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GUIDELINES

Journal of Transfusion Medicine
2022, vol. 15, no. 3, 171–182
DOI: 10.5603/JTM.2022.0014

ISSN 1689-6017
e-ISSN 2080-1505

Congenital/inherited thrombophilia in adults — characteristics, laboratory testing and management. Recommendations of the Hemostasis Group of the Polish Society of Hematology and Transfusiology 2022

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Key words: inherited thrombophilia, genetic testing, oral anticoagulation, venous thromboembolism

J. Transf. Med. 2022; 15: 171–182

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Abbreviations:

APC — activated protein C
APCR — APC resistance
aPTT — activated partial thromboplastin time
DOACs — direct oral anticoagulants
LMWH — low-molecular-weight heparin
MIM — Mendelian Inheritance in Man database (OMIM online Mendelian Inheritance in Man)
PAI-1 — plasminogen activator inhibitor type 1
PCR — polymerase chain reaction
TAFI — thrombin activatable fibrinolysis inhibitor
VKA — vitamin K antagonists
PE — pulmonary embolism
DVT — deep vein thrombosis
SVT — superficial vein thrombosis
VTE — venous thromboembolism

Introduction

Venous thromboembolism (VTE) is a disease that encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE). The VTE incidence in Europe is estimated at 104–183 cases annually per 100,000 adults with predominance of men (1.2:1). VTE episodes may be triggered by numerous inherited or acquired risk factors, the most important of which include: hospitalization (immobilization) for major surgery, acute medical diseases, active malignancy and contraceptive use. VTE episodes are most often the result of concomitant effect of 2 or more factors of diverse intensity.

Congenital thrombophilias are genetically determined hemostatic disorders which predispose to thromboembolic events. Testing for congenital thrombophilia aims to identify individuals at higher risk of VTE, with the purpose of reducing this risk and preventing complications, in particular - recurrence of thromboembolic events.

There are currently no sufficient high-quality data from clinical trials to support any specific diagnostic strategy. Expert opinions are inconsistent, and the guidance of scientific societies and expert groups vary. The recent years have witnessed a marked decrease in the number of indications for testing thrombophilia. This costly procedure is not recommended for unselected patients after the first VTE episode; abnormal results are unlikely to affect the management. Moreover, there is no safe and cost-effective method of long-term antithrombotic prophylaxis to be applied in most asymptomatic adults diagnosed with congenital thrombophilia. Awareness of the inherited predisposition to VTE may have psychological impact on the patient and his/her family but otherwise the benefits are not so obvious.

Based on the currently available data and expert opinions, testing for inherited predisposition to thromboembolic events (mostly VTE) involves search for 5 genetically-determined abnormalities:

— Factor V Leiden — mutation of the Factor V (F5) gene Leiden (NM_000130.4: c.1601G>A [p.Arg534Gln]);
— Prothrombin 20210A — mutation of the Prothrombin (F2) gene G20210A) (NM_000506.5:c. *97G>A)
— protein C deficiency (*PROC* gene mutations);
— protein S deficiency (*PROS1* gene mutations);
— antithrombin deficiency (*SERPINC1* gene mutations).

Thromboembolic events in a family history indicate the need to rule out dysfibrinogenemia (a separate document by the Hemostasis Group issued in 2019) and to consider performing factor IX and XI activity assays.

Extremely rare congenital thrombophilias involve plasminogen deficiency, increased levels of plasminogen activator inhibitor type 1 (PAI-1), and heparin II cofactor deficiency. Very few centers are prepared to perform such assays.

A higher risk for VTE is also associated with a blood type. The risk is higher for individuals with non-O blood type (> 70% of Polish citizens) which in partly due to higher factor VIII activity. Higher activity of factor VIII does not however affect the strategy for primary and secondary VTE prophylaxis.

In individuals suspected of thrombophilia it is not recommended to determine mutations/variants of the methylenetetrahydrofolate reductase (MTHFR) 677C>T gene: (NM_005957.5:c.1286A>C (p.Glu429Ala) (a separate document by the Hemostasis Group issued in 2019) or the genetic mutations/variants of PAI-1 4G> 5G: NM_000602.4:c.-820_-817G (4_5). In the European population these mutations/variants are quite common; in Poland they are detected in about 50% of the population. Confirmation of their presence in no way affects the management in any known clinical situation.

Table 1 presents the recommendations for inherited thrombophilia testing.

The Delphi technique was used to develop recommendations for thrombophilia testing. After 3 voting rounds, 100% agreement was reached regarding all recommendations included in the document. The recommended indications for testing thrombophilia are summarized in Table 2. Three phrases are used:

Table 1. Recommendations regarding the scope of thrombophilia testing

No.	Recommendation
1	We recommend performing the following screening tests for patients suspected of congenital thrombophilia: resistance to activated protein C, antithrombin activity (preferably with the thrombin method), protein C activity (preferably with a chromogenic substrate test), free protein S concentration (preferably with an immunoassay), and factor V Leiden and prothrombin G20210A mutations (preferably with PCR).
2	We suggest assessing fibrinogen level, factor VIII and factor XI activity, if no abnormalities are detected in screening tests for thrombophilia.
3	We suggest expanding the diagnostic panel by assays of rare types of antithrombin, protein C or protein S deficiency, including genetic tests, if no thrombophilia is detected in screening tests for congenital thrombophilia, in individuals suspected of inherited thrombophilia and family history of DVT and/or PE.
4	We do not recommend determining MTHFR 677C>T (c. 665 C>T) and 1298A>C (c. 1286A>C) gene polymorphisms, or the PAI-1 4G/5G genetic variants and 1299 H>R the factor V gene mutation in individuals suspected of congenital thrombophilia.

1. "we recommend" — the whole Group agrees that a strategy has more advantages than risks (according to the currently available evidence);
2. "we suggest" — research outcome and expert opinions regarding a given strategy are inconsistent so members of the Group have doubts concerning the approach in light of the available data. It is therefore recommended to apply a strategy/approach guided by the best interests of the patient;
3. "we do not recommend" — according to the available research data and/or expert opinions, the strategy/approach is of no clinical use.

Below we present a brief overview of the most important congenital thrombophilias and the related diagnostic and therapeutic issues.

Factor V Leiden

Characteristics

Factor V Leiden (MIM: 188055) results from a genetic mutation in the F5 gene NM_000130.4: c.1601G> A (p.; former nomenclature p.Arg506Gln) which leads to an amino acid change (Arg534Gln) in factor V protein and to activated protein C resistance (APCR). The reaction is 10 times slower than for normal factor Va Arg506, therefore factor Va Gln506 is partially resistant to APC. It is considered a mild risk factor for venous thrombosis.

Clinical features

Factor V Leiden is detected in 5% of the white population and is extremely rare in the black and yellow populations. On the evolutionary timescale it is placed some 20 000–30 000 years back, following separation of white and yellow races. The high prevalence of Factor V Leiden in Whites is most likely attributable to evolutionary advantages (lower risk of major bleeding eg. during childbirth).

Carriers of factor V Leiden most commonly present with DVT and superficial vein thrombosis (SVT). Relatively infrequent episodes of isolated PE as compared to deficiencies of the natural anticoagulants and the G20210A prothrombin mutation have been observed ("Factor V Leiden paradox"). Factor V Leiden is more frequently detected in individuals with leg ulcers in the course of chronic venous insufficiency (15–40% of patients). Such patients may also develop thrombosis of the cerebral, hepatic, portal and upper extremity veins. Approximately half of the population of factor V Leiden thrombophilia patients with DTV develop unprovoked VTE, typically after surgery, during pregnancy or hormonal contraception. The currently prevalent opinion is that for women with factor V Leiden, the incidence of pregnancy loss and other obstetric complications is no higher than for the general population.

In Europe, the percentage of patients after the first VTE episode and diagnosed factor V Leiden is estimated at 20–25% and approximates 40% for individuals with recurrent unprovoked incidents. The relative DVT and SVT risk in patients with heterozygous factor V Leiden is 4–8 and 4-fold higher, respectively. The risk of VTE in factor V Leiden carriers increases with age: in men ≥ 70 years of age it may be 6 times the baseline value. The annual risk of the disease in first-degree relatives of VTE patients who are carriers of factor V Leiden is estimated at about 0.45% (0.25% in the 15–30 age group and 1.1% in individuals > 60). Homozygous forms of factor V Leiden mutations have a 50–100 fold higher risk for VTE than the general population and it is estimated that more than 50% are likely to experience a clinically significant epi-

Table 2. Recommendations regarding indications for thrombophilia testing

No.	Recommendation
1	We recommend considering testing for congenital thrombophilia in individuals below 50 years with DVT and/or PE of unknown cause.
2	We recommend considering testing for congenital thrombophilia in individuals with DVT and/or PE in history, if the same disorders for unknown causes were reported in 1st degree relatives.
3	We recommend considering testing for congenital thrombophilia in individuals with recurrent DVT and/or PE for unknown cause, if the first episode occurred before the age of 50 years.
4	We recommend considering testing for congenital thrombophilia in individuals with a history of venous thrombosis of unknown cause at atypical sites (e.g. portal, abdominal, cerebral veins).
5	We recommend considering testing for congenital thrombophilia in women who developed DVT and/or PE during pregnancy and/or the puerperium.
6	We recommend considering testing for congenital thrombophilia in women with a history of DVT and/or PE during hormonal contraception or menopausal hormone therapy.
7	We recommend considering testing for congenital thrombophilia (with the exception of factor V Leiden and prothrombin G20210A mutation and after testing for antiphospholipid syndrome) in women with such obstetric complications in history as: spontaneous abortion for unknown causes, death of a morphologically normal foetus, preeclampsia, eclampsia, detached placenta; preferably after exclusion of foetal anatomical abnormalities, maternal endocrine/hormonal disorders, and chromosomal disorders in both parents.
8	We suggest testing for congenital thrombophilia in women with a positive family history of DVT and/or PE, prior to initiation of oral hormonal contraception with estrogens, hormone replacement therapy, or assisted reproduction.
9	We suggest testing for congenital thrombophilia in individuals below 50 years who experienced an arterial thromboembolic episode despite the absence of recognized cardiovascular risk factors.
10	We suggest testing for congenital thrombophilia in individuals who experienced an arterial thromboembolic episode after prior DVT and/or PE or have a positive family history of DVT and/or PE.
11	We suggest testing for congenital thrombophilia in the 1st degree relatives of patients with confirmed congenital thrombophilia, especially with antithrombin, protein C or protein S deficiency, if they are 18 years old or more.
12	We do not recommend performing tests for congenital thrombophilia in individuals with central retinal vein thrombosis, unless they have a positive personal or family history of DVT and/or PE for unknown reason, especially if it occurred at < 50 years of age.
13	We do not recommend performing tests for congenital thrombophilia or antiphospholipid syndrome in women diagnosed with primary infertility.
14	We do not recommend performing a routine testing for congenital thrombophilia in women planning OC or hormone replacement therapy who have a negative personal and family history of DVT and/or PE.
15	We do not recommend performing tests for congenital thrombophilia after the first DVT and/or PE episode related to major trauma or surgery, if the family history of DVT and/or PE is negative.

DVT — deep vein thrombosis; PE — pulmonary embolism

sode during their lifetime. The detection of factor V Leiden does not affect the patient survival.

Myocardial infarction associated with arterial thrombosis has more frequently been reported in factor V Leiden carriers, especially < 50 years of age, including patients with classic cardiovascular risk factors such as obesity, smoking, hypertension or diabetes. Data on the correlation of this mutation with myocardial infarction are however inconclusive. Large meta-analyses and prospective observational studies do not relate this mutation to ischemic stroke, even in the elderly individuals.

Small observational studies demonstrate that factor V Leiden in children is associated with cerebral infarction or venous thrombosis as well as central venous catheter (CVC) thrombosis.

Laboratory tests

Activated protein C resistance (APCR), the typical trait of factor V Leiden carriers, is measured with coagulation tests and genetic testing. Plasma

clotting tests are based on the relative prolongation of activated partial thromboplastin time (APTT) or other screening coagulation tests after plasma exposure to activated protein C (APC). Individuals with APCR have lower aPTT prolongation than normal (typically < 1.8). The other coagulometric tests make use of factor V deficient-plasma and provide information which confirms APCR in some patients with lupus anticoagulant, some pregnant women and in patients with inflammatory diseases. The coagulometric test for APCR is sensitive and specific. Abnormally low APCR results may be associated with venous thrombosis, regardless of the presence or absence of factor V Leiden mutations (other genetic causes, e.g. factor V Cambridge or factor V Hong Kong are to be considered), as well as with ischemic stroke. Some experts believe that the classic APCR test based on aPTT measurement in the patient's plasma provides relevant information and should be supplementary to the factor V-deficient plasma assay. Tissue factor-dependent APCR assay detects abnormalities in other essential components of the protein C pathway (e.g. thrombomodulin), although the significance of the results is unclear. The presence of platelets, their microparticles or antibodies to APC in plasma tested for APCR with aPTT tests may lead to false-positive results, so genetic testing is the only method to confirm factor V Leiden mutation. Oral anticoagulants may interfere with the APCR measurement and impede the detection of this abnormality.

Factor V Leiden is mostly detected with the polymerase chain reaction (PCR), the products of which are subjected to analysis of restriction fragment length polymorphism.

Coagulation tests are often used for plasma screening, and positive results are confirmed by PCR, which helps to differentiate between heterozygous and homozygous forms of factor V Leiden mutation. Factor V Leiden "pseudohomozygosity" (heterozygous Factor V Leiden mutation associated with partial type I V deficiency — 50%) is very low on APCR values in the plasma assay but appears as heterozygous in the PCR test.

Ultra-rare genetically determined factor V deficiencies or dysfunctions have also been described. They lead to familial thrombophilia and are diagnosed in high-tech coagulation and genetic laboratories. These mutations may also coexist with factor V Leiden mutation.

Prothrombin 20210A — mutation of the of the Prothrombin (F2) gene G20210A

Characteristics

The naturally occurring G→A transition at position 20210, (NM_000506.5: c.*97G> A) in the 3'UTR region of prothrombin gene (F2) enhances the translation and stability of prothrombin mRNA, which results in increased synthesis and release of prothrombin from hepatocytes. High concentration of prothrombin in plasma (around 130% of the normal range for heterozygotes) may be directly related to higher risk of DVT. At least three mechanisms are most likely responsible for higher thrombosis risk: increased thrombin production, promotion of thrombin-catalysed reactions, e.g. activation of factor V and XIII, and fibrinolysis impairment due to increased activation of the thrombin activable fibrinolysis inhibitor (TAFI).

Clinical features

Prothrombin gene mutation is mainly diagnosed in Whites: in Northern Europe it is detected rarely (1.5–2% of the general population) and occurs most frequently (approx. 5%) in Southern Europe and the Middle East. Individuals with the prothrombin G20210A mutation are at relatively higher risk of DVT and SVT (app. 2–5.5 fold and 4 fold, respectively). The mutation is also detected in patients with venous thrombosis at unusual sites; particularly hepatic, portal and cerebral veins. The reported risk for cerebral vein thrombosis was 10-fold higher. At still higher risk of VTE (100-fold higher) are carriers of this mutation who take oral contraceptives. Prothrombin gene G20210A mutation does not contribute to shortening of the survival.

Data regarding the relation of this mutation with ischemic stroke (particularly in women < 40 years) and myocardial infarction are inconclusive. A meta-analysis of patients with documented arterial events demonstrated that prothrombin 20210A mutation are a risk factor for such events, though mostly for individuals < 55 years of age.

Laboratory tests

Identification of mutations in the 3'- untranslated region of the prothrombin gene requires DNA analysis following PCR amplification of the appropriate region. Although prothrombin concen-

tration in plasma increases by 30% on average, in some patients it is still within the reference range and so the prothrombin activity or antigen assays are not sensitive enough to detect the mutation. Direct oral anticoagulants (DOACs), vitamin K antagonists (VKA), and other anticoagulants do not interfere with genetic testing.

Protein C deficiency (PROC gene mutations)

Characteristics

Protein C is one of the vitamin K-dependent glycoproteins synthesized in the liver. It circulates in plasma as a serine protease zymogen (APC). The endothelial cell protein C receptor (EPCR) accelerates protein C activation by the thrombin-thrombomodulin complex. APC is a strong enzyme that causes irreversible inactivation of factors Va and VIIIa with protein S as cofactor. Reduced levels of protein C zymogen inhibit thrombin production which leads to hypercoagulability. APC also inhibits the process of inflammation and apoptosis.

Type I protein C deficiency is defined as a simultaneous reduction of plasma antigen and anticoagulant activity, whereas in type II deficiency, the level of antigen in plasma is normal, but the circulating dysfunctional protein C molecules contribute to a low anticoagulant activity.

Clinical features

Heterozygous protein C deficiency is inherited in an autosomal dominant manner (MIM: 176 860) and occurs in 0.2–0.4% of healthy individuals and approximately 4–5% of patients with confirmed DVT. Protein C deficiency is responsible for a 6–8 fold increase of VTE risk. Protein C deficient individuals experience the first thrombotic event at the age of 45 (on average). The survival time of individuals with heterozygous protein C deficiency does not differ from that in the general population.

Clinical manifestations range from asymptomatic to massive venous thrombosis in early age. In adults, there is weak correlation between clinical symptoms and protein C activity and level. The most common clinical manifestations of protein C deficiency are DVT and SVT. The disease is estimated to develop before the age of 45 in 50% of individuals with heterozygous form of this mutation who come from families with a positive history of VTE: in half the cases, the disease will not be preceded by any other identifiable cause. Meta-analyses suggest a higher incidence of coronary events in protein C deficient individuals, including episodes of ischemic stroke.

Protein C deficiency due to biallelic PROC mutations is inherited in a recessive manner (MIM: 612,304). Protein C concentration < 1% of normal presents as purpura fulminans in the neonatal period and as massive venous thrombosis in affected infants. In protein C deficient patients who are on warfarin or acenocoumarol therapy, skin necrosis is rarely reported (mostly localized on the trunk) and usually observed in individuals affected with obesity. VKA in obese individuals rapidly reduces protein C activity to very low levels due to the short half-life of protein C (approx. 8 hours). The half-life of prothrombin, factor IX and factor X is much longer than that of protein C and the half-life of factor VII is comparable to that of protein C therefore, at the beginning of VKA therapy, a transient state of hypercoagulability may occur with skin necrosis as the symptom. VKA must then be discontinued and replaced by a heparin.

Laboratory tests

Protein C activity is usually evaluated with chromogenic substrate tests but also with coagulation assays measuring aPTT or factor Xa activity. Another method is based on the highly specific protein C activator Protac from the venom of the Agkistrodon contortrix snake. Immunoassays are used to distinguish between type I deficiency (lower antigen concentration and activity) and type II deficiency (normal antigen concentration and lower activity). The protein C concentration increases with age (4% per decade).

Protein C deficiency is to be suspected at activity < 70% of normal (no KA, no vitamin K deficiency or advanced liver disease) but the measurement should always be repeated after at least a month. If the result is abnormal, it is suggested to measure protein C activity in first-degree relatives. DOACs do not interfere with the measurement of protein C with the chromogenic method, but they do interfere with test results of coagulometric tests, as do heparins.

A particular challenge is to diagnose hereditary protein C deficiency (just like protein S deficiency) in patients on VKA therapy. Protein C antigen levels are comparable to those of other vitamin K-dependent coagulation factor antigens, providing accurate control ranges are established for the ratio of protein C to 2 other vitamin K-dependent factors. In most cases, a reliable diagnosis can be reached at least 2 weeks after discontinuation of warfarin or acenocoumarol (or a temporary switch

to low molecular weight heparin, LMWH). Warfarin should not be re-initiated before laboratory results become available so as to minimize the risk of warfarin-induced skin necrosis in patients who may be diagnosed as protein C deficient. If purpura fulminans is diagnosed in a neonate, the parents should also be investigated. Detection of PROC gene mutations confirms the congenital nature of the deficiency. More than 500 such mutations have been described so far.

Protein S deficiency (PROS1 gene mutations)

Characteristics

Protein S is one of the vitamin K-dependent glycoproteins synthesized in the liver. It is an APC cofactor for inactivation of coagulation factors Va and VIIIa. Typically, in human plasma, protein S circulates approximately 60% in complex with C4b-binding protein (C4BP) and about 40% as free PS (FPS) which acts as a cofactor for APC. Lower levels of free protein S may impair the inhibition of thrombin production and promote hypercoagulability.

PROS1 mutations resulting in quantitative or qualitative protein S deficiency may be inherited in an autosomal dominant (MIM: 612336) or recessive (MIM: 614514) manner. Type I protein S deficiency presents with low levels of total protein S (TPS) and free protein S (FPS) and reduced plasma levels of protein S activity. Type II, associated with dysfunctional molecules in circulation, is identified at normal levels of free protein S antigens in plasma and reduced protein S activity. Type III deficiency presents reduced levels of free protein S, at lower to normal levels of total protein S. More than 450 different *PROS1* gene mutations have been reported as the cause of protein S deficiency.

Clinical features

Protein S deficiency is reported in 2–3% of unselected VTE patients. It is more frequent in individuals < 50 years and in patients with VTE in family history. Despite diagnostic difficulties, patients with protein S deficiency are estimated to be at 1.5–10 fold higher risk of VTE which correlates well with the concentration of the protein in blood. DVT and PE are the most common forms of protein S deficiency-related thrombosis, Relatively common are SVT events and thrombosis at unusual sites. Half of the episodes are unprovoked. Arterial thrombosis (ischemic stroke in particular) has been reported in protein S-deficient individuals especially with cardiovascular risk factors (e.g. smoking). Warfarin-related skin necrosis, mostly on the torso/trunk, has been observed in obese patients with very low levels of protein S.

In asymptomatic relatives of protein S deficient patients, VTE episodes occurred at an annual frequency of 0.7–2.2%. and 50% were cases with major risk factors for thrombosis (surgery or hormonal contraception).

Laboratory tests

Screening tests for protein S deficiency currently rely on detection of free protein S antigen (as a parameter). Reference ranges vary by gender and age. Free protein S levels in plasma are determined with free protein S-specific monoclonal antibodies. The available protein S functional tests measure the anticoagulant activity of the APC cofactor with protein S deficient plasma used as a substrate. Evaluation of total and free protein S activity as well as total protein S antigen level is enough to determine the type of deficiency. Type I and III may be phenotypic mutations of the same disease, as for different carriers of the same *PROS1* gene mutation within the same family, the laboratory outcome may indicate both types. Type II deficiency occurs rarely (in approx. 5% of all S protein-deficient individuals) and is diagnosed at normal free protein S antigen level and reduced protein S activity. In healthy individuals there is a strong correlation between free S protein antigen level and anticoagulant activity. The lower limit of normal for free S protein level is different for women and men (55–60% vs 65–70%, respectively), though manufacturers of diagnostic kits sometimes recommend identical reference ranges.

Protein S is extremely sensitive to phase-dependent hormonal changes during the menstrual cycle. Mild protein S deficiency is often acquired. Oral contraceptives and hormone replacement therapy contribute to lower protein S level in plasma. Lower levels of free protein S are typical of pregnancy (reduced to 30%). They are also observed during VKA therapy, in disseminated intravascular coagulation, liver disease, nephrotic syndrome, inflammation and recent thrombosis. Protein S deficiency is also reported in autoimmune diseases or during viral infections.

DOACs do not interfere with laboratory assays for free protein S levels but they do interfere with haemostasis tests.

Lower protein S levels should always be re-tested (confirmed) after a minimum of one month;

warfarin or acenocoumarol therapy should be switched to LMWH and after 7–14 days, the test sample should be collected immediately prior to the coming LMWH injection. It is also advisable to screen family members. Congenital protein S deficiency can be confirmed with PCR tests, although the diagnosis is not easy and often requires new-generation sequencing and expert interpretation of the results.

Antithrombin deficiency (SERPINC1 gene mutations)

Characteristics

Antithrombin is a plasma protease inhibitor that inactivates thrombin and factors Xa, IXa and XIa in the intrinsic and common coagulation pathways by binding to the active site of these enzymes. The irreversible formation of antithrombin-coagulation factor (1:1) complexes is enhanced by heparin- or heparin sulfate present on the endothelium. Antithrombin deficiency is a blood disorder that impairs the regulation of physiological blood coagulation and leads to hypercoagulability. Antithrombin accounts for 80% of the antithrombin activity in plasma.

Genetically determined antithrombin deficiency (MIM: 613118, mutations in the *SERPINC1* gene) is inherited in an autosomal dominant or recessive manner. There are two main types of antithrombin deficiency. Type I is characterized by lower antigen concentration and activity regardless of the absence or presence of heparin. Type II is determined at normal antigen levels accompanied by defects that act on either the active site (forming a complex with the target enzyme's active site) or the heparin binding site (activation of antithrombin by heparin). Type II antithrombin deficiency is further classified into: type IIa with mutations affecting the reactive site, type IIb which includes mutations in the heparin binding site, and type IIc which involves pleiotropic mutations. To date, approximately 500 mutations associated with antithrombin deficiency have been described.

Severe antithrombin deficiency (< 5% of the normal) is rare, usually diagnosed as type IIb deficiency, and is associated with venous and arterial thrombosis already in early life.

Type I antithrombin deficiency occurs in 0.02% of the general population, and type II in 0.2% of the screened individuals.

Clinical features

Antithrombin deficiency is reported in approximately 1–3% of unselected patients aged < 70 with first venous thrombosis in documentation. The incidence is higher (> 5%) for patients with VTE in family history, especially if the events occurred before the age of 40. In patients with antithrombin deficiency, the overall risk of VTE (mostly venous thrombosis presenting with or without PE) is 10–20 fold higher than in the general population. There is no evidence of different severity of VTE symptoms between patients with type I heterozygous defects and patients with type II mutations at the thrombin binding site. Patients with type II heparin binding site mutations are at lower risk of thrombosis than those with other forms of heparin deficiency, yet biallelic mutations are associated with a high risk of VTE.

The most common symptom of antithrombin deficiency is DVT of the lower extremities that occurs at an early age. Antithrombin deficiency has been reported in patients with thrombosis at atypical sites such as the mesenteric, hepatic veins or cerebral venous sinuses. Arterial thrombosis is rare in patients with this deficiency (approx. 1%). Almost 70% of patients have their first thrombotic event before the age of 35, and 85% before the age of 50. Antithrombin deficiency increases the risk of obstetric failures, including miscarriages and fetal growth impairment. Cerebral ischemic stroke is reported in 1–7% of patients with confirmed antithrombin deficiency. Complete antithrombin deficiency is considered lethal.

Some patients with antithrombin deficiency were reported resistant to anticoagulant activity of heparin. Acute vascular thrombosis as well as several-day heparin therapy may sometimes reduce antithrombin levels to as low as ≤ 50% of normal (usually up to 65–80%). This may lead to misdiagnosis of congenital antithrombin deficiency. Other acquired conditions associated with lower antithrombin levels include liver disease, disseminated intravascular coagulation, nephrotic syndrome, asparaginase therapy, and preeclampsia.

Prospective studies of asymptomatic relatives of patients with antithrombin deficiency report the annual incidence of venous thrombosis to be 4%. Antithrombin deficiency is considered to be the most severe form of congenital thrombophilia.

It has also been demonstrated that the risk of VTE is higher for antithrombin activity within the range of 70–80% than 100%.

Laboratory tests

Nowadays, most laboratories rely on factor Xa or bovine thrombin to assess antithrombin activity and to avoid the inhibitory effect of heparin cofactor II on human thrombin. Experts of the International Society on Thrombosis and Haemostasis recommend the thrombin test as the first-choice assay for determining this deficiency. Screening tests for antithrombin deficiency rely on chromogenic substrate assay to evaluate the activity of this protein. Tests are performed in the presence of heparin as the defects may involve the heparin-binding site. If results of initial/preliminary tests are abnormal, the abnormality is identified by assessing the ability of the inhibitor to neutralize thrombin in the absence of heparin (progressive antithrombin activity). This test however is not easily accessible. In healthy individuals the plasma antithrombin reference range is usually 80–116%. Antithrombin antigen levels measured with eg. nephelometry or ELISA, help to differentiate between types I and II. Cross-immunoelectrophoresis with antithrombin antibody in the presence and absence of heparin can identify defects in the heparin binding site. In patients with type I and type II deficiency involving the thrombin binding site, antithrombin activity was within the 40–60% range. Antithrombin activity values within 60–80% range may result from other type II deficiencies, but are mostly caused by acquired antithrombin deficiency. An infrequent reason of lower antithrombin activity are disorders of glycosylation of the protein, which is mostly congenital, but also described in alcohol abusers.

The abnormal result indicates that the antithrombin activity assay should be repeated after a month and the patient's family should be included in the testing.

DOACs may interfere with the antithrombin activity assay: in patients on dabigatran therapy the thrombin-related results are overestimated and factor Xa tests are reliable. In patients on rivaroxaban or apixaban therapy, it is the other way round. The effect however, is slight and may be significant for type II antithrombin deficiency. For the test to be reliable, it is recommended to make a min. 24-hour interval since the last DOAC dose (providing renal function is normal).

Genetic testing confirms the diagnosis of hereditary antithrombin deficiency. Such tests, however, are not easily available in Poland.

Indications for diagnostic procedures

Specific recommendations are presented in Table 2, but some issues require additional comments.

Before pregnancy or the initiation of oral contraceptives, it is recommended to test for this specific deficiency the asymptomatic women who are 1st degree relatives of thrombophilia patients. If the proband is protein C, protein S or antithrombin-deficient, it is recommended to test 1st degree relatives.

It remains controversial whether to subject the closest relatives of thrombophilia patients to Factor V Leiden and the prothrombin G20210A mutation tests. Advocates of testing argue that there is a 50% chance of finding the same genotype in the closest relatives and a 2.5% chance of finding a homozygous variant of the mutation or double heterozygosity of factor V Leiden and prothrombin 20210A in a sibling, as one of the parents is an obligatory carrier and for the other, the risk for either of the mutations is estimated at 1:10 (in Whites). Although heterozygotes for these 2 common polymorphisms have a < 0.7% annual incidence of thrombosis, the cumulative incidence over 30–40 years may be significant and might be minimized/reduced with appropriate prophylaxis. For homozygotes and double heterozygotes the risk is markedly higher. Opponents of such assays underline the arguments of anxiety related to stigmatization, problems with health insurance (particularly in the US) and the high costs. It is estimated that 95% of factor V Leiden or a prothrombin mutation carriers will never experience a VTE event in their lifetime.

Most experts highlight the fact that before ordering thrombophilia testing, the patient should be informed of the pros and cons and be involved in shared decision making. A strong argument in favour of such a decision is a family history of VTE, especially with documented fatalities or early-age episodes.

The predominant opinion is that testing for thrombophilia is not required if distal DVT is the aftermath of trauma or surgery, the more so that the annual recurrence rate in this group of patients is only 1.5%. Likewise, tests for thrombophilia are not recommended if the event was associated with active malignant disease or device implantation (e.g., cardiac pacemaker).

Controversial are also tests for thrombophilia in individuals > 50 years with unprovoked VTE episodes or events associated with the use of selective estrogen receptor modulators.

For individuals < 50 years of age who experienced arterial thrombosis which occurred despite the absence of risk factors for atherosclerosis, evidence of atherosclerosis or any other diseases that might have been responsible for the event (e.g. myocardial infarction, stroke or peripheral arterial embolism), a personalized approach to thrombophilia testing should be considered. It is not clear whether detection of inherited thrombophilia affects the treatment strategy and prognosis if there are no VTE events in the history of a given patient or his/her family.

Prophylaxis of venous thromboembolism (VTE) in patients with inherited thrombophilia

Recommendations referring to the prophylaxis of thrombophilia patients are presented in Table 3.

Patients with VTE are recommended to lose weight (if required), to avoid immobilization, dehydration, injury, smoking and estrogens (women). Once congenital thrombophilia is diagnosed, the patient must be informed of the fact, also about the risk of thrombosis (during surgery, long air travel, pregnancy, and trauma), the bleeding risk during anticoagulation therapy, implications for the family and recommendations for thromboprophylaxis if VTE risk is high. Close surveillance of VTE in congenital thrombophilia patients is recommended on an outpatient basis in order to evaluate the effects of preventive measures and to keep the patient informed about any new available therapeutic options, including the potential benefits of DOACs. Large varicose veins are a risk factor for SVT and are indication for surgery or other therapeutic procedures with heparin prophylaxis.

Prophylactic doses of LMWH are recommended following surgery, lower limb fracture and other major injuries as well as during pregnancy (see Table 3 — recommendation 1 and 2) and for 6 weeks after delivery, during periods of immobilization and before long flights (> 4 hours). For antithrombin deficient women (and VTE), we recommend considering the use of antithrombin concentrate during surgery, pregnancy and the perinatal period.

Prophylaxis with oral anticoagulants is usually unwarranted for individuals with no history of thromboembolitic events and thrombophilia diagnosed during family counselling or in other circumstances; the risk of bleeding may be much higher than that related to vascular thrombosis. However, in situations of higher VTE risk, thromboprophylaxis is suggested (mostly with LMWH) as it may contribute to minimizing the incidence of VTE.

In the case of women with natural anticoagulant deficiency and obstetric failures (eg. fetal loss) in history but no VTE, LMWH therapy is recommended during pregnancy. Small observational studies demonstrate that this may improve prognosis. In the case of thrombophilia women with ≥ 2 miscarriages, stillbirth or preeclampsia in history, experts suggest LMWH prophylaxis throughout pregnancy and 6 weeks postpartum. Decisions should be individualized and the preferences of the patients taken into account.

DOACs should not be used during pregnancy.

Hormonal contraception

In women with no thromboembolic event in medical history (such event is contraindication to the use of estrogens), congenital thrombophilia is not *per se* a contraindication to oral hormonal contraception or menopausal hormone therapy. The therapy should however be administered with great caution and under supervision. Thromboembolic events in the family, especially at younger age, are an argument against the use of oral contraceptive in asymptomatic women, particularly those with natural anticoagulant deficiencies or with homozygous prothrombotic mutations. In such cases intrauterine systems are preferrable. In European women-carriers of factor V Leiden, the annual risk of fatal PE is estimated at 14 cases per 100,000 vs 3 cases per 100,000 of women with no such mutation. The risk factors for VTE are: smoking, obesity and higher thrombotic risk (e.g. following injury). Before making the decision about hormonal therapy, the patient should be informed about the potential risk of vascular thrombosis and ways of minimizing the risk. The preferences of the patient should also be considered. Plasma D-dimer concentration occasionally measured after 1 month of therapy, has little prognostic value as regards the pro-thrombotic effect of hormonal contraceptives.

Management

For all patients with congenital thrombophilia, as well as individuals with no congenital thrombophilia but 2 documented unprovoked VTE events, there are strong indications for long-term anticoagulation. An exception here are patients with recurrent major bleeds for no determined reason and unresponsive to therapy. According to recent recommendations, after the first VTE event most patients with congenital thrombophilia should be treated just like the whole population of patients with unprovoked VTE or VTE associated with weak risk factor. The majority of patients with

Table 3. Recommendations regarding prophylaxis of thrombophilia patients

No.	Recommendation
1	We recommend thromboprophylaxis with LMWH during pregnancy and puerperium in women with documented antithrombin, protein C or protein S deficiency or with homozygous factor V Leiden or prothrombin 20210A mutation, or combined thrombophilias.
2	We recommend that pharmacological thromboprophylaxis during pregnancy and puerperium should be considered in women with heterozygous for factor V Leiden or prothrombin 20210A mutation and with no history of thrombotic episodes especially in the presence of additional factors that increase the risk of DVT and/or PE.
3	We suggest considering LMWH prophylaxis in individuals with congenital thrombophilia and no documented thrombotic episodes who are not on anticoagulation therapy but at higher risk of DVT and/or PE.

LMWH — low-molecular-weight heparin; DVT — deep vein thrombosis; PE — pulmonary embolism

Table 4. Dosing of DOACs in patients with VTE and congenital thrombophilia

Anticoagulant	Initial therapy	Treatment (up to 3-6 months)	Secondary prevention (after 3-6 months)
Apixaban	2 × 10 mg/d for 7 days	2 × 5 mg/d, no dose reduction	2 × 2.5 mg/d or 2 × 5 mg/d at high risk of recurrence[a]
Dabigatran	2 × 150 mg/d after ≥ 5 days of unfractionated heparin or LMWH	2 × 150 mg/d[b]	2 × 150 mg/d (no specific criteria for dose reduction)[c]
Rivaroxaban	2 × 15 mg/d for 21 days	1 × 20 mg/d, no dose reduction[d]	1 × 10 mg/d or 1 × 20 mg/d[a]

[a]suggested doses for obese patients, with recurrent thromboembolic events, antithrombin deficiency, ≥ 2 congenital thrombophilias, etc.
[b]Summary of Product Characteristics (SPC): 2 × 110 mg/d for individuals ≥ 80, those taking verapamil, and are at increased risk of gastrointestinal bleeding
[c]SPC: 2 × 110 mg/d for individuals ≥ 80 and those taking verapamil
[d]SPC: 15 mg/d if the risk of bleeding exceeds the risk of VTE recurrence.

congenital thrombophilia should be on long term DOAC therapy (in Poland: apixaban, dabigatran and rivaroxaban) which is currently the preferred therapeutic option mainly due to lower risk of major bleeding (Table 4).

During secondary prevention of most patients with congenital thrombophilia, i.e. carriers of factor V Leiden or the prothrombin G20210A mutation (heterozygous variants), the dose of apixaban or rivaroxaban may be reduced. After 6 months since VTE episode, full-dose DOACs should be considered if VTE recurred during anticoagulant therapy as well as in patients with intermediate to high risk PE, with pulmonary thromboemebolic hypertension, and other concomitant risk factors (e.g. severe obesity). According to expert opinion, long-term anticoagulation for patients with VTE and antithrombin deficiency who are treated with DOACs as well as for individuals with homozygous form of factor V Leiden or prothrombin G20210A mutations (also others at high risk of VTE recurrence), should be based on standard-dose DOACs, with reduced-dose DOACs if bleeding events occur or the patient prefers such a change. An alternative approach are VKAs (in Poland: warfarin or acenocoumarol) with a target INR of 2–3 (due to low treatment cost).

Recurrent VTE during anticoagulant therapy require heparin doses for a period of ≥ 4 weeks (a 20% higher dose of LMWH if the incident occurred during drug administration), and then a switch to oral drugs, usually of the DOAC group (typically at full dose) or VKAs. the latter option is preferred for patients with additional risk factors for VTE recurrence, who have easy access to INR tests which facilitates long term treatment.

Long term (> 3 months) anticoagulation is not recommended as routine management in patients with congenital thrombophilia after the first VTE episode associated with major trauma or surgery. It may, however, be considered if the patient agrees to secondary prophylaxis and accepts the annual bleeding risk of 0.5–2%. The strategy is suggested for patients with antithrombin deficiency, individuals with homozygous form of factor V Leiden or prothrombin G20210A mutation, particularly after PE of intermediate-high risk PE.

Indefinite anticoagulation in post-VTE patients with congenital thrombophilia must be considered on the individual basis and potential complications of this strategy should undergo periodic evaluation.

Current reports do not demonstrate a relation/correlation between congenital thrombophilia and lower efficacy of DOACs after an unprovoked VTE event. The risk of VTE recurrence does increase

however, with irregular use of anticoagulants that have a half-life of approximately 12 hs. The patient therefore should be informed (both orally and in a written form) about the relevance of the regular intake of anticoagulants, the principles of drug discontinuation prior to minor invasive procedures and in the event of minor or moderate bleeding complications.

Long-term anticoagulation is not to be initiated in asymptomatic individuals with congenital thrombophilia diagnosed incidentally. Thromboprophylaxis with standard LMWH doses should be considered at higher VTE risk (e.g. major surgery, injury and pregnancy [see Table 3 — recommendations 1 and 2]), especially in patients with confirmed deficiency of natural anticoagulants.

The current publication should be considered as the expert opinion. The presented guidelines are to be considered as a support in everyday clinical practice, and they require a regular update based on the results of ongoing research.

Conflict of interest

Andrzej Mital — participated in clinical trials and received lecture honoraria from Pfizer and Bayer
Anetta Undas — received lecture honoraria from Pfizer, Bayer and Boehringer Ingelheim
Anna Klukowska — no conflict
Jacek Musiał — no conflict
Jacek Treliński — no conflict
Jerzy Windyga — participated in clinical trials and received lecture honoraria from AlfaSigma, Bayer, Pfizer, and Sanofi
Joanna Zdziarska — participated in clinical trials and/or received lecture and consultation honoraria from Sanofi
Krzysztof Chojnowski — participated in clinical trials and received lecture honoraria from Pfizer and Sanofi
Magdalena Łętowska — no conflict
Maria Podolak-Dawidziak — no conflict
Paweł Łaguna — no conflict
Tomasz Urasiński — participated in clinical trials and received lecture and consultation honoraria from Sanofi
Wojciech Młynarski — no conflict

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