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Inherited thrombophilia and venous thromboembolism: testing rules in clinical practice

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Testing for inherited thrombophilia in patients with venous thromboembolism is one of the most common genetic testing options prescribed by clinicians. Despite the large evidence base for the relationship of hereditary hemostasis disorders with the risk of venous thrombosis, most patients should not be tested. Performing tests in the acute phase of thrombosis or during anticoagulant therapy leads to erroneous results. The choice of anticoagulant therapy regimen and its duration are not specified by the presence of hereditary thrombophilia. The test results can be useful for increasing medication adherence of patient, determining the cause of thrombosis, especially at a young age or in atypical localization.

Key words: venous thromboembolism, inherited thrombophilia, anticoagulants.

Relationships and Activities: none.

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Venous thromboembolism (VTE) include deep vein thrombosis (DVT) and pulmonary embolism (PE). Blood clotting disorders (hereditary and acquired) play one of the leading roles in the pathogenesis of VTE. VTE is a multi-etiological disease and inherited hemostasis defects are only one of the links in pathogenesis. The fact that a person has a genetic susceptibility does not mean that a thrombosis will develop. Testing for heritable thrombophilia (HT) is the most commonly prescribed genetic testing by clinicians. It is easy to prescribe a test for HT, but the problem is in the further management of a patient. The stance on testing is most clearly articulated in the guidelines by Stevens SM, et al. (2016) — testing for HT should not be performed in most situations, and when performed, it should be used in a highly selective manner, and only in circumstances where the information obtained will influence a decision important to the patient [1]. Further, we will discuss what types of hereditary hemostasis disorders are advised to search in patients with VTE, using what methods and what to do with the result obtained.

Thrombophilia classification

Thrombophilia is a disorder of hemostasis and hemorheology, characterized by an increased predisposition to thrombosis or intravascular coagulation. The most common, but not all, thrombophilias are summarized in Table 1. Thrombophilia classification is based on selecting hereditary and acquired risk factors (RF) for thrombosis. This division is rather arbitrary since many thrombophilic conditions can be of a dual nature. For example, the deficiencies of natural anticoagulants — antithrombin (AT), proteins C and S (PC and PS), may have a genetic cause or may be a consequence of liver failure, sepsis, hemodialysis, chemotherapy, and many other diseases. Genetic disorders of hemostasis are defined as HT.

Among HTs, the most studied are AT deficiency, PC and PS deficiencies, factor V Leiden (FVL) mutation, and prothrombin G20210A mutation.

Rules of testing for HT

Foreign clinical guidelines recommend identifying only above HTs [1, 2]. In the Russian guidelines on VTE [3], in addition to above ones, significant HT include an elevated factor VIII levels and hyperhomocysteinemia. At the same time, experts from the American College of Medical Genetics and Genomics do not recommend the determination of polymorphisms of folate metabolism-related genes, since large meta-analyses do not confirm its association with the risk of venous and arterial thrombosis [4].

Table 2 summarizes information on diagnostic tests for HT. Polymerase chain reaction (PCR) is used to detect the FVL and the prothrombin gene 20210A mutations. Genetic testing can be performed at any time, regardless of the intake of anticoagulants, the presence of acute thrombosis and various comorbidities. Coagulation testing for activated PC resistance can also be used to identify the FVL. This test can give erroneous results with anticoa-

Table 1

Thrombophilia classification

Hereditary	Hereditary or acquired	Acquired
Factor V Leiden mutation Prothrombin G20210A mutation Factor IX Padua JAK2 V617F mutation MTHFR A1298C genetic polymorphism Fibrinogen gamma C10034T polymorphism FXIII Leu34Val polymorphism Gene polymorphisms — endothelial cell PC receptor — protein Z — protein Z-dependent protease inhibitor — thrombomodulin — lipoprotein(a) — ADAMTS13 — Calreticulin — TAFI — TFPI — ACE Blood group (not 0)	Antithrombin deficiency Protein C deficiency Protein S deficiency Increased activity of factors V, VIII, IX, X, XI, PAI-1, TAFI Dysfibrinogenemia Hyperhomocysteinemia Plasminogen deficiency TFPI deficiency Von Willebrand factor level Increased level of platelet-derived microvesicles Increased level of endothelial-derived microvesicles Increased level of microvesicles carrying tissue factor or annexin V	Antiphospholipid syndrome Myeloproliferative disorders Paroxysmal nocturnal hemoglobinuria Cancer Nephrotic syndrome Inflammatory bowel disease Behçet's disease Systemic lupus erythematosus Congestive heart failure Respiratory failure Pregnancy Obesity Age Prolonged immobilization Intake of estrogens, GCS Chemotherapy

Abbreviations: ADAMTS13 — a disintegrin and metalloprotease with thrombospondin type 1 motif 13, JAK 2 — Janus kinase 2, ACE — angiotensin-converting enzyme, TAFI — thrombin-activatable fibrinolysis inhibitor, PAI-1 — plasminogen activator inhibitor, TFPI — tissue factor pathway inhibitor, MTHFR — methylenetetrahydrofolate reductase, PC — protein C.

Table 2

Diagnostics of hereditary thrombophilia

Thrombophilia type	Method	Clinical assessment (yes/no)					
		Acute thrombosis (3 months)	Pregnancy	UFH	LMWH	Warfarin	DOAC
AT (Ag)	Immunological	No	No	No	No	No	No
AT (act.)	Coagulation	No	No	No	No	No	No
PC (Ag)	Immunological	?	?	Yes	Yes	No	Yes
PC (act.)	Coagulation	?	?	Yes	Yes	No	No
PS (Ag)	Immunological	No	?	Yes	Yes	No	Yes
PS (act.)	Coagulation	No	?	Yes	Yes	No	No
FVL	PCR	Yes	Yes	Yes	Yes	Yes	Yes
Prothrombin G20210A mutation	PCR	Yes	Yes	Yes	Yes	Yes	Yes

Abbreviations: Ag — antigen, act. — activity, AT — antithrombin, PC — protein C, PS — protein S, PCR — polymerase chain reaction, LMWH — low molecular weight heparin, UFH — unfractionated heparin, DOAC — direct oral anticoagulants.

gulants, acute thrombosis, pregnancy, and patients with antiphospholipid syndrome (APS).

Due to a large number of described variants of the PC, PS, and AT genes (>200 for each), the use of PCR is impossible. Special microarray-based diagnostic tests are created, but so far their use is limited. In routine practice, immunological (antigen level) and/or coagulation testing using the chromogenic substrate is performed to diagnose the deficiency of natural anticoagulants. In the acute phase of thrombosis, there may be significant deviations in the levels of PC, PS, and AT. Testing in this period is not recommended, since the adequate interpretation of the results is impossible. The testing results are significantly influenced by anticoagulant therapy. With warfarin therapy, it is inappropriate to perform both coagulation and immunological tests (PC and PS are vitamin-K-dependent factors); heparin therapy significantly affects the level of antithrombin. Administration of direct oral anticoagulants (DOACs) can distort the results of coagulation testing (Table 2). As a rule, all patients after an episode of VTE receive anticoagulants for at least 3 months. To reduce the number of laboratory errors, testing for natural anticoagulant deficiency is not performed during this period.

Due to the limitations of diagnostic tests, a two-stage approach for testing patients with thrombosis is reasonable [1]. First, tests for thrombophilia that can be reliably done on anticoagulation (FVL, prothrombin G20210A mutation, cardiolipin, and beta-2 Glycoprotein-I antibodies) are performed before stopping anticoagulation. If these tests are normal, anticoagulation is discontinued (warfarin for at least 2, or better for 4 weeks, DOAC — 5 half-lives, taking into account renal function) and the remaining thrombophilia tests (PC, PS, and AT) are performed. It is prohibited to conduct the second stage of testing earlier than 3 months. after an episode of thrombosis. When testing healthy individuals, a two-stage approach is not required; it is recommended to perform testing before pregnancy and estrogen intake.

It is assumed that a significant part of patients with VTE are carriers of other genetic variants [5]. The most frequently mentioned types of other HT are listed in Table 1. One of the leading Russian experts, Momot AP (2015), offers to significantly expand the test panel [6]. However, an extended search for HT, in addition to significant material costs, may pose a difficult question for a clinician — what to do with the results?

The introduction of new-generation genome-wide association studies and sequencing has led to the development of a panel containing 55 genetic markers associated with VTE [7]. The use of genetic tests made it possible to significantly reduce the cost of analysis, to eliminate the limitations associated with the acute phase of thrombosis/anticoagulation therapy. The development of a polygenic risk score can significantly improve the clinical value of testing, but this issue requires further study.

Testing for HT in clinical practice

All testing algorithms are limited to the most common HTs, which determines only a small part of VTE situations.

Numerous studies have established an increased risk of VTE in patients with thrombophilia, but the maximum risk corresponds to the first episode of thrombosis. The impact on the risk of recurrent VTE is significantly lower (Table 3). This pattern significantly reduces the clinical value of testing [8]. Colucci G, et al. (2020) recommend an assessment

Table 3

Relative risk of VTE for different types of HT [10]

Thrombophilia type	Prevalence in the general population, %	Prevalence in the patients with VTE, %	General population	
			OR for first episode of VTE	OR for second episode of VTE
AT deficiency	0,02-0,2	1	50	2,5
PC deficiency	0,2-0,4	3	15	2,5
PS deficiency	0,03-0,1	2	10	2,5
FVL, heterozygotes	5	20	7	1,5
FVL, homozygotes	0,02	1,5	80	-
Prothrombin G20210A mutation, heterozygotes	2	6	3-4	1,5
Prothrombin G20210A mutation, homozygotes	0,02	<1	30	-
FVL + prothrombin G20210A mutation, heterozygotes	0,01	-	50	2,5

Abbreviations: AT — antithrombin, VTE — venous thromboembolism, OR — odds ratio, PC — protein C, PS — protein S.

of thrombophilic status taking into account the clinical and family history in all patients with VTE, while HT testing is advisable to perform only in certain cases [9].

Testing patients with VTE for HT, in most cases, does not affect the treatment tactics and does not improve the prognosis of the disease. For example, the study by Kozak PM, et al. (2019) revealed that a doctor's decision to test for HT, but not the test result, is associated with a high risk of recurrent VTE, despite the high probability of long-term anticoagulant therapy [11]. Data on 3590 patients with the first episode of VTE were analyzed; testing was carried out in 747 patients. Among the patients who underwent testing, the risk of recurrent VTE was significantly higher — 46,1% compared to 28,5% ($p<0,001$). These patients were more likely to receive long-term anticoagulant therapy (53,9% compared to 37,1%), and there was no difference in bleeding-related hospitalization rate. At the same time, the detection of HT was not associated with an increased recurrent VTE rate, duration of anticoagulant therapy, bleeding, and overall mortality. In some cases, the test results can lead to the non-justified prescription of anticoagulants and ethical problems [12, 13].

Expansion of the test panel due to the introduction of new generation sequencing technologies has favorable prospects. A large number of new genetic variants associated with thrombosis and bleeding are identified, the clinical significance of which requires further study [14, 15].

Testing in the acute phase of VTE in clinical practice

In the acute period of thrombosis (first 3 months), the testing is not recommended according to all clinical guidelines. The selection of the anticoagulant agent is specified by the clinical situation and does not depend on the HT [2]. It is reasonable to test only for APS since the result will affect the drug selection. In the TRAPS study, rivaroxaban was inferior to warfarin in patients with APS [16]. European [2] and UK [17] guidelines do not recommend the use of DOACs in patients with APS. Currently, there are no comparative data on the effectiveness of DOAC and warfarin for VTE treatment in patients with HT. Several published clinical observations show conflicting results [18-23]. Despite no evidence base, experts do not recommend limiting the DOAC use in patients with HT [24]. This is due to the fact that the common HTs listed above are relatively widespread and presumably 2-4% of patients included in clinical trials on DOAC could have HT. With significant activated PC resistance (homozygous carriage of FVL) or with deficiency of PC and PS (vitamin K-dependent anticoagulants), it is assumed that the use of DOAC may be safer than warfarin. An extremely rare but very dangerous complication — warfarin-induced skin necrosis — develops at the start of therapy due to massive thrombosis in response to a decrease in the level of natural vitamin K-dependent anticoagulants.

Testing 3 months after the VTE

There are following indications for testing in this period: the justification of extended anticoagulation, the search for etiological factors of thrombosis, and family genetic counseling.

Justification of extended anticoagulation

The determinant in the therapy duration is the assessment of recurrent VTE risk (Table 4). A low risk of recurrence is characteristic of VTE provoked by reversible major risk factors.

In *VTE provoked by reversible major risk factors*, the recurrence incidence does not differ in persons

Table 4

Assessment of the risk of recurrent VTE [2]

Risk of recurrent VTE	Provoking factors	Examples
Low (<3%/y)	VTE provoked by major reversible RF	Surgery with general anaesthesia >30 min In-hospital bed rest ⩾3 days (acute illness or exacerbation of chronic illness) Bone fracture
Moderate (3-8%/y)	VTE provoked by transient RF No RF detected	Surgery with general anaesthesia <30 min In-hospital bed rest <3 days (acute illness or exacerbation of chronic illness) Taking estrogen-containing medication Pregnancy/postpartum period Out-of-hospital bed rest for ⩾3 days due to acute illness Lower limb trauma (without fracture) with limited mobilization ⩾3 days Autoimmune diseases in the acute phase Inflammatory bowel disease
High (>8%/y)		Active cancer APS History of VTE episode not provoked by major reversible RF

Abbreviations: APS — antiphospholipid syndrome, VTE — venous thromboembolism, y — year, RF — risk factor.

with and without HT [25, 26]. After an episode of provoked VTE, anticoagulants are prescribed for 3 months (6 months for proximal DVT) even if thrombophilia is established — 2020 UK guidelines [17], 2019 ESC guidelines [2], 2016 American College of Chest Physicians (ACCP) guidelines [27], 2015 Russian guidelines on VTE [3]. Experts do not recommend testing for HT in patients of any age, since this leads to an unreasonable prolongation of anticoagulant therapy and an increase in bleeding risk.

Unprovoked VTE/provoked by transient risk factors. Patients with PC/PS, AT deficiency, FVL, prothrombin G20210A mutation are candidates for indefinite anticoagulation after the first VTE episode not associated with a major reversible RF [2]. In the 2015 Russian guidelines on VTE [3], the same recommendation is given, with the exception of the separation depending on homo- and heterozygous carriers.

However, routine testing for HT is not recommended. All patients, regardless of the testing results, with VTE unprovoked/provoked by transient RF and a low/moderate bleeding risk, should be prescribed extended anticoagulation; with a high bleeding risk — 3-month anticoagulation (2019 ESC [2], 2016 ACCP [27]).

Testing for HT may be recommended in certain cases where a patient with a low/moderate bleeding risk wants to discontinue anticoagulation to increase medication adherence [17]. A negative test result for HT should not be a reason to discontinue anticoagulation in patients without a high bleeding risk.

Thus, testing for HT should not be performed to determine indications for extended anticoagulation; the duration is specified by the clinical setting. A positive test result can be used to increase medication adherence of a patient.

Identification of etiological factors of VTE

Testing for HT is recommended in patients in whom VTE occurs at a young age (aged <50 years) and in the absence of an otherwise identifiable risk factor [2]. In patients over 60 years genetic screening should not be performed [9].

Family genetic counseling

HT significantly increase the risk of first VTE episode, especially in provoked cases — pregnancy, estrogen intake, etc. Family genetic counseling is recommended in all cases of unprovoked VTE in patients <50 years of age. Routine testing in first-degree relatives of patients is not recommended [17].

Connors JM (2017) recommends considering the testing for HT in patients with VTE (unprovoked or provoked by minor factors) if there are women of childbearing age in the family (first-degree relative) [28]. It seems reasonable to us to test not a patient in such cases, but his relatives.

Testing of patients with rare types of venous thrombosis

Testing guidelines are presented in the 2012 British Committee For Standards In Haematology (BCSH) guidelines [29]. In most cases, routine testing is not recommended, since there is no clinical data on the need to correct antithrombotic therapy in case of HT. After the first episode of central venous sinus thrombosis, the need for testing is not confirmed, but extended therapy can be considered in the case of AT/PC/PS deficiency. The common causes of splanchnic-vein thrombosis are cirrhosis, myeloproliferative disorders, paroxysmal nocturnal

hemoglobinuria, Janus Kinase 2 gene mutations; the need for HT testing has not been determined. In patients with upper-extremity DVT, retinal vein thrombosis, venous thrombosis within the genitourinary system, testing is not recommended. Connors JM (2017) recommends considering testing for HT in cases of central venous sinus and splanchnic-vein thrombosis [28].

Conclusion

Thus, testing for "common types of HT in patients with VTE should not be performed in most cases. In the first 3 months, only genetic tests can be performed; assessment of PC, PS, and AT — after the anticoagulant discontinuation. Testing is not used to select an anticoagulant and therapy duration, and should not be performed in patients with VTE provoked by major reversible RF. In other cases, it can be used to increase medication adherence in case of refusal of anticoagulation, to identify the cause of VTE in young patients, and as part of family genetic counseling. The rationale for testing for other types of HT is for further study.

Relationships and Activities: none.

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