular basis for the phenotype of APC-R in the large majority of affected individuals (2, 3). This mutation, which is associated with a significant increase in thrombotic risk (3–5), has been found in about 50% of selected families with thrombophilia and in 20% of consecutive patients with thrombosis. A consequence of this advance has been a conceptual change in how thrombophilia is viewed, which has implications for its diagnosis and treatment.

Pathogenesis and genetic basis of thrombophilia

Thrombophilia’s predisposing defects do not necessarily cause continuous clinical impairment; they only weaken the ability to cope with fluctuations induced by interactions with the environment. A list of potential genetic risk factors that have been associated with thrombosis is shown in Table 1.

The term inherited thrombophilia reflects the presence of an inherited factor that, per se, predis-
Fig. 1. Schematic representation of the two principle anticoagulant pathways that are important in the regulation of coagulation proteinase activity. a) Coagulation regulation by antithrombin; b) Coagulation regulation by protein C/protein S. To the left of each diagram is a simplified view of the coagulation cascade, illustrating the positive “procoagulant” feedback loops through which thrombin activates factors V and VIII. To the right, are the “anticoagulant” pathways which prevent excessive activation of coagulation. These pathways involve antithrombin (which directly inhibits the coagulation proteinases such as factor Xa and thrombin), and protein C/protein S (which inactivate factor Va and factor VIIIa). Protein S normally forms a complex with C4bBP and it is only the free form of protein S that acts as a cofactor for protein C.
Table 1: Causes of inherited thrombophilia*

<table>
<thead>
<tr>
<th>Acquired</th>
<th>Inherited</th>
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<tr>
<td>Antithrombin deficiency</td>
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<td>Protein C deficiency</td>
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<td>Protein S deficiency</td>
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<td>APC-R/factor V 506 Arg → Gln</td>
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<tr>
<td>Dysfibrinogenaemia</td>
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<td>Thrombomodulin</td>
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| * To date, there is no firm evidence that these conditions are linked to inherited thrombophilia. The development of thrombosis is often believed to be caused by interaction between genetic and acquired factors (the best recognized of the latter being advancing age, immobilization, major surgery, orthopaedic surgery, neurosurgery, pregnancy, puerperium, use of estrogen-containing hormones, malignancies, and antiphospholipid syndrome).

Antithrombin deficiency is a heterogenous disorder. The subclassification of antithrombin deficiency was originally based mainly on the results of functional and immunological assays of plasma. Subsequently, as understanding of the mutations in the antithrombin gene increased, the nomenclature was modified (6, 7). Currently, two types of antithrombin deficiency are recognized: type I (identified by a concordant reduction of both functional and immunological antithrombin); and type II (identified by a variant antithrombin molecule, which has a defect in the reactive site (II RS), a defect affecting the heparin binding site (II HBS), or multiple functional defects (pleiotropic effect) (II PE)). Clinically antithrombin deficiency is heterogeneous, with mutations causing type II HBS deficiency being of much less risk than those causing the other subtypes (7, 8).

Recurrent mutations occur in all types of antithrombin deficiency, but especially in type II HBS; of the 21 distinct repeat mutations, nine involve a CpG dinucleotide (a “hotspot” for mutation). Only preliminary data are currently available that throw light on whether these recurrent mutations are the result of independent mutations or a founder effect (identity by descent) (9).

**Protein C deficiency**

Protein C is a vitamin-K-dependent plasma glycoprotein that is the precursor of the serine-proteinase-activated protein C (APC). Protein C is synthesized in the liver as a single chain (62 kDa), which is converted into two chains by removal of a dipeptide (Arg157-Thr158) probably in the Golgi complex. In plasma most protein C occurs in the double chain form (41 kDa heavy chain and 21 kDa light chain); the concentration of protein C in plasma is normally 65 nmol/l but is reduced during treatment with oral anticoagulants.

Protein C deficiency is a heterogeneous disorder (10, 11). A phenotypic subclassification has been proposed, based on the results of functional and immunological assays of protein C. In type I protein C deficiency there is a concordant reduction in protein C activity and in the level of protein C antigen, while in the type II deficiency there is evidence for the presence of an abnormal protein C molecule (reduced protein C activity, normal protein C anti-
gen). A further classification of the type II protein C deficiency can be made by comparing the results of different functional tests (clotting test vs chromogenic test).

In total, 160 different mutations have been reported to cause type I or type II protein C deficiency. Surprisingly, ca. 60% of the mutations causing the type I deficiency are missense mutations. Probably these amino acid substitutions lead to changes in the interactions with other residues and thus interfere with protein folding, a condition associated with rapid intracellular degradation of the protein.

Protein S deficiency

Protein S is a vitamin-K-dependent plasma glycoprotein (70 kDa) that is synthesized in the liver, but also in endothelial cells, megakaryocytes and Leydig cells in the testis. Its concentration in plasma is 25 μg/ml, but is lower during treatment with oral anticoagulants.

Consistent with the subclassifications used for other hereditary deficiencies, type I deficiencies/defects result in a reduction of total protein S antigen (and of free protein S antigen and protein S activity). Type II deficiency defines the presence of a functionally abnormal protein S molecule (normal levels of total protein S antigen and of free protein S antigen but reduced protein S activity). Type III protein S deficiency is defined by normal total protein S antigen but reduced free protein S antigen and activity. Although this phenotype seems to be rather prevalent, it is not yet clear whether it is caused by a hereditary defect and if so, whether it is linked to the protein S locus.

Genetic analysis of the protein S genes of symptomatic protein S deficient probands has been hampered by the structural complexity of the protein S gene and by the existence of the highly homologous pseudogene. In three separate studies mutations were only found in 50–60% of the patients, although all coding and flanking regions had been amplified and sequenced (12–14).

The majority of genetic lesions that cause a type I deficiency are single nucleotide substitutions, insertions, and deletions; so far, at least 33 unique events have been reported. Four different mutations have been reported to cause a type II protein S deficiency (15, 16); two in the propeptide, one in the first EGF domain and one in the second EGF domain.

Factor V Arg506 → Gln mutation

Factor V is a single-chain plasma glycoprotein procofactor that is synthesized in the liver and in megakaryocytes. Its concentration in human plasma is 20 nmol/l and in platelets, 4 μg per 10⁶ platelets. During blood coagulation factor V is converted into factor Va by meizothrombin and/or factor Xa.

More recently it has been reported that factor V is not only a pro-cofactor in the prothrombinase reaction but also a cofactor in the inactivation of factor VIIIa by APC (17). A recent review provides more information on the structure and function of human factor V (18).

In 1994, the single-point mutation in the factor V gene was identified as the genetic defect that caused the phenotype of APC-R in the vast majority of affected individuals (3, 5, 19). This mutation involves a G → A transition of nucleotide 1691 in exon 10, which predicts the synthesis of a variant factor V molecule (factor V 506 Arg → Gln or factor V Leiden). The mechanism through which the mutation leads to the APC-R phenotype is still the subject of detailed biochemical studies.

So far, the factor V 506 Arg → Gln mutation is the only genetic defect that has been identified in APC-R families. Its frequency is relatively high in Caucasian populations (up to 6%) but is much lower in African and Asian populations (down to 0%) (20). Evidence for a founder effect in the spread of this disorder was obtained from the results of haplotype analysis of 53 Dutch carriers of the mutation (3).

Other candidates?

There are a number of other genetic defects or isolated deficiencies that have been implicated in contributing to the risk of thrombosis in families with thrombophilia. In most cases these are based on observations of case families. Sometimes genetic defects have been identified but no data on genotype-phenotype relationships are currently available (21).

Hereditary dysfibrinogenemia is characterized by a prolonged plasma thrombin time. Clinical symptoms vary from none, to mild bleeding, to venous or arterial thrombosis. The phenotype may follow recessive or dominant inheritance. Recently the evidence for a causal relationship between an isolated dysfibrinogenemia and venous thrombosis has been critically reviewed and discussed (22).

Mild hyperhomocysteinaemia has recently been found in 19% of patients with juvenile venous thrombosis, and family studies showed that in most cases this phenotype was inherited (23).

Thrombomodulin (TM), a further component of the protein C anticoagulant pathway (24), is a transmembrane protein synthesized by endothelial cells and acts as a receptor for thrombin and as cofactor of thrombin in the activation of protein C.
By analogy with protein C and protein S deficiencies, it might be expected that deficiencies or defects in thrombomodulin may be associated with an increased risk of thrombosis. However, to date, information is still very limited on the co-segregation of these mutations with thrombophilia in the proband families.

Plasminogen deficiency and dysplasminogenemia, have frequently been reported to be associated with thrombophilia. However, studies reveal that in most families with a type I plasminogen deficiency (parallel reduction of plasminogen activity and antigen) only the proband suffers from thrombotic disease (25), which can be taken as evidence against its causative role in thrombosis. The frequency of plasminogen deficiency among the general population seems to be slightly lower (0.4%) than among cohorts of patients with thrombosis (1–3%) (26, 27).

Since reduced plasminogen levels may cause thrombophilia, it seems reasonable to propose that an inherited elevated level of histidine-rich glycoprotein (HRG) in plasma is also a risk factor for thrombosis. HRG (a non-enzymatic protein) forms a 1:1 complex with plasminogen in plasma (via its lysin-binding sites) and thus reduces the free plasminogen concentration to around 50% (28). Complex formation with HRG interferes with the binding of plasminogen to fibrin. Although several families with thrombophilia and high HRG levels have been reported (29, 30), there is still no formal evidence for its association with thrombosis.

A further potential risk factor of thrombosis — tissue factor pathway inhibitor (TFPI) deficiency — has been investigated, but no mutation has been found in the TFPI genes of 30 symptomatic probands of families with thrombophilia (P.H. Reitsma & R. M. Bertina, unpublished observations).

Finally, a phenotype has recently been identified as a risk factor for thrombosis in large patient-control studies: elevated factor VIII levels (31). The heritability of this phenotype and the possible underlying molecular defects have not yet been reported.

Epidemiology of inherited thrombophilia

Venous thrombosis has an overall annual incidence of 1 per 1000 population. It is rare among the young, and becomes more frequent with advancing age. The true prevalence of hereditary thrombophilia has not yet been determined. All the genetic abnormalities that cause a tendency to venous thrombosis are clearly not known, since only in about half the patients from families selected on the basis of a high number of unexplained thromboses is an underlying defect found (32). Hence, the prevalence of hereditary thrombophilia in the general population will be higher, possibly twice as high as in estimates from large prevalence studies on known genetic defects, and the high prevalence of hereditary thrombophilia will make it an important factor in the overall incidence of thrombosis.

The prevalence of protein C and antithrombin deficiencies has been investigated in a very large study of almost 10000 blood donors (33, 34) (Table 2). Repeated testing of the levels of these proteins, coupled with family studies and DNA analysis, led to prevalence estimates of 1 in 500 for protein C deficiency and 1 in 5000 for type I antithrombin deficiency. These values lie in the same range found in a previous study of over 5000 blood donors, where 1 in 250 were considered to be protein C deficient (35). If the approximate prevalence of protein C deficiency is taken to be 1:350, the prevalence of severe (homozygous or compound heterozygous) deficiency is $1:700 \times 1:700 = 2 \times 10^{-6}$.

For APC-R, the groups that have been studied are smaller than those used in the blood donor studies. However, since the prevalence of this abnormality is ten times greater than that of the other inhibitor deficiencies, the estimates for its prevalence are as reliable. Such estimates for Caucasians lie in the range 3–7% (4, 36, 37), which, since they are based on self-selected individuals without a history of cardiovascular disease or venous thrombosis, are under- rather than over-estimates. The prevalence of the homozygous factor V 506 Gln mutation has been estimated to be approximately 1:5000 (4).

Approximately 5% of consecutive patients with objectively confirmed deep-vein thrombosis are accounted for by protein C, protein S, and antithrombin deficiencies combined, while APC-R is present in 20% of consecutive patients with deep-vein thrombosis (4, 38).

Among selected patient groups, the prevalences of protein C, protein S, and antithrombin deficiencies are mostly in the range 5–10%, much higher than the levels found in population studies, and also somewhat higher than those among consecutive unselected patients. The higher prevalences among patients with thrombosis than among healthy individuals, and among thrombophilic individuals than among unselected patients also indicate that these deficiencies indeed lead to venous thrombosis and venous thrombophilia. APC-R appears to account for half of all cases of hereditary thrombophilia, and as shown in Table 2 clearly emerges as the most important cause of hereditary thrombosis and perhaps of venous thrombosis in general.
Memorandum

Table 2: Prevalence of the major thrombophilic clotting abnormalities

<table>
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<th>% prevalence of:</th>
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<tr>
<td>Protein C deficiency</td>
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**Healthy individuals**
- Tait et al. (n = 9669) (ref. 33, 34) 0.2
- Miletich et al. (n = 5422) (ref. 35) 0.4
- Svensson & Dahlbäck (n = 130) (ref. 36) —
- Rosendaal et al. (n = 474) (ref. 4) —
- Ridker et al. (n = 704) (ref. 37) —

**Consecutive patients with first deep venous thrombosis**
- Heijboer et al. (n = 277) (ref. 64) 3
- Koster et al. (n = 474) (ref. 65) 3
- Rosendaal et al. (n = 471) (ref. 4) —

**Thrombophilic patients**
- Briët et al. (n = 113) (ref. 66) 8
- Scharrer et al. (n = 158) (ref. 67) 9
- Ben Tai et al. (n = 107) (ref. 68) 6
- Taberner et al. (n = 204) (ref. 69) 1
- Griffin et al. (n = 25) (ref. 32) —

* DNA confirmed.
* Type I antithrombin deficiency.

Clinical manifestations of inherited thrombosis

The commonest clinical manifestation of inherited thrombosis is deep vein thrombosis of the lower limbs, with or without pulmonary embolism, which accounts for approximately 90% of all the thrombotic episodes (Table 3). Unusual sites of venous thrombosis, such as the mesenteric or cerebral veins, account for less than 5% of the total episodes in patients with antithrombin, protein C or protein S deficiencies; in patients with APC-R, thrombosis seems to occur less frequently at such sites.

A total of 50–60% of individuals from families with antithrombin, protein C, and protein S deficiencies have a history of thrombosis at diagnosis with a 50% recurrence rate; in approximately 80% of patients the first thrombotic episode occurs before 40 years of age. For antithrombin deficiency, the overall risk of venous thrombosis is considered to be greater than with protein C or protein S deficiency (39), but contradictory results have been obtained (40).

In patients with antithrombin, protein C and protein S deficiencies, 32–50% of the venous thrombotic episodes occur when other risk factors are concomitantly present (surgery, pregnancy, immobilization) (40–43). In individuals with APC-R, the need for the presence of such risk factors to trigger thrombotic episodes appears to be greater (62%) than for the other thrombophilic syndromes (44).

The following risk factors are often associated with the occurrence of thrombosis: pregnancy, puerperium, and surgery. Among women with antithrombin deficiency, the frequency of thrombosis during pregnancy and puerperium is 37–44%: in instances of protein C or protein S deficiency, 12–19% (45); in APC-R, 28% (46). Thrombotic episodes occur most frequently during puerperium, accounting for 60–75% of all such episodes that complicate pregnancy. Retrospective analysis of a large number of antithrombin, protein C or protein S deficient individuals produced an overall frequency of venous thrombosis complicating surgery of 22%, with no significant differences arising because of the

Table 3: Clinical features of patients with inherited thrombophilia arising from defects in anticoagulant pathways

<table>
<thead>
<tr>
<th>Venous thromboembolism (&gt;90% of cases)</th>
<th>Deep vein thrombosis of lower limbs (common)</th>
<th>Pulmonary embolism (common)</th>
<th>Superficial thrombophlebitis</th>
<th>Mesenteric vein thrombosis (rare but characteristic)</th>
<th>Cerebral vein thrombosis (rare but characteristic)</th>
<th>Family history of thrombosis</th>
<th>First thrombosis usually at &lt;45 years of age</th>
<th>Frequent recurrences*</th>
<th>Neonatal purpura fulminans (homozygous protein C and protein S deficiency)</th>
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<td>* Less evident in patients with APC-R, who appear to be less severely affected clinically.</td>
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type of deficiency or surgical procedure. Intake of oral contraceptives is associated with an increased thrombotic risk, particularly for women with antithrombin deficiency and APC-R (46).

Homozygous antithrombin deficiency is extremely rare and almost exclusively reported in patients with HBS defects; such individuals have a severe thrombotic history of early onset, often affecting their arteries (47). Homozygous type I antithrombin deficiency has a poor prognosis: in one report, two brothers with this defect died within 3 weeks of birth (48). Homozygous protein C deficiency is associated with unusual phenotypic and clinical expressions (reviewed in 49). Among patients whose protein C levels are too low to be measured, purpura fulminans, caused by thrombosis of small vessels with cutaneous and subcutaneous ischaemic necrosis, may occur soon after birth or during the first year of life. Among those patients with very low but measurable protein C levels (5–20%), clinical manifestations are milder and generally similar to those for heterozygous deficiency. Homozygous protein S deficiency has rarely been reported, but is also associated with neonatal purpura fulminans (50). In view of the high frequency of the mutant factor V among the general population, homozygous APC-R is relatively frequent (ca 1:5000) (4).

Management of inherited thrombophilia

Acute events

The management of acute venous thrombosis or pulmonary embolism involving patients with inherited thrombophilia is generally not different from that of other patients. Thrombolytic therapy can be used for patients with massive acute venous thrombosis or pulmonary embolism. An intravenous bolus of 5000 units of heparin should be initiated followed by an infusion of 1400 units per hour (51), or if a weight-adjusted regimen is used, a bolus of 80 units per kg body weight followed by an infusion of 18 units per kg per hour (52).

The infusion of 50 units of antithrombin concentrate per kg body weight (one unit is the amount of antithrombin present in 1 ml of pooled normal human plasma) will usually raise the plasma antithrombin level to approximately 120% in a congenitally deficient individual whose baseline level is 50% (53). Plasma levels should be monitored to ensure that they remain above 80%; administration of 60% of the initial dose at 24-hour intervals is recommended to maintain inhibitor levels in the normal range (53).

After an episode of venous thrombosis or pulmonary embolism, patients are usually given oral anticoagulants for 3–6 months. Recent data indicate that the risk of recurrence is greater for patients with permanent rather than temporary risk factors for thrombosis (54, 55) and it is therefore appropriate that patients with inherited thrombophilia be given warfarin for at least 6 months at an international normalized ratio (INR) of 2.0–3.0.

Following 6 months of anticoagulant therapy for an acute thrombotic event, the relative benefit conferred by such long-term therapy in preventing future thromboembolic complications versus the potential side-effects, cost, and inconvenience for the patient should be assessed. Unfortunately there are a paucity of reliable data on the magnitude of the thrombotic risk or the benefit of anticoagulant therapy for patients with antithrombin, protein C, or protein S deficiencies since these are relatively uncommon disorders. Because of the high frequency of APC-R among patients presenting with a first episode of venous thrombosis, reliable data are just emerging on the risk of recurrence (37). Here however, we give only general guidelines for managing patients with the various hereditary defects that predispose to thrombosis rather than provide rigid recommendations.

Inherited thrombotic disorders

When a heterozygous patient with one of the hereditary thrombotic disorders is identified, family studies should be conducted since approximately half the first-degree relatives will be affected. Affected asymptomatic individuals should be counselled about the implications of the diagnosis and given advice about those symptoms that require immediate medical attention. Among women of child-bearing age, oral contraceptives are generally contraindicated because of the increased thrombotic risk associated with their use, although individual circumstances need to be considered. The replacement dose of estrogens administered to postmenopausal women is much lower than the contraceptive dose and has not been shown to increase the risk of venous thrombosis among the general population (56). Since no data are available that indicate that postmenopausal estrogen replacement therapy increases the risk of thrombosis among patients with a hereditary thrombotic disorder, such therapy is not absolutely contraindicated.

All individuals with inherited thrombotic disorders should be carefully evaluated prior to surgical, medical, or obstetric procedures that carry an in-
creased thrombotic risk and should then receive appropriate prophylactic anticoagulation regimens. If specific concentrates are available for the patient's deficiency state, under certain circumstances these might also be administered to raise the plasma levels of the protein to the normal range during the perioperative period.

Among patients with an inherited thrombotic disorder, the occurrence of two or more spontaneous thromboembolic episodes often leads to the continuation of oral anticoagulants for life, even though some workers hold that the risks of bleeding could exceed those of recurrence of thrombosis.

**Management of pregnancy**

The management of pregnancies among women with hereditary thrombotic disorders poses special problems. The incidence of thrombotic complications during pregnancy and the postpartum period appears to be greater for women with antithrombin deficiency than protein C or protein S deficiencies (57). Recent data indicate that 60% of women who develop a first episode of venous thrombosis during pregnancy have APC-R. During pregnancy, adjusted-dose heparin administered subcutaneously is the anticoagulant of choice because its efficacy and safety for the fetus are established (58). Centres with greater experience in using low molecular weight heparins, however, might find that they are advantageous since laboratory monitoring may not be required. Patients with a history of thrombotic episodes should receive treatment throughout pregnancy, while women with antithrombin deficiency but who have not yet experienced thrombosis should probably be treated. Treatment of asymptomatic women with other hereditary thrombotic disorders should be considered on an individual basis.

Both the dose and duration of heparin therapy in pregnancy are uncertain since appropriately designed clinical trials have not been performed. Patients considered to be at high risk should receive full-dose heparin subcutaneously every 12 hours for the duration of pregnancy. The dose of heparin should be adjusted to ensure that the 6-hour post-injection activated partial thromboplastin (APTT) level is 1.5 times the control value. For women considered to be at intermediate risk, lower doses of heparin can be used (5000–10000 units subcutaneously every 12 hours) and therapy can be started during the second or third trimester and continued for approximately 6 weeks into the postpartum period. Low-risk patients can be observed closely throughout pregnancy with duplex ultrasound imaging of their leg veins at regular intervals.

**Coumarin-induced skin necrosis and neonatal purpura fulminans**

A clear association has been established between coumarin-induced skin necrosis and hereditary protein C deficiency, with about a third of patients with such necrosis having hereditary protein C deficiency (59). This complication has also been described in a patient with homozygous protein S deficiency (60). Since coumarin-induced skin necrosis is a rare complication, therapy has been guided primarily by understanding of its pathogenesis; such therapy should consist of immediate discontinuation of warfarin, administration of vitamin K, and infusion of heparin at therapeutic doses. Fresh frozen plasma has been used, but improved results can be expected with the administration of a highly purified protein C concentrate, which facilitates the rapid and complete normalization of plasma protein C levels (61).

Management of neonatal purpura fulminans in association with homozygous or doubly heterozygous protein C deficiency is more complicated and heparin therapy as well as antiplatelet agents are not effective (62). Administration of a source of protein C appears to be critical in the initial treatment of such patients, and fresh frozen plasma has been used with success to treat infants. However, the half-life of protein C in the circulation is only about 6–12 hours (63), and administration of plasma on a frequent basis is limited by the development of hyperproteininaemia, hypertension, loss of venous access, and the potential for exposure to infectious viral agents. Warfarin has been administered to infants without the redevelopment of skin necrosis during the phased withdrawal of fresh frozen plasma infusions (62), and has been used chronically to control thrombotic diathesis.

**General overview**

Currently, mutations in four genes are clearly linked to increased risk for venous thromboembolism. Many discrete mutations cause antithrombin, protein C, and protein S deficiencies that diminish the capacity to balance procoagulant activity. One specific mutation in factor V (506Arg → Gln) has a similar impact by rendering this procoagulant factor resistant to proteolytic degradation. Roughly 50% of cases of familial thrombophilias can be explained by these four established risk factors for thrombosis. Apparently a number of other genetic risk factors have escaped detection, so far; it is, however, unlikely that these will include plasminogen, heparin co-factor II, tissue factor pathways inhibitor, or β2-glycoprotein-1 deficiencies. Other risk factors will
need further evaluation (e.g. dysfibrinogenaemia, thrombomodulin defects and inherited hyperhomocysteinaemia). Increasingly it is becoming apparent that coinheritance of more than one relatively mild thrombophilic risk factor results in more severe clinical expression.

Greater attempts are being made to quantify genetic and acquired risk factors. Risk estimates depend heavily on how study subjects are selected and do not necessarily apply to individuals chosen differently. In particular, results from studies of families with a marked tendency to thrombophilia probably overestimate the risk for individuals who have had a single thrombotic event. Finally, when gene–gene and gene–environment interactions are required to bring about thrombosis, within-family and between-family differences may well be comparable.

Laboratory evaluation of thrombophilia should involve the use of assays with the highest sensitivity and specificity for the genetic defect being detected. Such assays can be immunological or functional — the former may not detect cases with truly functional defective proteins. A practical approach should be taken and the selection of analytical procedures should be governed by the aim of the investigation as well as of locally determined factors such as prevalence of the genetic defects to be detected and availability of technical support. Based on available scientific information, the laboratory evaluation should include the determination of the levels of protein C, total and free protein S, and antithrombin, as well as use of a functional APC-R test that is sensitive and specific for the presence of the factor V 506Gln allele. For protein C, assays that are based on its activation by the protein C activator Protac and determination of the active enzyme with synthetic substrate fulfil the required quality criteria. At present, no functional protein S assays can be recommended for general screening of thrombophilic patients. Immunological assays of total as well as of free protein S are, however, recommended. Recently published results indicate that free protein S in the best marker for genetically determined protein S deficiency, but further studies are required before a recommendation can be made only to determine free protein S. Functional assays for antithrombin that are based on heparin-stimulated inhibition of factor Xa are recommended for screening thrombophilic patients. For initial screening of APC-R, functional tests are recommended; such tests can be improved by diluting the patient plasma in factor-V-deficient plasma. Since assays for protein C and protein S have distinctly lower sensitivity and specificity for detecting the presence of inherited deficiency during the acute thrombotic episode and oral anticoagulation, at present it is recommended that the laboratory investigation for these components be performed after discontinuation of therapy.

The clinical manifestations of the defects of naturally occurring anticoagulant systems (antithrombin, protein S, and protein C deficiencies; and APC-R) are similar. In heterozygotes, the typical manifestations are venous thromboembolism (e.g. deep-vein thrombosis of the legs, pulmonary embolism, and superficial thrombophlebitis). Visceral and cerebral vein thrombosis are rarer but quite typical for inherited thrombophilia. Patients with homozygous defects usually have more severe clinical manifestations with an earlier age of onset. Some manifestations are quite typical, e.g. skin necrosis and widespread neonatal thrombosis in cases of protein C and protein S deficiencies. Preliminary data suggest that some homozygous defects (anti-thrombin type II HBS deficiency) may be also associated with an increased risk for arterial thrombosis in the young, but more data on this and other homozygous deficiencies are warranted to establish any relationship with arterial disease.

When a symptomatic patient with inherited thrombophilia due to a known genetic defect is identified, family studies should be conducted since approximately half the first-degree relatives will be affected. Asymptomatic individuals who carry the genetic defect should be counselled on the implications of the diagnosis and on symptoms that require medical attention. In general, management of symptomatic individuals with the genetic defect is similar to that for symptomatic patients without an identifiable genetic defect. An exception is provided by patients with neonatal purpura fulminans in association with homozygous or doubly heterozygous protein C deficiency, for whom administration of a source of protein C is critical during initial treatment. Since future thrombotic events in patients with inherited thrombophilia cannot be accurately predicted and there is a risk of bleeding associated with anticoagulant therapy, recommendations on long-term treatment are best carried out individually at the present time.

Once an individual is defined as having hereditary thrombophilia, as many family members as possible need to be examined for the particular defect detected in the proband and a pedigree constructed. Family members who are affected should be counselled about the risk of thrombosis. Evaluation of the potential risk of giving birth to severely affected neonates is usually carried out for families between which intermarriage is practised, and consequently counselling, extensive carrier detection, and prenatal diagnosis are carried out. Targets for antenatal diagnosis of hereditary thrombophilias are those
families into which infants have seen born with severe thrombosis caused by homozygosity or compound heterozygosity for protein C, protein S, or antithrombin deficiency, as well as the consanguineous families mentioned above. For such target families attempts need to be made to detect the responsible mutation(s), and to devise a simple method for their detection, e.g., polymerase chain reaction and restriction analysis or Southern analysis. This is followed by an extensive search of family members of child-bearing age for carriers, who are then counselled. Antenatal diagnosis is based on analysis of DNA obtained by chorionic villus sampling or amniocentesis.

Deep vein thrombosis and pulmonary embolism have a lower incidence in developing than in developed countries, possibly because of a combination of racial and environmental factors. A few studies with complete laboratory evaluation on inherited thrombophilia have, however, been carried out in developing countries; the results indicate that there is a higher chance of finding an underlying genetic defect (protein C, protein S, and antithrombin deficiency) in patients with thrombosis in such countries. Preliminary data on APC-R suggest that this defect is rare among Asians, Africans, and Chinese.

Conclusions and recommendations

- Recent reports support the hypothesis that familial thrombophilia is a multiple gene disorder and that its penetrance is higher among carriers of multiple gene defects. Prophylactic and therapeutic measures therefore need to be adjusted to the number of independent risk factors present in an individual. Hence, efforts should be intensified towards identifying those genetic risk factors that so far have escaped detection, so that they can be included in diagnostic screening procedures.

- Guidelines need to be developed for the use of specific laboratory tests in screening procedures aiming at identifying individuals who carry a genetic risk factor for venous thrombosis. Collaborative international investigations with standardized recruitment protocols should be encouraged.

- More specific recommendations need to be developed for the classification of hereditary protein S deficiency. In this respect, it needs to be established whether type III protein S deficiency is an independent risk factor for venous thrombosis or a different phenotype of type I protein S deficiency.

- At present, general screening of the population for the protein C, protein S, and antithrombin genetic defects is not recommended because of their low prevalence in populations and because of the low predictive value of a positive test. Studies should be carried out to determine whether general screening for APC-R (factor V:506Gln allele), e.g. before surgery or hospitalization and use of oral contraceptives, is beneficial for decision-making on therapeutic and prophylactic regimens.

- Heterozygous protein C, protein S, or antithrombin deficiencies should not be the target of antenatal diagnosis. In view of the very low expected frequency of severe cases of such homozygous or compound heterozygous defects in the general population, it is not recommended that population screening for carriers be carried out.

- Recommendations for screening for hereditary thrombophilia at the national level in health care services can be made only after adequate data on the epidemiology, risk of thrombosis, and results of therapeutic intervention are available.

- In developing countries, where thrombotic disorders in general appear to have a low prevalence, all patients with venous thromboembolism should be screened in order to determine the proportion with hereditary thrombophilia. Family studies should be carried out on all patients for whom a genetic defect is documented.

- Since facilities for screening may be available only in reference centres, treatment should be initiated without delay, where appropriate, and tests performed in the reference centre after use of anticoagulants has been discontinued.

- Accurate data are needed on the frequency and impact of thrombophilia in developing countries. For this purpose, individual laboratories should be identified in different regions and these should develop the necessary expertise to screen for and document the genetic defect responsible, in association with WHO Collaborating Centres.

- Data on the thrombotic risk in patients and asymptomatic family members with thrombophilia should be collected to determine whether the risk profile is different in developed and developing countries. Careful documentation of the risk of haemorrhage when anticoagulants are taken is necessary to determine the risk: benefit ratio for therapeutic intervention involving patients with thrombophilia.

- In order to increase awareness about inherited thrombophilia in developed and developing
countries, a WHO Collaborating Centre should be
designated to improve diagnosis, clinical recognition,
and treatment of related thrombosis. Such a centre
should serve as a reference centre for an appropriate
WHO region and improve education of both health
professionals and the general public.

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Résumé
La thrombophilie héréditaire
A l'occasion d'une réunion conjointe qui s'est tenue
tôt à Genève du 6 au 8 novembre 1995, l'OMS et la
Societé internationale de thrombose et d'hémo-
stase (ISTH) ont examiné les problèmes posés par
la thrombophilie héréditaire, fait état des diverses
possibilités de lutte contre la maladie, et formulé
une série de recommandations pour le diagnostic,
le traitement et la recherche concernant cette affection.
L'hypothèse que la thrombophilie familiale est une
maladie multigénique et que sa pénétrance est plus grande chez les porteurs d'anomalies
multigéniques est confortée par des observations récentes. On peut donc s'attendre à ce que les mesures prophylactiques et thérapeutiques re-
flectent par nécessité le nombre de facteurs de risque indépendants présents chez un individu donné. Il est par suite souhaitable de faire porter les efforts sur l'identification des facteurs de risque génétiques qui n'ont pas encore été identifiés, de manière à pouvoir en tenir compte dans la démarche diagnostique.

La prédistribution à la thrombose ne s'explique pas chez 85% des familles atteintes. Ce vide explicatif a favorisé l'utilisation des méthodes génétiques et épidémiologiques et il en est résulté des découvertes majeures dans l'étude de la thrombose familiale au cours des trois dernières années. C'est tout d'abord la résistance à la protéine C activée
(APC-R) qui a été identifiée, puis une mutation du
gène du facteur V (1691G→A dans l'exon 10, con-
duisant au remplacement 506Arg→Gln) considérée
comme la base moléculaire du phénotype APC-R chez la plupart des individus atteints. Cette mutation, identifiée à une augmentation considérable du risque thrombotique, est présente chez 50% des
familles sélectionnées atteintes de thrombophilie,
et chez 20% des cas consécutifs de thrombose.
Cette découverte a eu pour conséquence de modi-
fier la perception de la thrombophilie, et donc des répercussions sur son diagnostic et son traitement.
Il serait bon de recueillir des données sur le
risque thrombotique, à la fois chez des patients et chez les membres asymptomatices des familles
atteintes, pour déterminer si le profil de risque est différent dans les pays développés et en
développement. Il est nécessaire de documenter soigneusement le risque d'hémorragie chez les
sujets sous anticoagulants pour évaluer le rapport
risque/avantage de l'intervention thérapeutique en
cas de thrombophilie.
On ne pourra formuler des recommandations concernant le dépistage de la thrombophilie au
niveau national que lorsqu'on disposera des données appropriées sur l'épidémiologie, le risque de thrombose et les résultats des interventions thérapeutiques.

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Memorandum


