Inherited Thrombophilia: Pathogenesis, Clinical Syndromes, and Management

By Valerio De Stefano, Guido Finazzi, and Pier Mannuccio Mannucci

It has been known for centuries that inherited defects of blood coagulation cause lifelong bleeding disorders, but the existence of the counterpart, inherited thrombotic disorders, has been appreciated for only a few decades. In 1965 the first kindred with antithrombin (AT) deficiency was described and for many years the deficiency of this naturally occurring anticoagulant protein was the only identified cause of inherited thrombophilia. Inherited thrombophilia was defined as a genetically determined tendency to venous thromboembolism which characteristically occurs at a young age (before 40 to 45 years) without apparent causes and tends to recur.

Since the early 1980s there has been an explosion of new knowledge, with the identification first of protein C (PC) deficiency and then of protein S (PS) deficiency as additional causes of inherited thrombophilia. However, altogether these three defects only account for less than 15% of selected cases of juvenile and/or recurrent venous thrombosis and for less than 10% of unselected cases. In 1993 it was observed that plasma samples from certain individuals with inherited thrombophilia were resistant to the anticoagulant action of activated PC (APC), the protease generated by the thrombomodulin–PC anticoagulant pathway to inactivate activated factor V and VIII (Va and VIIa). APC resistance is associated with a point mutation in the factor V gene that causes a hypercoagulable state by slowing the inactivation of factor Va by APC. It is the most frequent cause of inherited thrombophilia, accounting for 20% to 30% of cases. Another step forward was made in 1994, when mild hyperhomocysteinemia was found in 19% of patients with juvenile venous thrombosis and family studies showed that in most cases the abnormality was inherited. While the genetic lesions for deficiencies of AT, PC, PS, and APC resistance can be found in single genes encoding the defective proteins, inherited hyperhomocysteinemia may be caused by defects in several genes encoding different enzymes involved in the metabolism of the amino acid.

This review will summarize the biochemical and molecular basis of inherited thrombophilia, describe the clinical manifestations, and provide guidelines for diagnosis and management. The review will be restricted to the more frequent and well-established thrombophilic syndromes mentioned above (Table 1). Other causes of inherited thrombophilia are much rarer, such as dysfibrinogenemia, or not firmly established, such as abnormalities of the fibrinolytic system (plasminogen, plasminogen activator inhibitor, histidine-rich glycoprotein), of heparin cofactor II, and of thrombomodulin (Table 1).

INHERITED THROMBOPHILIA CAUSED BY DEFECTIVE ANTICOAGULANT SYSTEMS

The AT System

Biochemistry. AT is a single-chain glycoprotein with a molecular mass of approximately 60 kD, comprising 432 amino acid residues and four oligosaccharide side chains. The protein is synthesized by the liver and its plasma concentration is approximately 125 µg/mL (2.3 µmol/L), with a plasma half-life of 65 hours. AT belongs to the serine protease inhibitor (serpin) superfamily and inactivates thrombin and other coagulation enzymes, including factors Xa, IXa, XIa, and XIIa. Protease inactivation by AT involves the formation of a complex between the active site of the protease and the reactive center of AT, formed by 393Arg and 394Ser. The inhibition of serine proteases by AT is markedly accelerated by heparin and proteoglycans present in the vessel wall. Heparin promotes the formation of a ternary complex with AT and proteases, in which the active site of the protease is brought into close contact with the reactive site of AT. This reduces the half-time of thrombin inhibition in plasma from approximately 40 seconds to 10 milliseconds. Similar rate enhancements have been observed for the inactivation of factors Xa and IXa. The heparin-binding domain of AT has two regions, encompassing amino acids 41 to 49 and 107 to 156. Both regions consist of clusters of basic amino acids and are adjacent to each other when the molecule assumes its tertiary structure.
Frequency and phenotypes. In the general population the frequency of symptomatic AT deficiency has been estimated to be between 1:2,000\(^{18}\) and 1:5,000.\(^{19}\) However, asymptomatic deficiency may occur as frequently as 1:600.\(^{20}\) In unselected patients with a history of venous thromboembolism, the frequency is 1.1%\(^{21}\); in selected patients, 2.4% (range 0.5% to 4.9%) in our cumulated analysis of 1,705 reported cases\(^ {22-25}\) (Table 2). The pattern of inheritance is autosomal dominant. The majority of affected individuals are heterozygotes, with AT levels between 40% and 70% of normal, and homozygotes are extremely rare.

Two types of AT deficiency can be distinguished phenotypically. In type I, both AT activity and antigen are low in plasma, indicating that the protein is not produced by the mutant allele. In type II, low AT activity contrasts with normal antigen levels, indicating a functional impairment of the molecule. Type II can be further subdivided into three subtypes: those characterized by abnormalities primarily affecting the reactive site, those affecting the heparin-binding site, and those with pleiotropic effects on both heparin binding and protease inhibition.\(^ {26}\)

Molecular basis. In many cases of AT deficiency, the underlying molecular defect has been identified and mutations are reported in a recently updated database\(^ {6}\) (Table 3). Major deletions of the AT gene, located at chromosome 1q23-25, are relatively rare and account for fewer than 10% of cases of type I deficiency.\(^ {28}\) In this type most mutations are single nucleotide changes, short insertions or deletions, which may directly affect mRNA processing or result in premature termination of AT synthesis or in unstable or nonsecreted molecules (Table 3). Type II deficiency is usually caused by point mutations causing single amino acid substitutions leading to a dysfunctional protein (Table 3). The phenotypic behavior of the mutant proteins can provide information about the region of the gene affected. Mutations involving the reactive site occur in two distinct clusters: residues Gly392, Arg393, and Ser394 or residues Ala382 and Ala384. In the former group, AT is unable to interact with thrombin,\(^ {27,28}\) whereas in the latter, although the mutant protein is inactive as an anticoagulant, it is still a substrate for serine proteases.\(^ {28}\) The majority of mutant proteins involving the heparin-binding site are caused by missense mutations in the positively charged residues Arg47 and Arg129.\(^ {29}\) Perhaps their replacement by noncharged residues impairs the ionic interaction of AT with negatively charged heparin oligosaccharides. Other mutations involving the heparin-binding site affect affinity to heparin by altering the conformation of the heparin-binding surface.\(^ {29}\) AT mutants with pleiotropic effects have substitutions in residues 402-407, which are in or adjacent to strand 1C close to the carboxyl terminal of AT.\(^ {26}\) These mutants show impaired protease inhibition and reduced heparin affinity. Perhaps their defective interaction with thrombin results from the close proximity of strand 1C to the reactive site, whereas their altered affinity for heparin may result from conformational linkage between the reactive and heparin-binding sites of the mutant molecule.\(^ {26}\) Many of these mutant proteins are detected in plasma at low concentrations, indicating that strand 1C is essential for the structural integrity and stability of AT.

### Table 1. Causes of Inherited Thrombophilia

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>General Population</th>
<th>Unselected Patients</th>
<th>Selected Patients</th>
<th>With Venous Thrombosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT deficiency</td>
<td>0.02-0.17</td>
<td>1.1</td>
<td>0.5-4.9</td>
<td></td>
</tr>
<tr>
<td>PC deficiency</td>
<td>0.14-0.5</td>
<td>3.2</td>
<td>1.4-8.5</td>
<td></td>
</tr>
<tr>
<td>PS deficiency</td>
<td>—</td>
<td>2.2</td>
<td>1.4-7.5</td>
<td></td>
</tr>
<tr>
<td>APC resistance</td>
<td>3.6-6</td>
<td>21</td>
<td>10-64</td>
<td></td>
</tr>
</tbody>
</table>

*Data from Bertina et al.\(^ {138}\)

### Table 2. Frequency (%) of Inherited Thrombophilic Syndromes in the General Population and in Patients With Venous Thrombosis

<table>
<thead>
<tr>
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<th>General Population</th>
<th>Unselected Patients</th>
<th>Selected Patients</th>
<th>With Venous Thrombosis*</th>
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<tr>
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<td>21</td>
<td>10-64</td>
<td></td>
</tr>
</tbody>
</table>

*Age <45 years and/or recurrent thrombosis.

### Table 3. Molecular Basis of Inherited Thrombophilia Caused By Impaired Anticoagulant Mechanisms

<table>
<thead>
<tr>
<th>Genetic Defect</th>
<th>No. of Different Mutations*</th>
<th>Most Frequent Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT deficiency</td>
<td>&gt;79</td>
<td>Type I: Whole or partial gene deletions (&lt;10% of cases)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short insertions or deletions (frameshift mutations)</td>
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<tr>
<td></td>
<td></td>
<td>Single nucleotide changes (nonsense or missense mutations leading to premature stop codons)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type II: Missense mutations (leading to amino acid substitutions)</td>
</tr>
<tr>
<td>PC deficiency</td>
<td>&gt;160</td>
<td>Type I: Frameshift mutations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Missense mutations</td>
</tr>
<tr>
<td>PS deficiency</td>
<td>&gt;13</td>
<td>Type II: Missense mutations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frameshift mutations</td>
</tr>
<tr>
<td>APC resistance</td>
<td>1</td>
<td>Missense mutation in the factor V molecule</td>
</tr>
</tbody>
</table>

*Data from Bertina et al.\(^ {138}\)
The PC System

Biochemistry. PC is a vitamin K-dependent glycoprotein with a molecular mass of 62 kD (carbohydrate 23%), a plasma concentration of 3 to 5 µg/mL (48 to 80 nmol/L), and a half-life of 6 to 8 hours.31-33 PC is synthesized in the liver as a 461-amino acid single-chain polypeptide, composed of a leader sequence, a propeptide recognition site for the vitamin K-dependent gamma-carboxylase, a light chain, and a heavy chain.33 Mature double-chain PC results from intracellular proteolytic removal of the propeptide and cleavage of the single chain. The light chain contains a gamma-carboxyglutamic acid (Gla) domain, with nine Gla residues. The Gla domain is connected to two epidermal growth factor (EGF)-like domains. The heavy chain contains the serine protease domain, with an N-terminal activation peptide linked to the catalytic domain.33

PC is activated slowly by thrombin with cleavage of an Arg169-Leu170 bond and release of the activation dodoprotein from the heavy chain.33 Faster activation is induced by the complex formed by thrombin with an endothelial receptor, thrombomodulin. The high-affinity binding of thrombin to thrombomodulin produces a 20,000-fold increase in the rate of PC activation.33 APC inactivates membrane-bound factor Va through an initial cleavage at Arg506, which is required for optimal exposure of the other cleavage sites at Arg306 and Arg679.34-36

The principal cofactor of APC is PS, a vitamin K-dependent single-chain glycoprotein with a molecular mass of 69 kD (carbohydrate 7%). The plasma concentration of PS is 20 to 25 µg/mL (260 to 330 nmol/L)37 and the half-life is 42 hours.38 PS is produced by the liver but has been localized also in endothelial cells, megakaryocytes, and Leydig cells.33,35 It is synthesized as a precursor of 676 amino acids, with a leader sequence cleaved before secretion, and is composed of a hydrophobic signal peptide and a carboxylase recognition site homologous with those of the other vitamin K-dependent proteins. The mature form of PS (635 amino acids) consists of a Gla-domain with 11 Gla-residues, a serine protease domain, with an N-terminal activation peptide, followed by a connecting peptide, a thrombin-sensitive region, four EGF-like domains, and a carboxy-terminal region, homologous with the sex hormone–binding globulin (SHBG).37

PS acts by enhancing the affinity of APC for negatively charged phospholipids, forming a membrane-bound APC-PS complex that renders factors Va and VIIIa more easily accessible to APC-mediated cleavage. PS circulates in plasma as a free form and as a noncovalent complex with C4b-binding protein (C4b-BP), a component of the complement system that binds to PS in the SHBG-like region.37 PS has no APC cofactor activity when complexed to C4b-BP; 60% to 65% of the total PS circulates in the complexed form in equilibrium with free PS, which is the only form involved in APC anticoagulant activity.37

Frequency and phenotypes. Defects of the PC anticoagulant system associated with thrombophilia are usually transmitted with an autosomal dominant pattern of inheritance. Recently a thrombomodulin point mutation has been identified in a thrombophilic patient and his son.39

The frequency of PC deficiency is 3.2% in unselected patients with venous thrombosis31 and 3.8% in selected patients (range, 1.4% to 8.6% in our cumulative analysis of 1,705 published cases)32-35 (Table 2). In the general population, extrapolation from data obtained for cohorts of thrombotic patients gave a frequency between 1:16,000 and 1:36,000.40 A much higher frequency was found in healthy subjects, with a rate of 1:200 to 1:300.41 This frequency is similar to that of 1:500 to 1:700 found in a recent study of 9,648 blood donors and confirmed by PC gene analysis.42 Two phenotypes of PC deficiency can be distinguished. In type I, PC antigen and activity are decreased to the same extent, whereas in type II low activity contrasts with normal antigen levels.

The frequency of PS deficiency is roughly similar to that of PC deficiency: 2.2% for unselected patients with venous thrombosis32 and 3.0% for selected patients (range, 1.4% to 7.5% in our cumulative analysis of 1,705 cases)32,35 (Table 2). There are no data on the frequency of PS deficiency in the general population: extrapolation from cohorts of thrombotic patients gives a frequency of 1:33,000.40 PS deficiency can be distinguished phenotypically as a quantitative deficiency, with low plasma levels of free PS antigen and activity (type I) and a functional deficiency, with normal levels of free and total PS antigen (type II). Type I has two phenotypic subtypes, characterized by either normal or low levels of total PS antigen, with low levels being the result of an equimolar relationship between C4b-BP and PS.43

The frequency of APC resistance in unselected patients with primary venous thromboembolism is 21%;8,9 our cumulative analysis of 775 selected patients gave an average frequency of 22% (range, 17% to 37%) for the mutant factor V gene (cumulated analysis of 536 cases).37-40 The high frequency of APC resistance in patients with venous thromboembolism mirrors the high rate of carriage of the mutant factor V gene in populations of European descent (from 2% to 10% and possibly more)7,9,11,15,25 (Table 2). This suggests that the mutation may have conferred some evolutionary advantages, but the nature of such advantages is only speculative. The mutation seems to have a lower frequency in Africa and Asia than in European populations.33

Molecular basis. The human PC gene has been localized at chromosome 2q13-q14; it spans over 11 kb and comprises 9 exons.40 Recently, a database of mutations has been updated (Table 3). In type I, deletions and insertions occur relatively rarely (approximately 10%) and most of the mutations are of the missense variety; approximately one third of the single point mutations occur in a CpG dinucleotide.44 Most mutations lead to premature termination of protein synthesis or disruption of protein folding with loss of stability. In type II, missense mutations account for approximately 10% of the database entries and are located mostly in the Gla-domain and protease domains. Other mutations causing type II are located in the propeptide sequence or at the thrombin cleavage site.45

Two homologous genes for human PS have been localized
at chromosome 3. The active α gene is located on the region 3p11.3-3q11.2, spans over 80 kb, and comprises 15 exons; the β gene, a nonexpressed pseudogene, is also localized at chromosome 3 close to the α gene. Relatively few mutations of the PS gene have been identified, probably because of the technical difficulties related to the size of the gene and the presence of a pseudogene. Large deletions of the α gene have been described, but the majority of PS defects are point mutations leading to premature termination of synthesis or incorrect protein folding. In rare instances amino acid substitutions produce a dysfunctional PS by affecting the propeptide cleavage site or the EGF-like domains (Table 3).

The factor V gene, located at chromosome 1q21-25, contains 25 exons spanning over 80 kb. APC-resistant individuals were originally screened for mutations affecting the factor Va cleavage site (Arg506-Gly507) or the APC-binding region of factor V (Arg1865-Ile1874). A single-point mutation in the factor V gene leading to the substitution of Arg506 by Gln and causing resistance to APC inactivation of factor Va is found in 80% to 100% of the patients investigated (Table 3). APC resistance in patients lacking this mutant factor V may be explained by the relatively poor specificity of the functional assay used to measure APC resistance (see Diagnosis of Inherited Thrombophilia) or by some hitherto unrecognized molecular alterations causing APC resistance.

**INHERITED THROMBOPHILIA CAUSED BY HYPERHOMOCYSTEINEMIA**

**Biochemistry**

Homocysteine is a sulfydryl amino acid derived from metabolic conversion of methionine. Its intracellular metabolism occurs through remethylation to methionine or transulfuration to cysteine (Fig 1). There are two remethylation pathways. In that catalyzed by methionine synthase, the methyl group is donated by methyltetrahydrofolate and cobalamin acts as a cofactor. In the other pathway, betaine is the methyl donor and the reaction is catalyzed by betaine-homocysteine methyltransferase. In the transulfuration pathway, homocysteine is transformed by cystathionine-β-synthase into cystathionine, with pyridoxine acting as a cofactor (Fig 1).

Homocysteine is oxidized in plasma to the disulfides homocysteine-homocysteine (homocystine) and homocysteine-cysteine (mixed disulfide). Homocysteine and the two disulfides exist both in free and protein-bound forms and are referred to globally as total homocysteine, which has a concentration range in normal plasma of 5 to 16 μmol/L.

**Phenotypes**

Several inherited or acquired conditions can cause severe (>100 μmol/L), moderate (25 to 100 μmol/L), or mild (16 to 24 μmol/L) hyperhomocysteinemia. The most frequent cause of severe hyperhomocysteinemia is homozygous deficiency of cystathionine-β-synthase, which has a frequency in the general population of approximately 1:200,000 to 1:335,000. Affected individuals have severe mental retardation, ectopic lens, skeletal abnormalities, and premature arterial vascular disease and venous thromboembolism. A smaller number of cases (5% to 10%) are associated with inherited defects of the remethylation pathway. Homozygous deficiency of the wild form of methylenetetrahydrofolate reductase is the most common of these defects, characterized by neurological defects, psychomotor retardation, seizures, premature vascular disease, and thromboembolism.

Mild or moderate hyperhomocysteinemia is found in individuals with gene defects and in some individuals with acquired diseases. In the general population heterozygous cystathionine-β-synthase deficiency has a high frequency of 0.3% to 1.4%. A common defect of the remethylation pathways is the presence of a thermolabile mutant of methylenetetrahydrofolate reductase, which has approximately 50% the normal enzyme activity and a frequency of 5% in the general population in the homozygous state. The most com-
INHERITED THROMBOPHILIA

Table 4. Clinical Features of Patients With Inherited Deficiencies of AT, PC, PS, and APC Resistance

| Venous thromboembolism (>90% of cases) | Deep vein thrombosis of lower limbs (common) | Pulmonary embolism (common) | Superficial thrombophlebitis | Mesenteric vein thrombosis (rare but characteristic) | Cerebral vein thrombosis (rare but characteristic) | Frequent family history of thrombosis* | First thrombosis usually at young age (<40 yr)* | Frequent recurrences* | Neonatal purpura fulminans (homozygous protein C and protein S deficiency) |

*All these features are less evident in patients with APC resistance, who appear to be less severely affected clinically.

mon causes of acquired hyperhomocysteinemia are nutritional deficiencies of cobalamin, folate, or pyridoxine, the essential cofactors for homocysteine metabolism. Elevated concentrations of homocysteine are not unusual in the elderly, even in the presence of normal serum vitamin levels. Chronic renal insufficiency and compounds interfering with the metabolism of folate, such as methotrexate and anticonvulsants, or with that of cobalamin, such as nitrous oxide, may also cause mild or moderate hyperhomocysteinemia.

Pathogenetic Mechanisms

The mechanisms by which hyperhomocysteinemia contributes to atherogenesis and thrombogenesis are understood partially. In vivo studies in baboons have shown that homocysteine causes endothelial cell desquamation, smooth-muscle-cell proliferation, and intimal thickening. In vitro studies have shown that homocysteine-induced endothelial injury requires copper and oxygen and is prevented by catalase but not by superoxide dismutase, which suggests that production of hydrogen peroxide is responsible for the toxic effects on endothelial cells. Other effects of homocysteine on endothelial cells include the following: activation of factor V and interference with PC activation and thrombomodulin expression; inhibition of tissue plasminogen activator; impaired generation of nitric oxide and prostacyclin, which are potent antiaggregating and vasodilating agents; induction of tissue factor activity, and suppression of the expression on the vessel wall of the anticoagulant substance heparan sulfate. However, it must be emphasized that most in vitro effects of homocysteine are seen when plasma concentrations are very high, usually at least one order of magnitude greater than the concentrations found in patients with homozygous homocystinuria.

CLINICAL MANIFESTATIONS

Clinical Manifestations in Heterozygotes

The clinical manifestations of defects of the proteins involved in the naturally occurring anticoagulant systems (AT, PC, and PS deficiencies; mutant factor V causing APC resistance) are similar. Venous thromboembolism is typical (Table 4). The most common manifestation, deep-vein thrombo-

sis of the lower limbs with or without pulmonary embolism, accounts for approximately 90% of all thrombotic episodes. Unusual sites of venous thrombosis, such as the mesenteric or cerebral veins, account for less than 5% of the total episodes in patients with AT, PC, or PS deficiencies. For unknown reasons superficial thrombophlebitis is more frequent in patients with PC deficiency, PS deficiency, or APC resistance than in AT-deficient patients. There is no evidence that heterozygosity for AT, PC, and PS deficiencies or APC resistance increase the risk of arterial thrombosis.

Venous thromboembolism develops in 60% to 80% of individuals with AT, PC, or PS deficiency, most frequently before the age of 40 to 45 years and recurs in approximately half of the patients. In AT deficiency, the overall risk of thrombosis is considered greater than in PC or PS deficiency. On the other hand, individuals with APC resistance may have a lesser tendency to thrombosis than those with AT, PC, and PS deficiencies, as indicated by the higher prevalence of asymptomatic individuals beyond the young age11 and by the frequent occurrence of the first episode of thrombosis at advanced age. Even though APC resistance per se seems to be less severe than the other defects, its presence markedly increases the risk of thrombosis in patients with AT, PC, and PS deficiencies. Because of the high prevalence of APC resistance in the general population, its combination with other genetic defects is not exceptional.

Approximately half of the venous thrombotic episodes occur in association with circumstantial risk factors (surgery, pregnancy, immobilization). In AT deficiency, the frequency of thrombosis during pregnancy and the puerperium is between 31% and 44%, in PC or PS deficiency between 10% and 19%, and in APC resistance 28%. Thrombotic episodes occur most frequently during the puerperium, accounting for 60% to 75% of all episodes complicating pregnancy. Pregnant women with a history of thrombosis have a twofold increased risk of thrombosis compared to women with no previous thrombosis. A retrospective analysis of a large number of AT-, PC-, or PS-deficient individuals gave a frequency of postoperative venous thrombosis of 21% in abdominal surgery and 37% in high-risk orthopedic or cancer surgery, with no obvious differences between different thrombophilic defects. Even though there is no study comparing the risk of thrombosis triggered by pregnancy or surgery in thrombophilic patients with that in the general population, the aforementioned high frequencies indicate that these patients are at high risk and that prophylaxis in these situations is warranted (see Guidelines for Management). Intake of oral contraceptives is associated with a markedly increased thrombotic risk, particularly in women with AT deficiency or APC resistance.

Dysfunctional defects of AT, PC, and PS carry a similar risk of thrombosis to the corresponding quantitative defects. A notable exception is type II AT deficiency caused by abnormalities of the heparin-binding site, with a prevalence of thrombosis in heterozygotes of only 6%, contrasting with 52% to 68% in patients with other types of AT deficiencies.
Clinical Manifestations in Homozygotes

Homozygous AT deficiency is extremely rare and almost exclusively reported in patients with heparin-binding defects. These individuals have a severe thrombotic history of early onset, often affecting arteries. Homozygous type I AT deficiency appears to be incompatible with life: in one report, two brothers with this defect died within 3 weeks of birth. The frequency of homozygous PC deficiency has been estimated as 1:160,000 to 1:360,000 and the deficiency is associated with peculiar phenotypic and clinical expressions. In patients with unmeasurable PC, purpura fulminans, caused by thrombosis of small vessels with cutaneous and subcutaneous ischemic necrosis, may occur soon after birth or in the first year of life. In patients with very low but measurable PC (5% to 20%), clinical manifestations are similar in type to those for heterozygous deficiency. In heterozygous relatives of patients with homozygous PC deficiency, the frequency of thrombosis is 5%, which is much lower than a frequency of approximately 50% found in homozygotes from kindreds without any homozygous members. The reasons for this discrepancy are still unclear, although the presence or absence of concomitant thrombophilic traits, particularly one as frequent as APC resistance, may be one explanation.

Homozygous PS deficiency has been reported rarely, and is associated with neonatal purpura fulminans. Because of the high frequency of the mutant factor V in the general population, 1:5,000 individuals are expected to have homozygous APC resistance. The overall thrombotic risk for homozygotes is estimated to be 11-fold higher than for heterozygotes and 80-fold higher than for normal individuals. For homozygotes, the probability of a thrombotic episode before the age of 33 years is twice that for heterozygotes (40% vs 20%). Whether homozygotes have an increased risk of arterial thrombosis remains to be elucidated.

Hyperhomocysteinemia

Case-control, cross-sectional, and prospective studies have clearly shown that mild or moderate hyperhomocysteinemia is an independent risk factor for stroke, myocardial infarction, peripheral arterial disease, and extracranial carotid artery stenosis. High plasma homocysteine concentrations are associated with inherited enzymatic defects or with low plasma levels of folate or vitamin B12, particularly in elderly individuals. This indicates that hyperhomocysteinemia per se, whether inherited or acquired, is associated with an increased risk for arterial diseases.

Mild or moderate hyperhomocysteinemia has been associated also with venous thrombosis in the young and recurrent venous thrombosis, and has been shown to have a high frequency (10%) in patients with a first episode of venous thrombosis. In young patients with venous thrombosis, family studies showed that most index cases had at least one first-degree relative with hyperhomocysteinemia. These data suggest that the abnormality was inherited in the majority of cases, although testing for specific gene lesions was not performed. Heritability of hyperhomocysteinemia in venous thrombosis occurring in the young is consistent with the knowledge that homocysteine levels exhibit significant heritability, particularly in young individuals.

In hyperhomocysteinemia the venous thromboembolic manifestations associated with hyperhomocysteinemia do not appear to differ from those of other thrombophilic syndromes. In a series of 67 hyperhomocysteinemic patients, deep-vein thrombosis, with or without pulmonary embolism, was the most frequent clinical manifestation, accounting for 64% of the thrombotic episodes; less common clinical manifestations were superficial thrombophlebitis (24%) and thrombosis of cerebral or mesenteric veins (12%) (Cattaneo M, Mannucci PM, unpublished observations, 1996). Most thrombotic episodes were associated with other risk factors such as oral contraceptive intake, trauma/surgery, pregnancy, the puerperium, and immobilization.

**DIAGNOSIS OF INHERITED THROMBOPHILIA**

The goal of diagnosis is to identify accurately the defects in affected individuals, both in those who have already developed thrombotic symptoms and in those who are still asymptomatic.

**Candidates for Diagnosis**

For inherited bleeding disorders, laboratory diagnosis is simplified by the availability of global screening tests, such as the prothrombin time and the activated partial thromboplastin time (APTT), that pinpoint individuals who are candidates for further specific testing. Unfortunately, no such global test is yet available for screening thrombophilic patients, so that several assays must be performed (Table 5). These assays are neither simple nor inexpensive and should be performed in selected individuals.
The selection begins with the collection of the clinical history of the index case. Acquired causes of thrombosis such as malignancy, myeloproliferative disorders, systemic lupus erythematosus, and the antiphospholipid syndrome must be excluded. A family history of thrombosis is an important selection factor. However, a negative family history does not exclude inherited thrombophilia because the defect may be caused by a fresh mutation (which is rare) or because affected relatives are still asymptomatic. The latter situation may occur more frequently in defects characterized by relatively low clinical penetrance, such as APC resistance and hyperhomocysteinemia. Important selection criteria are unexplained episodes of venous thromboembolism occurring in patients less than 40 to 45 years old, recurrent thrombosis, and thrombosis at unusual venous sites. However, APC resistance should be considered also in older patients with primary venous thromboembolism.\(^5\) Thrombosis during the neonatal period is an indication for diagnostic screening, particularly for PC deficiency. Plasma homocysteine should be measured in young patients with arterial or venous thrombosis.

An emerging issue is whether or not diagnostic screening should be considered in healthy individuals with no personal or family history of thrombosis, before or during their exposure to thrombotic risk factors such as pregnancy, oral contraception, major surgery, or prolonged immobilization in bed during hospitalization. Although AT, PC, and PS deficiencies are too infrequent in the general population to justify screening, APC resistance is so frequent that testing for this abnormality may have clinical relevance in some circumstances. For instance, a woman in her thirties with APC resistance, when considering oral contraception, can be advised of the 35-fold increased risk of venous thromboembolism; more vigorous antithrombotic prophylaxis may be considered in bedridden elderly individuals with APC resistance because they have an increased risk of developing venous thromboembolism that is a frequent cause of death. Unfortunately, firm recommendations cannot be made until prospective clinical trials have assessed the medical and economical desirability of screening. Screening of family members of index cases may be useful, not only to confirm the inherited nature of the defect but also to identify asymptomatic individuals who may benefit from antithrombotic prophylaxis during exposure to circumstantial risk factors.

### Diagnostic Methods

The laboratory tests chosen to confirm a diagnosis of congenital thrombophilia should be specific, limited in number and, most importantly, their results should be clinically relevant. Which test should be performed and which of methods should be chosen? Table 5 shows a two-step diagnostic procedure designed to answer these questions.

The first step is to exclude or confirm the most frequent and well-established causes of inherited thrombophilia. Functional assays should be chosen because they can detect both quantitative and qualitative defects, whereas immunoassays only identify the former. For AT deficiency, there are facile assays that measure the heparin cofactor activity of antithrombin on synthetic substrates.\(^{97\text{-}98}\) An international standard for AT is available. Several functional assays can be used to diagnose PC deficiency. These differ in the PC activator used (thrombin, thrombin plus thrombomodulin, snake venoms that specifically activate PC) and in the detection method (cleavage of synthetic substrates, prolongation of global coagulation tests such as the APTT). When compared in a multicenter study, several of these assays had acceptable precision and diagnostic accuracy.\(^{99}\) Functional assays based on the use of snake venoms as PC activators and synthetic substrates should be preferred to coagulation-based assays that can give spuriously low values in the presence of APC resistance.\(^{100}\) However, some dysfunctional PC defects may not be detected by amidolytic assays.\(^{101}\) An international standard for PC is available.

The diagnosis of PS deficiency is more complex. Functional assays have been described and some methods are commercially available.\(^{58\text{-}102\text{-}104}\) Unfortunately, the methods require special reagents not in routine use,\(^{104}\) and APC resistance interferes with the assays to give spuriously low PS values.\(^{102\text{-}105\text{-}107}\) Hence, current functional assays are not recommended. Immunoassays of total PS, in particularly enzyme immunoassays based on polyclonal or monoclonal antibodies (MoAbs) are sufficiently accurate for diagnostic purposes.\(^{108}\) Because there are thrombophilic patients with normal or borderline total PS but low free PS, the latter should be measured. For this, plasma is mixed with polyethylene glycol, which precipitates the complex between PS and C4b-binding protein, so that free PS antigen can be measured in the supernatant.\(^{109}\) Recent assays, based on MoAbs reacting almost exclusively with free PS, have made possible the direct measurement of this fraction in plasma without the need for a precipitation step.\(^{109}\) Free PS assays appear to have higher diagnostic sensitivity and specificity than total PS assays, because total PS levels of normal individuals and those with the genetic defect show considerable overlap.\(^{14}\)

APC resistance was originally diagnosed with a functional assay based on the capacity of patient plasma to “resist” the prolongation of the APTT caused by added APC.\(^*\) A version of this assay is now commercially available.\(^{111}\) Currently, several other assays are being proposed, based on different coagulation or synthetic substrates. So far, there is little comparative experience with them. The effective use of an APTT-based functional assay in the first step of screening for inherited thrombophilia depends on the sensitivity and specificity of the assay. When all variables, such as handling and storage conditions of plasma, instrumentation and choice of reagents are strictly controlled, APTT assays have a sensitivity and a specificity of 85% to 90% or better.\(^{79\text{-}112}\) Accordingly, usually they discriminate well between normal and APC-resistant individuals with mutant factor V. However, a number of patients referred for thrombophilia screening (approximately 5% to 10%) may have borderline results.\(^{112}\) In these instances a search for the factor V mutation is necessary for an accurate diagnosis. Another reason to recommend the functional assay in the first diagnostic step for inherited thrombophilia is that patients with APC

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<th>Table 5: Two-Step Diagnostic Procedure for Thrombophilia</th>
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resistance but without the factor V mutation are at increased risk of thrombosis. A screening test based exclusively on DNA analysis would miss the diagnosis in these patients. Recent data indicate that predilution of test plasma with factor V–deficient plasma gives the APTT-based assays greater sensitivity and specificity for the presence of mutant factor V, with no overlap among results for individuals without the mutation, heterozygotes and homozygotes (B. Dühl- back, unpublished observations, 1996).

Diagnosis of PC and PS deficiency and APC resistance is difficult in patients on oral anticoagulant therapy because assay results are influenced by the administration of vitamin K antagonists. Even though assay modifications have been proposed to circumvent this problem, their diagnostic accuracy has not been fully validated. For the diagnosis of APC resistance one can look for the mutant factor V gene that is not affected by oral anticoagulant therapy. When clinical reasons demand testing for PS and PC deficiencies, oral anticoagulants should be replaced for 7 to 10 days with therapeutic doses of standard heparin by subcutaneous administration or low-molecular-weight heparin, to allow plasma levels of vitamin K–dependent proteins to return to normal. Assays should be performed 12 to 24 hours after stopping heparin, when it will have been cleared from the circulation and will not affect results.

Hyperhomocysteinemia is diagnosed by measuring plasma levels of total homocysteine by ion-exchange chromatography, gas chromatography–mass spectrometry, or high-performance liquid chromatography with electrochemical or fluorescent detection. Patients with homozygous deficiency of cystathionine-β-synthase have very high plasma homocysteine levels, usually exceeding 100 μmol/L. Patients with heterozygous deficiencies may have normal or borderline-high levels of fasting homocysteine. Measurement of total plasma homocysteine 4 to 8 hours after an oral methionine load (3.8 g/m² body surface area or 0.1 g/kg body weight) improves the discrimination between normal individuals and heterozygotes for cystathionine-β-synthase. Another way to improve the identification of heterozygotes is to measure the enzyme activity in cultured fibroblasts from skin biopsy specimens. Recently, molecular probes have become available for the identification of mutations in the genes encoding for cystathionine-β-synthase and methylene-tetrahydrofolate reductase.

The assays recommended in the first diagnostic step should establish whether or not the individual under scrutiny has one of the common causes of inherited thrombophilia. When there are abnormal results, the more specialized coagulation laboratory may characterize the defect more fully by proceeding to a second step (Table 5). For instance, in AT deficiency immunoassays will help to distinguish type I from type II; further tests (such as two-dimensional immunoelectrophoresis with and without heparin, AT assays with or without heparin) will distinguish between the various subtypes, before the molecular defect is ultimately unraveled through DNA analysis. A similar approach can be applied to PC and PS deficiencies. For APC resistance, the coagulation test results may be confirmed by the demonstration of mutant factor V by DNA analysis. At the moment, the test for mutant factor V is too complex to be recommended for the clinical coagulation laboratory in the first step of diagnostic screening. In addition, this test would miss cases of APC resistance caused by molecular abnormalities other than the Arg506 to Gln mutation.

What strategy should be followed when the aforementioned screening tests are normal and yet the index case has a strong personal and family history of juvenile/recurrent idiopathic thrombosis? The thrombin time is a simple method to detect congenital dysfibrinogenemia, which can be confirmed by measuring plasma fibrinogen both with a clotting and an immunologic assay. It is important to be aware that with the recent dramatic progress in the identification of the causes of inherited thrombophilia, it is likely that further causes will be unraveled in the next few years. Hence, it is essential to keep a record of these patients and to store their plasma and DNA.

GUIDELINES FOR MANAGEMENT

Management of inherited thrombophilia encompasses primary prophylaxis of thrombosis in asymptomatic individuals, secondary postthrombotic prophylaxis of recurrences, and treatment of acute thrombotic episodes (Table 6). Because data from controlled randomized clinical trials are not available, guidelines are based on data stemming from small series of AT-, PC-, and PS-deficient individuals. An attempt will be made to extend these guidelines to encompass APC resistance and hyperhomocysteinemia.

Primary Prophylaxis

In individuals with inherited thrombophilia, thrombotic manifestations do not occur continuously, except in some newborns with homozygous PC deficiency. Hence, lifelong prophylaxis should not be considered for asymptomatic individuals who are not exposed to thrombotic risk factors. Even though thrombotic episodes may occur spontaneously, the cost and risk of lifelong anticoagulant therapy, and the likelihood of poor patient compliance, makes this approach unrealistic. These views are supported by the fact that mortality is not higher in AT- and PC-deficient patients than in the general population, and that at the moment there is no way to identify those asymptomatic individuals destined to develop thrombosis. On the other hand, prophylactic measures should be implemented during circumstances exposing patients to thrombotic risk (such as surgery, prolonged immobilization in bed, pregnancy, and the puerperium). The fact that prophylaxis is beneficial is shown by a large retrospective study of 238 patients. This study indicates that exposure to risk factors without prophylaxis is frequently complicated by thrombosis, whereas knowledge of an inherited thrombophilia and implementation of pharmacologic prophylaxis reduces morbidity. The main benefit is obtained in individuals less than 40 years old in whom the rate of thrombosis decreases from 77% to 33%.

Before and during surgery, all thrombophilic patients should receive the prophylactic regimen currently recommended in nonthrombophilic patients over 40 years of age,
ie, subcutaneous unfractionated heparin (5,000 U three times a day). When the risk of thrombosis is exceptionally high, as in orthopedic or cancer surgery, transfusional therapy with plasma fractions containing the deficient protein may be considered as an additional therapy. AT concentrates have been used successfully, but there has been relatively little experience with PC concentrates. However, the benefit of concentrates should be weighed against their cost and the potential risk of transmitting bloodborne infections. AT levels should be brought to normal postinfusion, knowing that the infusion of 0.7 U/kg of AT usually attains an increase of 1% in plasma. Because AT has a long half-life of approximately 65 hours, concentrates may be administered on alternate days. The scheme of primary prophylaxis recommended before surgery should be adopted for thrombophilic patients immobilized either in bed, particularly after trauma, or during prolonged car or air journeys.

Because pregnancy and the puerperium are accompanied by a high thrombotic risk, particularly in AT-deficient patients, vigorous prophylaxis is recommended. Two main approaches have been attempted: ie, subcutaneous heparin throughout pregnancy, or a sequential regimen based on subcutaneous heparin during the first trimester (when oral anticoagulants carry a teratogenic risk), oral anticoagulants at intermediate intensity (INR 2.0-3.0) during the second trimester, and heparin again from the 37th week until delivery. It is not clear which approach is more efficacious because there is no comparative study. Our analysis of 36 cases published in the literature indicates that during 24 pregnancies handled with heparin alone there were 6 thrombotic complications, whereas none of the 12 pregnancies handled with the sequential regimen had complications. Although these data are insufficient to recommend firmly the sequential regimen, they do suggest that the current prophylactic dosage of heparin (5,000 U three times a day), which does not prolong the APTT, is inadequate in AT-deficient women during pregnancy. In them, heparin should be started with larger doses (20,000 to 25,000 U/d) and adjusted to prolong the APTT up to 1.3 to 1.5 times the normal value.

Because in PC- or PS-deficient women and in those with APC resistance the risk of thrombosis associated with pregnancy appears to be lower than in AT-deficient women, the regular scheme of subcutaneous unfractionated heparin should suffice.

The high incidence of thrombosis during the puerperium warrants antithrombotic prophylaxis for at least 4 weeks after delivery. Different regimens based on oral anticoagulants (international normalized ratio [INR] 2.0 to 3.0), subcutaneous unfractionated heparin (15,000 U/d), or a low-molecular-weight heparin (2,500 U/d) have been proposed, but the available data are insufficient to help us in making a choice. Because anticoagulants may engender a risk of bleeding, in the early postpartum period an alternative is replacement therapy, given with a scheme similar to that recommended for surgery. However, for PC deficiency the short half-life of the protein makes this approach of less practical use than for AT-deficient women.

Secondary Prophylaxis

Should a patient who has a thrombophilic defect be prescribed lifelong oral anticoagulant therapy after the first thrombotic episode? No prospective study can answer this question. Hence, the risks and benefits of lifelong anticoagulation should be weighed on an individual basis and take into account such factors as the age of the patient, the type and degree of the defect, the site of the first thrombotic event, and, most importantly, other concomitant inherited or acquired risk factors for thrombosis. We offer lifelong oral anticoagulants to patients who have had more than one episode of thrombosis. Usually this therapy is not recommended after the first thrombotic episode if this developed in association with surgery, pregnancy, or other circumsitual thrombotic risk factors. On the other hand, if the first thrombotic episode was life-threatening (such as pulmonary embolism, visceral or cerebral vein thrombosis), occurred in patients with more than one genetic defect, or if thrombotic risk factors are sustained, lifelong oral anticoagulation may be considered.
There are no clear guidelines on the intensity of anticoagulation. Based on the current recommendations adopted for nonthrombophilic patients, the INR range should be 2.0 to 3.0. Limited clinical experience would suggest that lower INR values may be insufficient to prevent recurrences in symptomatic patients.\textsuperscript{131,132}

**Management of Acute Thrombosis**

The management of acute thrombosis is the same for patients with or without inherited thrombophilia. Deep-vein thrombosis should be treated with heparin and oral anticoagulants, but thrombolytic drugs may be used for massive pulmonary embolism. Heparin should be administered by continuous intravenous infusion or subcutaneously, at doses adjusted to maintain the APTT ratio within the range 1.5 to 2.5. Oral anticoagulants are generally started after 1 to 2 days of full heparinization, and when the target INR range (usually 2.0 to 3.0) has been reached, often in 5 to 6 days, heparin is withdrawn. In patients with congenital AT deficiency, replacement therapy is not more effective than anticoagulant therapy and is not cost-effective.\textsuperscript{133} It may be necessary during life-threatening events or in the very rare instances when the APTT is not prolonged despite very high doses of heparin (above 60,000 to 70,000 U/d). In these cases, the recommended concentrate dosage is similar to that recommended for surgery (see above).

There are a few special problems in the management of inherited thrombophilia. Skin necrosis during the initiation of oral anticoagulant therapy is a rare complication that may occur particularly in homozygous PC-deficient patients. This complication is caused by a temporary procoagulant imbalance that develops during the early phase of vitamin K antagonist intake when high circulating levels of procoagulant vitamin K--dependent factors with long half-lives (such as prothrombin) contrast with the rapidly lowered levels of PC with a short half-life.\textsuperscript{32} To avoid or reduce the risk for subjects with half-normal levels of PC compatible with heterozygote, oral anticoagulants should be started with small, progressively increasing daily doses (eg, 1 to 2 mg warfarin) and administered with heparin.\textsuperscript{134} In patients with severe (homozygous) PC deficiency, replacement therapy with plasma concentrates throughout the initial phase of oral anticoagulation seems to be a more practical and safer approach, maintaining PC levels above 50\% until stable anticoagulation is reached.\textsuperscript{135}

Another special problem is purpura fulminans in newborns homozygous for PC deficiency. Before PC concentrates became available, the recommended treatment was plasma, infused until purpuric lesions healed.\textsuperscript{136} More recently, encouraging results have been obtained with purified PC concentrates.\textsuperscript{137} For long-term treatment of homozygous newborns two methods can be used: PC replacement therapy or oral anticoagulants. Warfarin is the first-choice treatment and should be administered daily at about 0.15 to 0.40 mg/kg until a maintenance dose is found that keeps the INR between 2.5 and 4.4.\textsuperscript{138}

There is little information about the usefulness of anti-thrombotic prophylaxis in hyperhomocysteinemia. Supplementary vitamins, in particular folic acid but also pyridoxine and cobalamin, can lower mildly elevated levels of homocysteine irrespective of initial serum vitamin levels.\textsuperscript{61} Although it is not known whether this strategy will also decrease the thrombotic risk, in view of its low cost and high safety it should be recommended to patients with hyperhomocysteinemia associated with thrombosis. Recommended daily doses of vitamin supplements are: 1 mg folic acid, 0.4 $\mu$g cobalamin, and 100 mg pyridoxine.

In general, the recommendations given in these guidelines are based on the results of small and noncontrolled series of patients, and as such should be considered tentative. More precise treatment guidelines are needed based on large multicenter studies.

**NOTE ADDED IN PROOF**

Fermo et al have recently confirmed that moderate hyperhomocysteinemia may have pathogenetic significance in venous and arterial occlusive disease in the young and should be included among the causes of inherited thrombophilia (Ann Intern Med 123:747, 1995). Mandel et al have shown that the coexistence of hereditary homocystinuria and mutant factor V increases the risk of arterial and venous thrombosis (N Engl J Med 334:763, 1996).

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