

Screening for inherited thrombophilia: indications and therapeutic implications

VALERIO DE STEFANO, ELENA ROSSI, KATIA PACIARONI,
GIUSEPPE LEONE

Institute of Hematology, Catholic University, Roma, Italy

Background and Objectives. In recent years knowledge concerning inherited and acquired causes of thrombophilia has increased greatly. The most common inherited traits (deficiency in antithrombin, protein C, or protein S, factor V Leiden, prothrombin G20210A) and mild hyperhomocysteinemia are diagnosed in at least 40% of patients with venous thromboembolism (VTE).

Information Sources. The authors work in this field, contributing to multicenter clinical and laboratory investigations and to peer-reviewed journals with original papers. The material examined in this review includes articles published in journals covered by MedLine.

State of the Art. The associated risk for VTE is different according to genotype, being higher among the carriers of natural anticoagulant deficiencies and homozygotes for factor V Leiden. The overall prevalence of thrombophilic traits in the general population being near to 10% renders the probability of carrying multiple defects not excessively rare, with a further increase in thrombotic risk of up to 20-fold. Thus, clinical penetrance is heterogeneous, producing either mild or severe venous thrombotic manifestations, which can be unprovoked or associated with circumstantial risk factors and occur in either young or advanced age. More recently, inherited thrombophilia has been focused on as an important determinant of complications of pregnancy and puerperium. As expected, inherited thrombophilia produces an increased risk of VTE, particularly during puerperium. Moreover it is well established that thrombophilic women have an increased risk of late and/or recurrent fetal loss; whether they are at higher risk of pre-eclampsia, fetal growth restriction, and *abruptio placentae* is debated. Overall, 40% of women with obstetric complications other than VTE carry a thrombophilic trait. Yet, as a rule VTE and obstetric complications seem to occur in different individuals, probably because of the presence of unknown factors favoring one or other of these clinical manifestations.

Conclusions and Perspectives. Inherited thrombophilia is now viewed as a multicausal model, the clinical event being the result of gene-gene and gene-environment age-dependent interactions; the associated clinical manifestations can be heterogeneous as regards severity as well as type of event (VTE or obstetric complication). Therefore the criteria for screening affected individuals who have suffered from the above complications or their rel-

Correspondence: Prof. Valerio De Stefano, Istituto di Ematologia, Università Cattolica, Largo Gemelli 8, 00168 Roma, Italy.
Phone: international +39.06.30154180. Fax: international +30.06.3051343. E-mail: v.destefano@eudoramail.com

atives should not be very stringent. The patient's genotype could be a main determinant of the features of primary or secondary prophylaxis used in the affected individual.
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In Western countries the incidence of venous thromboembolism (VTE) per year is about 1 per 1.000 individuals.^{1,2} The term *thrombophilia* describes a tendency to develop thrombosis on the basis of inherited or acquired disorders of blood coagulation or fibrinolysis leading to a prothrombotic state. Familial thrombophilia was first described in 1956 on a clinical basis by Jordan and Nandorff.³ Inherited deficiency of antithrombin III (AT), protein C (PC) and its co-factor, protein S (PS), were the first identified causes of thrombophilia.⁴⁻¹⁰ In recent years two common gene polymorphisms were recognized as additional causes of hypercoagulability: factor V G1691A (factor V Leiden), resistant to the anticoagulant action of activated protein C,¹¹⁻¹⁴ and prothrombin G20210A, associated with increased levels of circulating prothrombin.¹⁵ Mild hyperhomocysteinemia is also an established risk factor for thrombophilia.¹⁶⁻¹⁸ *Inherited thrombophilia* is defined as "a genetically determined tendency to venous thromboembolism. Dominant abnormalities or combinations of less severe defects may be clinically apparent from early age of onset, frequent recurrence or family history. Milder traits may be discovered only by laboratory investigation".¹⁹ Thus venous thromboembolic disease is now viewed as a multicausal model, the thrombotic event being the result of gene-gene and gene-environment interactions (for instance dietary habits or circumstantial risk factors such as surgery, trauma, pregnancy and puerperium, and oral contraceptive intake) (Figure 1).²⁰

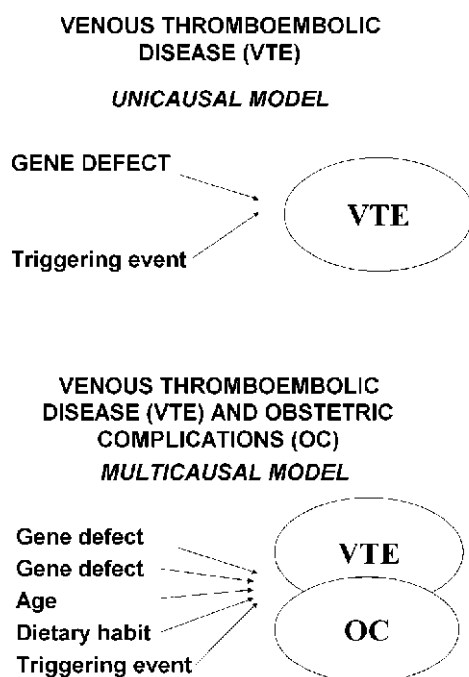


Figure 1. Pathogenetic models of the clinical manifestations associated with inherited thrombophilia.

Epidemiology of inherited thrombophilia and associated risk of venous thromboembolism

Deficiency of natural coagulation inhibitors

Overall, the rare deficiencies of natural coagulation inhibitors (AT, PC, and PS) are detectable in less than 1% of the general population and in less than 10% of unselected patients with VTE (Table 1).^{8,9,21}

Among carriers of such deficiencies the risk of VTE is 5 to 8-fold higher than that in the general population²²⁻²⁵ and the annual incidence of VTE is 1% to 2% (Table 1).²³⁻²⁷ Approximately half of the thrombotic events occur in association with circumstantial risk factors;^{8,23-27} the first event more frequently occurs before 45 years old.^{8,26}

Factor V Leiden

Factor V Leiden is present almost exclusively among Caucasians, with a prevalence of 5% in the general population with European ancestry and 18% among patients with VTE (Table 1).^{28,29} The risk of VTE is 2 to 7-fold higher among heterozygotes and 40 to 80-fold higher among homozygotes (Table 1);^{23,24,32-35} the annual incidence of VTE is 0.19% to 0.67%.^{23,24,32-36} Pregnancy and oral contraceptive intake increase the risk, as do minor events (prolonged travel, short illness, minor surgery).³⁷ The first event occurs in a relevant number of cases after 45 years old.^{29,38}

Prothrombin G20210A

The prothrombin 20210A allele is present in 2% of healthy individuals and in 7% of patients with VTE (Table 1).^{15,39-41} The risk of VTE is 2 to 3-fold higher among heterozygotes (Table 1).^{15,34,41} The risk of spontaneous VTE increases with age, being up to 19-fold higher after 60 years.⁴¹

Mild hyperhomocysteinemia

Homocysteine is a sulfhydryl amino acid derived from metabolic conversion of methionine. Mild hyperhomocysteinemia is a risk factor for arterial and venous occlusion (Table 1).¹⁶⁻¹⁸ Acquired factors (low intake of pyridoxine, cobalamin, folate) can produce mild hyperhomocysteinemia interacting with gene factors, such as the C677T polymorphism in

Table 1. Epidemiology of inherited thrombophilia and associated risk of venous thromboembolism (VTE).

Thrombophilic trait	Caucasian general population (% carriers)	Unselected patients with VTE (% carriers)	Selected patients with VTE (% carriers)	Relative risk of VTE (Case-control studies)	Relative risk of VTE (Family studies)	Annual incidence of VTE (%)
AT deficiency	0.02-0.16 ^{8,9,21}	1.9 ⁹	4.3 ⁹	5 ²²	5-8 ²³⁻²⁵	1-2 ²³⁻²⁷
PC deficiency	0.2-0.4 ^{8,9}	3.7 ⁹	4.8 ⁹	6.5 ²²	5-8 ²³⁻²⁵	1-2 ²³⁻²⁷
PS deficiency	-	2.3 ⁹	4.3 ⁹	1.7 ²²	5-8 ²³⁻²⁵	1-2 ²³⁻²⁷
Factor V Leiden	4.8 ²⁹	18.8 ²⁹	18.1 ²⁹	7 ^{30,31} (heterozygotes) 40-80 ³⁰ (homozygotes)	2 - 4 ^{23,24,32-35}	0.19-0.67 ^{23,24,32-36}
PT G20210A	2.0 ⁴⁰	7.1 ⁹	7.3 ⁴¹	2-3 ^{15,41}	2 ³⁴	0.13 ³⁴
FV Leiden +PT 20210A	0.01 (expected)	-	2.2 ⁴⁴	20 ⁴⁴	6 ³⁴	0.57 ³⁶
Hyperhomocysteinemia	5 ^{16#}	13-25 ¹⁶	10-25 ¹⁶	2.5 ¹⁸	-	-
MTHFR 677 TT	13.7 ¹⁷	-	13.9 ¹⁷	1 ¹⁷	-	-

⁹AT, PC, and PS deficiencies are considered as a whole. [#]Levels above the 95th percentile of the normal values as arbitrary cut-off for mild hyperhomocysteinemia.

the methylenetetrahydrofolate reductase gene. Homozygous carriers can develop hyperhomocysteinemia especially in the presence of low folate levels. Among Caucasians the prevalence of the TT genotype is 13.7%, quite similar to that found among patients with VTE,¹⁷ suggesting that search for this genotype is not useful *per se* (Table 1).

Combined defects

The risk of a first thrombotic event further increases in the presence of combined defects. This has been firmly established by a number of family studies showing a higher prevalence of VTE among carriers of combined thrombophilic traits in comparison within carriers of either trait.^{8,20,36,42-44} According to the frequency in the general population, the expected prevalence of double heterozygotes for factor V Leiden and prothrombin G20210A is 1:1,000 (Table 1); in a pooled analysis double heterozygosity was present in 2.2% of 2,310 cases with VTE, with a 20-fold increase in risk (Table 1).⁴⁴ The annual incidence of VTE among double heterozygotes has been reported to be 0.57%.³⁶ Combination of mild hyperhomocysteinemia with factor V Leiden or prothrombin G20210A has been reported to produce a 20 to 50-fold increase in the risk of VTE,⁴⁵ although review of the published series shows contradictory evidence.¹⁷ A specific factor V gene haplotype (HR2) is present in 8% of normal subjects and is an additional cause of resistance to activated protein C.⁴⁶ Its role as an independent risk factor for VTE is uncertain;⁴⁷⁻⁴⁹ yet double carriers of the HR2 haplotype and factor V Leiden have an increased plasma resistance to activated protein C and an increased risk of VTE in comparison with heterozygotes for factor V Leiden;^{46,47,49,50} the rare state of homozygosity for HR2 produces a 5.5-fold increase in the risk of VTE.⁴⁹

Additional plasma abnormalities associated with an increased risk of venous thrombosis

Increased levels of circulating fibrinogen, lipoprotein(a), factor VIII, factor IX, or factor XI raise the risk of VTE by 2-to-4 fold.⁵¹⁻⁵⁵ Recently, increased levels of thrombin activatable fibrinolysis inhibitor (TAFI) have been reported to produce a 1.7-fold increase the risk of VTE.⁵⁶ Moreover the level of APC-resistance was found to be related to the risk of VTE independently of the presence of factor V Leiden.^{57,58}

Clinical manifestations

In most cases the clinical manifestations of inherited thrombophilia consist of deep venous thrombosis (DVT) of the legs with or without pul-

monary embolism; yet the risk of superficial thrombophlebitis⁵⁹ is also increased as is that of potentially fatal events such as splanchnic or cerebral venous thrombosis.⁶⁰⁻⁶² AT, PC, and PS deficiencies, factor V Leiden, and prothrombin G20210A are present in about 40% of the patients with a first unprovoked DVT of the legs before the age of 45 years; in patients with unprovoked DVT after 45 years old or in patients with DVT provoked by pregnancy, puerperium, oral contraceptives, surgery, trauma, plastering or bed rest the diagnostic yield accounts for about one third of all the cases.⁶³

As a rule such defects are not associated with an increased risk of arterial occlusion;⁶⁴ some studies suggest that prothrombin G20210A could play a role in the pathogenesis of arterial occlusion among young patients without any traditional vascular risk factor.^{65,66}

Inherited thrombophilia and risk of obstetric complications

Thrombotic risk during pregnancy and puerperium among women with inherited thrombophilia

Before the knowledge of factor V Leiden and prothrombin G20210A mutations some relatively large series of pregnant women with AT, PC, or PS deficiency were analyzed, retrospectively.⁶⁷⁻⁷⁰ A pooled analysis of such studies showed a rate of VTE complicating pregnancy and puerperium of 37.8% among AT-deficient women, 14.7% among PC-deficient women, and 17.1% among PS-deficient women. The frequency of VTE was higher during puerperium (61.5% of all the events).⁷¹ A family study reported an 8-fold increase in the risk of VTE during pregnancy and puerperium among the affected women with a deficiency of natural coagulation inhibitors.⁷²

The prevalence of factor V Leiden among women with VTE during pregnancy or puerperium has been reported to range from 8% to 78%.⁷¹ Yet only three studies enrolled a relevant number of cases (70 to 119 women).⁷³⁻⁷⁵ The risk of VTE during pregnancy or puerperium associated with factor V Leiden is increased by 4.5 to 16.3-fold.⁷¹ Accordingly, the absolute risk is relatively low, ranging from 3.6 to 13.0 events per 1,000 deliveries (assuming an incidence of 0.8 events per 1,000 deliveries in the general population).⁷¹ In homozygotes for factor V Leiden the rate of DVT was 4.2% during pregnancy and 4.7% during puerperium; the rate of superficial thrombophlebitis was 8.0% during pregnancy and 3.8% during puerperium.⁷⁶ In a multicenter family study homozygosity for factor V Leiden was

associated with a 41.3-fold increase in the risk of VTE in comparison with in normal relatives, with an absolute risk during pregnancy and puerperium of 3%.⁷⁷ The risk of VTE during pregnancy or puerperium associated with prothrombin G20210A has been estimated to be increased by 2.9-fold.⁷⁵

Complications of pregnancy other than thrombosis among women with inherited thrombophilia

Obstetric complications such as severe pre-eclampsia, fetal loss, fetal growth retardation, and *abruptio placentae* are associated with inadequate placental perfusion. Overall, 38% of 110 women with obstetric complications have been reported to have inherited thrombophilia.⁷⁸

Pre-eclampsia

Pre-eclampsia is estimated to complicate 2.6% of births.⁷⁹ Scarce data are available about the risk of pre-eclampsia associated with deficiency of natural anticoagulants; a study reported a 10.7-fold increase in risk in PS-deficient women.⁸⁰

In 12 published series of women with a history of pre-eclampsia or HELLP syndrome (with a total of 1,458 patients) factor V Leiden was diagnosed in from 4% up to 26% of the cases.⁷¹ Accordingly, the association of factor V Leiden with a higher risk of pre-eclampsia is uncertain, since not all the studies reported positive results.⁷¹ Investigations on the role of prothrombin G20210A also produced contradictory results.⁷¹ A recent systematic review of the literature evidenced that factor V Leiden does not seem to be associated with the development of pre-eclampsia *per se* but that it favors progression to the severe forms of disease. Prothrombin G20210A was not found to be associated with either mild or severe pre-eclampsia.⁸¹

Fetal loss

In Western countries up to 13% of pregnancies end in fetal loss,⁸² in the large majority of cases as miscarriage (early fetal loss) and in 0.5% - 0.8% of pregnancies as stillbirth (late fetal loss).⁸³ Recurrent fetal loss is a common health problem as well, with two or more losses affecting approximately 5% of women in the fertile age and three or more fetal losses affecting 1% to 2% of women.⁸⁴ Among women with inherited thrombophilia (AT, PC, and PS deficiency or factor V Leiden) the overall risk of stillbirth (late fetal loss after the 28th week of gestation) was 3.6-fold increased and that of miscarriage (fetal loss before the 28th week of gestation) 1.3-fold increased.⁸⁵ A 2.0-fold increase in the risk of fetal loss among carriers of AT, PC, and PS deficiency and factor V Leiden has also been

reported in several family studies.⁸⁶⁻⁸⁸ A number of case-control studies confirmed that factor V Leiden is a weak but well-established risk factor for unexplained recurrent fetal loss (two or more events) and for one or more late fetal loss, with a 2- to 7-fold increase in risk.⁷¹ In contrast, most reports denied a significant association between prothrombin G20210A and fetal loss;⁷¹ in a series of 67 women, prothrombin G20210A was associated with a significant 3.3-fold increased risk of late fetal loss.⁸⁹

Fetal growth retardation

Carrier status of the prothrombin 20210A allele has been reported to produce a 4.6 to 5.9-fold increase in risk of unexplained fetal growth retardation (FGR).^{78,90} Factor V Leiden was associated with a 6.9-fold increase in the risk of FGR.⁹⁰ A large investigation on 1,100 live neonates born from 755 women confirmed that carrier status of factor V Leiden or prothrombin G20210A was associated with a 1.7-fold increase in the risk of having a baby under the 10th growth centile.⁹¹ A study on 375 children reported a 4-fold increase in the risk of FGR among babies with multiple or homozygous defects.⁹² A recent investigation denied any association of factor V Leiden and prothrombin G20210A with FGR among 493 neonates and their mothers.⁹³

Abruptio placentae

The risk of severe *abruptio placentae* is 4.9 to 11.8-fold higher among carriers of factor V Leiden^{78,94} and 8.9-fold higher among carriers of the prothrombin G20210A allele.⁷⁸

Multiple clinical manifestations among women with inherited thrombophilia

The prevalence of obstetric complications was recently evaluated in 395 women with a history of VTE, in 51% of the cases with inherited thrombophilia; 313 women were investigated as a control group.⁹⁵ Pregnancy-induced hypertension, pre-eclampsia, or eclampsia occurred in 7.6% of cases and in 2.6% of controls, with a 3.1-fold increase in risk associated with a history of previous VTE. Late fetal loss (after the 24th week of pregnancy) occurred in 4.3% of cases and in 3.2% of controls; miscarriage occurred in 21.8% of cases and in 21.3% of controls. Analysis of the patients after stratification according to the presence or the absence of inherited thrombophilia did not reveal any difference in the rate of obstetric complications between the two subgroups. It was, therefore, concluded that a general predisposition to thrombosis, not linked to any specific abnormality,

is associated with an increased risk of pre-eclampsia.⁹⁵ Thus VTE and obstetric complications seem to occur in different individuals with inherited thrombophilia, probably because of the presence of unknown factors favoring one or other of the clinical manifestations (Figure 1).

Screening for inherited thrombophilia

Candidates for screening

Candidates to be screened for inherited thrombophilia should not be selected on the basis of stringent criteria (Table 2). The identification of gene polymorphisms widely present in the general population and associated with a moderate thrombotic risk contrasts with the paradigm of inherited thrombophilia as cause of severe and/or recurrent venous thromboses in young age, in many cases without an apparent cause and with a high clinical penetrance among members of the same kindred. Yet such signs remain as those associated with a higher probability of diagnosis. For example, in patients with venous thrombosis before 18 years old, the prevalence of thrombophilic defects has been reported to be 54% (deficiency of natural coagulation inhibitors 18.3%, factor V Leiden 31.8%, prothrombin G20210A 4.2%), whereas in adult individuals the diagnostic yield is one third of cases.⁹⁶ Yet it is not rare that the proband patient suffered from a superficial thrombophlebitis possibly induced by a minor event, or had no family history of thrombotic events, or had a VTE event at an advanced age. Therefore all patients with venous thrombosis are potentially candidates, independently of the age at which the event occurs, the circumstances of thrombosis, and the severity of the clinical manifestations.

A possible exclusion criterion is the contemporary presence of a high-risk disease for thrombosis such as neoplasia, since in those situations the presence of thrombophilic polymorphisms associated with a moderate risk for VTE is not considered a significant additive risk factor.⁹⁷ However such a criterion does not seem absolute, especially in the case of hematologic neoplasia.^{61,98,99}

Patients with arterial thrombosis are not candidates for a search for inborn thrombophilic defects, except homocysteine dosage. The appropriateness of checking pediatric patients or adult patients without traditional risk factors for artery disease for inherited thrombophilia is debatable.⁶⁴⁻⁶⁶

Potential candidates for screening are women who have suffered from complications, other than venous thromboembolism, of a pregnancy (pre-eclampsia, late and/or recurrent fetal loss, fetal growth retardation, *abruptio placentae*).

Table 2. Candidates for screening for inherited thrombophilia.

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- All patients with venous thromboembolism, independently of the age of onset (before or after 45 years), the circumstances of thrombosis (provoked or unprovoked), and the severity of the clinical manifestations.
 - As a rule, patients with cancer may be excluded. Yet patients with hematologic neoplastic diseases and venous thromboembolism are potential candidates.
 - All women with complications of a pregnancy other than venous thromboembolism:
 - one or more episodes of late fetal loss
 - two or more episodes of early fetal loss
 - Women with pre-eclampsia, fetal growth retardation or abruptio placentae are potential candidates.
 - All asymptomatic individuals who are first-degree relatives of a diagnosed carrier of a thrombophilic trait. This should be accompanied by accurate information and counseling.
 - Asymptomatic women with a family history of venous thromboembolism are potential candidates for screening before use of oral contraceptives, hormone replacement therapy, or pregnancy.
-

This indication seems well established as regards the search of factor V Leiden among women with fetal loss or severe pre-eclampsia, whereas the remaining ones need to be confirmed by further investigations.

Diagnostic panel

The inclusion of a large number of individuals with VTE (or obstetric complications) in a diagnostic panel for inherited thrombophilia needs to be counterbalanced by a stringent selection of the laboratory tests. Screening should be limited to those traits that are more frequent or carry a higher thrombotic risk. Validation of new possible assays should be conducted by case-control studies carried out by specialized centers (Table 3). A first-line diagnostic panel should include AT heparin cofactor assay (functional amidolytic method), PC assay (functional clotting or amidolytic method), and PS assay (total and free fraction, measured by immunologic methods).⁸ The APC-resistance plasma assay should be included in order to identify kindreds with genetically determined APC-resistance not related to factor V Leiden.¹⁰⁰ Analysis of DNA should include the search for factor V Leiden and the prothrombin G20210A. Genotyping for the C677T polymorphism in the methylenetetrahydrofolate reductase gene is of poor use-

Table 3. Screening for inherited thrombophilia (first line panel).

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- Antithrombin heparin cofactor activity (amidolytic method)
 - Protein C (clotting or amidolytic method)
 - Protein S (total and free antigen fractions)
 - APC-resistance plasma assay
 - Factor V Leiden
 - Prothrombin G20210A
 - Homocysteine
-

fulness,¹⁷ while homocysteine measurement is recommended. Applying this panel, at least one third of the patients with VTE can be diagnosed as carrying inherited thrombophilia; homocysteine measurement allows identification of at least a further 10% of patients with thrombophilia,¹⁸ achieving an overall diagnostic yield of 40%.

The timing of screening for inherited thrombophilia

A timely determination of AT is recommended in patients with acute DVT, since the diagnosis of AT deficiency could influence treatment of acute events (see *below*); the results of the other assays as a rule do not influence the management of acute events. Interpretation of the results obtained from plasma assays should consider possible drug-induced alterations: lowered AT levels during prolonged heparin treatment; lowered PC and PS levels during oral anticoagulant treatment; lowered AT, PC, and PS levels during L-asparaginase treatment; lowered PS levels and the presence of acquired APC-resistance during hormonal treatment; poor performance of the APC-resistance assay during heparin treatment. Moreover results can be altered by a number of physiopathologic conditions: lowered AT levels due to liver disease, nephrotic syndrome, inflammatory bowel diseases, pregnancy; lowered PC and PS levels due to liver disease; lowered PS levels due to pregnancy; acquired APC-resistance due to pregnancy or to antiphospholipid antibodies.

A diagnosis of AT, PC, or PS inherited deficiency should be validated by the identification of another proband's relative with low levels of inhibitor, such defects being due to a large number of gene mutations not identifiable on a routine basis.

Treatment of acute events

As a rule the management of acute thrombosis is the same for patients with or without inherited thrombophilia. The presence of AT deficiency

should be ruled out soon, because of the frequent need to achieve therapeutic aPTT levels with higher dosages of heparin.^{101,102} In the case of life-threatening events the use of purified AT concentrates should be considered.⁸ In the rare cases of severe (homozygous) PC deficiency, replacement therapy with PC concentrates should be added during the transition from heparin to oral anticoagulants, in order to maintain PC levels above 50% until stable anticoagulation is reached.^{103,104}

Secondary prophylaxis

After a first DVT the duration of secondary prophylaxis with oral anticoagulants (INR target 2 to 3) should be established weighing the risk of major hemorrhagic complications against the risk of a new spontaneous venous thromboembolic event. To a certain extent, the risk of recurrence can be individually predicted considering the circumstances of the first event, whether spontaneous or provoked in association with circumstantial risk factors (use of oral contraceptives, pregnancy and puerperium, surgery) as well as the genotype of the patient (Table 4). Other factors to be considered are the family history of the proband and the severity of the first event.

Patients with standard risk of recurrence

Patients with inherited thrombophilia and with a previous provoked VTE have no higher risk of spontaneous recurrence than do patients with a normal genotype; this has been confirmed even in patients with an unfavorable genotype, such as double carriers of factor V Leiden and prothrombin G20210A.¹⁰⁵ Therefore in such patients secondary oral anticoagulant treatment should be continued for as long as it would be in patients with provoked VTE and unknown cause of thrombophilia.

It is under debate whether heterozygosity for factor V Leiden alone or prothrombin G20210 is associated with an increased risk of recurrence; in most retrospective as well as prospective studies the risk of recurrence after a first spontaneous DVT did not differ from that in patients with a normal genotype.¹⁰⁵⁻¹¹¹ Yet in two prospective patient cohorts (one of them being formed of men older than 45 years) a significant increase in the risk of recurrence was found among heterozygotes for factor V Leiden or prothrombin G20210A.¹¹²⁻¹¹⁵

Patients with increased risk of recurrence

Patients who have suffered from two or more venous thromboembolic events that occurred spontaneously are candidates for life-long oral anticoagulant treatment (INR target 2 to 3).⁸

Table 4. Secondary prophylaxis with oral anticoagulants after venous thromboembolism (VTE) in patients with inherited thrombophilia.

Oral anticoagulant prophylaxis for as long as in patients with a normal genotype.

- First unprovoked deep venous thrombosis with or without pulmonary embolism affecting individuals with isolated heterozygosity for factor V Leiden or for the prothrombin G20210A or with moderate hyperhomocysteinemia.
- First provoked deep venous thrombosis with or without pulmonary embolism (all genotypes).

Oral anticoagulant prophylaxis for an indefinite duration (after evaluation of the individual hemorrhagic risk).

- Two or more recurrent unprovoked episodes of deep venous thrombosis with or without pulmonary embolism (all genotypes).
- First unprovoked deep venous thrombosis with or without pulmonary embolism affecting individuals with severe thrombophilia (AT, PC, or PS deficiency, homozygosity for factor V Leiden, combined defects)
- First life-threatening thrombotic episode (massive pulmonary embolism, cerebral venous thrombosis, splanchnic venous thrombosis), in particular if unprovoked (all genotypes).

Uncertain indications for oral anticoagulant prophylaxis of indefinite duration to be given on an individual basis (all genotypes).

- Two or more recurrent unprovoked episodes of superficial thrombophlebitis
- Two or more recurrent provoked episodes of deep venous thrombosis with or without pulmonary embolism
- Two or more recurrent episodes of deep venous thrombosis with or without pulmonary embolism, of which only one unprovoked
- Diagnosis of severe thrombophilia in individuals with not recent occurrence of unprovoked deep venous thrombosis with or without pulmonary embolism.

Patients with a first spontaneous VTE carrying multiple or homozygous defects are considered to have an increased risk of recurrence and are potential candidates for life-long secondary prophylaxis.^{105,110,116-118}

The carriers of deficiencies of natural coagulation inhibitors (AT, PC, PS) are estimated to have a higher risk of thrombosis than the carriers of factor V Leiden and prothrombin G20210A;^{23,24} accordingly, they have been reported at higher risk of recurrence after a first spontaneous DVT¹¹⁹ and are potential candidates for life-long prophylaxis. There is preliminary evidence that the risk of recurrence is increased in the presence of high levels of factor VIII¹²⁰ and in the presence of increased levels of homocysteine.¹²¹ Even in the absence of *ad hoc* studies, vitamin intake aimed to reduce the homocysteine levels is warranted in individuals with moderate hyperhomocysteinemia.¹⁶ Moreover, long-term anticoagulation should be considered for patients with a first life-threatening event (splanchnic venous thrombosis, cerebral venous thrombosis, massive pulmonary embolism). In such patients therapeutic options should consider the

genotype and the circumstances of the acute event, as above described; in the absence of *ad hoc* studies, life-long treatment should be preferred for those patients with a severe impairment of the function in the sites of thrombosis.

However in a number of clinical situations no clear guidelines can be established and the recommended duration of secondary antithrombotic prophylaxis should be decided on an individual basis, considering also the patient's wishes and compliance: this is the case of individuals with recurrent provoked venous thrombotic episodes, or recurrent superficial thrombophlebitis, or spontaneous VTE occurring many years before a diagnosis of severe inherited thrombophilia.

Antithrombotic prophylaxis during risk circumstances

As a rule, women with a previous venous thrombosis receive s.c. low molecular weight heparin (75 to 150 U/kg b.w. once daily) during pregnancy; heparin or oral anticoagulants (INR 2-3) are administered during the puerperium. This is especially warranted in the case of a first event that occurred spontaneously or during circumstances such as oral contraceptive use or pregnancy. A recent investigation confirmed that women with a previous spontaneous VTE and/or inherited thrombophilia (in particular, factor V Leiden) are at increased risk of recurrence during pregnancy and puerperium.¹²² Pregnant women with AT deficiency should receive special care; different strategies have been proposed. In general, heparin should be administered at dosages higher than usual. A review of the published cases treated with unfractionated heparin suggested the need to achieve a 1.3 to 1.5-fold prolongation of the aPTT, since such a prolongation was not present among the cases having had VTE.⁸ Antithrombotic prophylaxis with low molecular weight heparin (LMWH) throughout pregnancy in AT-deficient women failed in 2 cases of 7 (28%) treated with a high-dose regimen (10,000 to 17,500 IU daily given by two administrations) and in 5 cases of 12 (42%) treated with a low-dose regimen (2,500 to 7,500 IU once daily).¹²³ An alternative approach consists of administering s.c. heparin during the first trimester (when oral anticoagulants carry a teratogenic risk), oral anticoagulants (INR 2 to 3) during the second trimester, and heparin again from the 37th week until delivery.⁸ Infusion of AT concentrate has been reported in the peripartum period in order to normalize circulating AT levels and to allow a reduction in the heparin dosage.^{124,125} As regards

antithrombotic prophylaxis during risk situations other than pregnancy, no evidence-based recommendations can be attempted and clinical practice derives only from the estimate of the thrombotic risk during such situations as well as from the opinions of the expert investigators involved in this field. Such recommendations apply both to individuals with previous VTE as well as to asymptomatic individuals. Individuals undergoing high-risk surgery should receive prophylaxis as usual, since the presence of deficiencies of natural inhibitors or factor V Leiden seems to add little to the postoperative thrombotic risk.^{68,126} In the case of AT-deficient individuals undergoing high-risk surgery, replacement with AT concentrate should be considered.⁸ Heparin prophylaxis should be adopted also for all individuals with inherited thrombophilia undergoing low-risk surgery or in the case of situations such as plastering of the legs, bed immobilization, and prolonged air journeys.

Primary prophylaxis

The relatively high frequency of some thrombophilic polymorphisms in the general population raised the question whether generalized screening before hormonal treatment or before pregnancy could have a clinical relevance. So far indiscriminate screening in such situations is considered not cost-effective and unlikely to compete for resources with other medical interventions.²⁹ It could be worthwhile to investigate women with a family history positive for VTE,²⁹ yet such an approach has been reported as unsatisfactory as a criterion for screening before prescribing oral contraceptives.¹²⁷

Family screening

Laboratory screening of asymptomatic relatives of index patients with inherited thrombophilia has a diagnostic yield of 50%, such traits being genetically dominant. Recently the usefulness of family screening focused on identifying the asymptomatic relatives of a known thrombophilic individual has been debated.¹²⁸⁻¹³⁰ Several arguments have been made to suggest caution in screening asymptomatic relatives: the risk of labeling individuals as carriers of a genetic disease, generating anxiety and possibly leading to insurance and employment discrimination, the low incidence of VTE among carriers of the most common thrombophilic traits (factor V Leiden and prothrombin G20210A), the cost of screening which is not negligible. On the other hand, the identification of asymptomatic carriers may enable prevention to be assured by adopting antithrombotic measures during risk sit-

uations and avoiding hormonal treatments; moreover, the knowledge of the thrombophilic trait can lead to signs and symptoms of VTE not being underestimated, allowing early recognition of unprovoked events. A multicenter retrospective study conducted in 1994 on patients with deficiencies of AT, PC, and PS showed that primary prophylaxis given to asymptomatic individuals during risk events produced a decrease in both incidence per year of first thromboses (0.7/100 pt-years after diagnosis *versus* 1.7/100 pt-years before diagnosis of inherited thrombophilia) and incidence per year of recurrences (1.3/100 pt-years after diagnosis *versus* 4.8/100 pt-years); moreover the rate of first thrombosis among individuals younger than 40 years decreased from 77% before diagnosis to 33% after diagnosis.²⁶ No information concerning this issue is available for individuals with factor V Leiden or prothrombin G20210A; however a large multicenter family study reported that in half the subjects with factor V Leiden or prothrombin G20210A and previous VTE a circumstantial risk factor triggered the thrombotic event.³⁴ Therefore it can be suggested that prophylaxis during risk situations could halve the incidence of first VTE. Since the incidence per year of secondary DVT among carriers of factor V Leiden is about 0.1% to 0.4%,^{34,35} to avoid one secondary DVT from 250 to 1,000 affected individuals should be identified by screening 500 to 2,000 relatives of index patients. In Italy the cost of a DNA assay for gene polymorphism is € 64 (=approximately US\$ 58) (National Health Service tariff), so that screening such a number of individuals would cost US\$ 29,000 to 116,000. The overall cost of acute and long-term treatment of one DVT has been estimated to be US\$ 26,000,¹³¹ so that in this case family screening would not be cost-effective. In contrast, family screening for the defects with higher clinical penetrance (AT, PC, and PS deficiency) is definitively cost-effective. Since the incidence per year of secondary DVT in these individuals is 0.6-0.7%,^{24,27} to avoid one secondary DVT 150 carriers should be identified by screening 300 relatives of index patients. According to the tariffs of the National Health Service in Italy the cost would be € 837 for AT deficiency (US\$ 753), € 2,727 (US\$ 2,454) for PC deficiency, and € 2,944 (US\$ 2,649) for PS deficiency. According to these figures, screening for factor V Leiden and prothrombin G20210A could be limited to the kindreds with a higher clinical penetrance, in which more members besides the proband had suffered from VTE; in fact in such kindreds the incidence per year of VTE is near to that estimated in kindreds with deficiencies of AT, PC and

PS, likely for the presence of still unidentified prothrombotic risk factors.^{33,132} On the other hand, it can be expected that the cost of DNA assays will decrease significantly, thus probably also rendering cost-effective screening for thrombophilic traits with low clinical penetrance.

Primary prophylaxis during risk manifestations

Independently of a previous history of VTE, all individuals with inherited thrombophilia should be evaluated for antithrombotic prophylaxis during pregnancy and puerperium. In fact, assuming a general incidence of 0.8 venous thromboembolic events per 1,000 pregnancies, the expected rate of VTE should be 1 per 150 among women with AT, PC or PS deficiency (with a higher risk among AT-deficient women), 1 per 125 pregnancies among heterozygotes for factor V Leiden, 1 per 500 pregnancies among heterozygotes for prothrombin G20210A, and 1 per 30 pregnancies among homozygotes for factor V Leiden.^{71,72,75,76} In conclusion, antithrombotic prophylaxis is warranted in all women with inherited thrombophilia during the post-partum period (4 to 6 weeks after delivery), which is the period at higher thrombotic risk;⁷⁵ administration of heparin throughout the pregnancy should be considered for asymptomatic women with genotypes at higher risk (AT deficiency, and perhaps PC and PS deficiency, and homozygosity for factor V Leiden) (Table 5). A special effort should be made in asymptomatic women with AT deficiency to rule out a type II defect affecting the heparin-binding site, since such women do not have an increased risk of VTE.¹³³ Even though a direct comparison is not available, in AT-deficient women the administration of unfractionated heparin seems more efficacious than LMWH.^{8,123} The need for extended prophylaxis during pregnancy for asymptomatic women carrying heterozygosity for factor V Leiden, prothrombin G20210A, or both is uncertain.

Prophylactic measures to be adopted during other risk situations are essentially the same as those described for patients with previous VTE in the section concerning secondary prophylaxis.

Inherited thrombophilia and oral contraceptive use

The risk of VTE associated with oral contraceptive use has recently been reviewed.¹³⁴ The risk is 3- to 6-fold higher among users of *low-dose* oral contraceptives (30 to 40 µg of ethinylestradiol) than among non-users, so that the absolute baseline risk of 1 event per 10,000 individuals per year among

Table 5. Primary antithrombotic prophylaxis in asymptomatic relatives of proband patients with inherited thrombophilia.

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- Contraindication to oral contraception or hormone replacement therapy (in particular for women with severe thrombophilia, i.e. AT, PC, or PS deficiency, homozygosity for factor V Leiden, combined defects)
 - Prophylaxis with low molecular weight heparin in the case of:
 - surgery
 - bed immobilization
 - plastering of the arms or of the legs
 - long air journeys (more than 4 hours)
 - Prophylaxis with low molecular weight heparin:
 - in the puerperium (all genotypes)
 - throughout the whole pregnancy (severe thrombophilia)
 - In the case of AT deficiency, prophylaxis during pregnancy and puerperium should employ high-dosages of low molecular weight heparin or alternatively high-dosages of unfractionated heparin (with 1.3 to 1.5-fold prolongation of the aPTT) (see text).
 - The indication for primary antithrombotic prophylaxis with low molecular weight heparin throughout the pregnancy in women with isolated heterozygosity for factor V Leiden or for the prothrombin G20210A is not yet certain.
-

women in fertile age is increased to 3 to 6 events per 10,000 person-years.¹³⁴

Women with AT, PC or PS deficiency should be advised about the risk associated with the use of oral contraceptives; in particular the incidence per year of VTE among AT-deficient women users was reported to be 27.5% in comparison with the 3.4% among AT-deficient women non-users.¹³⁵ The risk of VTE is 35-fold higher among women users heterozygous for factor V Leiden than in non-carriers and non-users, with an absolute incidence of VTE among user carriers of factor V Leiden of 28.5 per 10,000.¹³⁶ The risk of carriers of factor V Leiden using a desogestrel-containing contraceptive (third-generation product) is as much as 50 times higher the risk of non-carrier, non-users.¹³⁷ The further increase in risk associated with the use of third-generation products has been denied by a Danish case-control study, which estimated a 65-fold increase in risk among carriers of factor V Leiden using first- and second-generation products and a 30-fold increase in risk among carriers using a third-generation product.¹³⁸ Other investigations reported a magnitude of increase in risk among user carriers of factor V Leiden of 10- to 20-fold;^{44,139,140} in contrast, a Spanish family study found no increase in risk among user

carriers of factor V Leiden in comparison with the risk among non-carrier, non-users.¹⁴¹ Women homozygous for factor V Leiden suffered their first VTE during the use of oral contraceptives in 80% of cases.¹⁴² Such data can be explained by the fact that the use of oral contraceptives induces *per se* a significantly decreased sensitivity to activated protein C, in particular in users of third-generation contraceptives, further enhancing the plasma resistance to activated protein C in women with factor V Leiden.¹⁴³ Oral contraceptive use by women heterozygous for prothrombin G20210A is associated with a 3- to 16-fold increase in the risk of VTE^{44,139,140} and a 150-fold increase in the risk of cerebral venous thrombosis.⁶⁰ Women carrying both factor V Leiden and the prothrombin G20210A and who use oral contraceptives have a 17-fold increase in the risk of VTE in comparison with non-carrier, non-users.⁴⁴ The early risk of VTE among first-users mirrors the existence of a subgroup of women at immediate risk when exposed to oral contraceptives; accordingly, women with thrombophilia (AT,PC,PS deficiency, factor V Leiden, prothrombin G20210A) have a 19-fold increased risk during the first 6 months of use and an 11-fold increased risk during the first year of use, in comparison with the risk during later, prolonged use.¹⁴⁴ In conclusion women with genotypes presumably at higher risk (deficiency of natural coagulation inhibitors, homozygosity for factor V Leiden, combined defects) should be advised against the use of oral contraceptives; in the remaining cases, with an absolute risk not exceeding 30 per 10,000 person-years, the risk should be carefully weighed against benefit on an individual basis. A previous history of VTE, especially if unprovoked or secondary to oral contraceptive use or pregnancy, constitutes a strong contraindication for women with inherited thrombophilia. Further data to discourage oral contraceptive use can derive from a family history of VTE and the presence of other concomitant risk factors such as relatively older age, obesity, superficial varicose veins; the potential benefits of hormonal treatment for cure of gynecologic pathologies should also be considered.

Inherited thrombophilia and hormone replacement therapy

Hormone replacement therapy (HRT) after menopause is associated with a 2.1-fold to 3.6-fold increased risk of VTE;¹⁴⁵ the risk was increased for users of oral as well as users of transdermal preparations.¹⁴⁵ The risk of VTE during HRT has been reported to be 15-fold increased among women with factor V Leiden.¹⁴⁶ Until more information is

available clinical guidelines should be similar to those applied to women with inherited thrombophilia who ask for oral contraception. In particular a previous history of VTE should constitute a contraindication to replacement therapy, according to a randomized trial demonstrating, among women with previous VTE, a high rate of recurrence (8.5% per year) in those receiving HRT versus 1.1% per year in those receiving placebo.¹⁴⁷

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