An update on common inherited thrombophilic conditions

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Abbreviations
VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; PT, pulmonary thrombosis; COVID-19, coronavirus disease 2019; APCr, activated protein C resistance; tPA, tissue plasminogen activator; BSH, British Society for Hematology; KCT, Kaolin Clotting Time; dRVVT, Diluted Russell Viper Venom Time; ISTH, International Society of Thrombosis and Hemostasis; DIC, disseminated intravascular coagulation; DDAC, direct oral anticoagulant; APTT, activated partial thromboplastin time.

Abstract
The term thrombophilia refers to a number of inherited and/or acquired conditions that may be associated with an increased tendency to develop thrombosis. In this sense, hereditary thrombophilia is a genetic disorder of hemostatic proteins that is present at birth and contributes to individuals being at increased risk for venous thrombosis. Three important questions arise from the still ongoing debate about the benefits and limitations of testing for hereditary thrombophilia, which can be summarized as follows: “which tests?”, “when?”, and “how?”. This article attempts to answer these questions by providing an up-to-date overview of the most common and clinically significant inherited thrombophilic disorders (i.e., antithrombin, protein S and protein C deficiencies, activated protein C resistance, factor V Leiden, and prothrombin gene mutationG20210A), their prevalence, clinical severity, and diagnostic approach, while also highlighting the potential advantages and limitations of testing for inherited thrombophilia.

Keywords: thrombosis; thrombophilia; risk factors; testing
Introduction

Venous thromboembolism (VTE) is a heterogeneous clinical picture which may include deep vein thrombosis (DVT), pulmonary embolism (PE)/pulmonary embolism (in situ) thrombosis (PT), or both [1]. According to the most recent statistics from the American Heart Association (AHA) [2], the annual incidence is 0.2% for DVT, 0.12% for PE, and thus 0.32% for both conditions. The cumulative lifetime risk of venous thrombosis increases with age, so that the cumulative risk of developing this condition is as high as 8% after 45 years of age. Venous thrombosis has a recurrence rate of about 13%, an immediate mortality rate of nearly 5%, and a 1-year death rate of approximately 20%. It is also associated with significant costs to the health care system, estimated at around US $ 12 000–15 000 per each episode. Last but not least, the epidemiological trend of VTE has not reversed in recent years, as shown by the AHA statistics, but there has been instead an almost linear increase [2], which has intensified further by the burden of coronavirus disease 2019 (COVID-19) during recent years [3].

The pathogenesis of VTE is complex and essentially multifactorial, as no single risk factor is sufficient in itself to trigger thrombosis [4]. Risk factors can be divided into inherited (or congenital) and acquired, and each single factor has a different influence on the cumulative risk of developing an acute thrombotic episode. A paradigmatic example of this pathogenesis is the situation in which various risk factors, whose effect can be reflected by the size of a drop, fall into a cup up to a certain point when the liquid in the cup overflows [4]. In practice, this means that the accumulation of these risk factors alters the physiological pro- and antithrombotic balance, and drives the patient to a certain point when the occurrence of thrombosis becomes almost inevitable. Another key element that must be emphasized is that the acute thrombotic episode is certainly the result of a sum of risk factors, but its acute onset is very often caused by an acute precipitating condition (e.g., trauma, surgery, immobilization, etc.) [5]. In practice, the risk factors are the bullets from a gun, while other conditions may act as triggers that cause the gun to fire.

Thrombophilia

Although there are many definitions of thrombophilia, one of the most common is that this term is used to describe a number of inherited and/or acquired conditions that are associated with an increased predisposition to develop thrombosis [6]. In this sense, inherited thrombophilia is described as a genetic disorder of certain hemostatic proteins that is already present at birth, and which would contribute to place the subjects at enhanced risk of venous thrombosis [6]. Three important questions arise from the still ongoing debate about the benefits and limitations of testing for inherited thrombophilia, which can be summarized as follows: “which tests?”, “when?”, and “how?”. This article attempts to answer these questions by providing an up-to-date overview of the most common and clinically significant inherited thrombophilic disorders (i.e., antithrombin, protein S and protein C deficiency, activated protein C resistance (APC), factor V Leiden, and prothrombin gene mutation G20210A), their prevalence, clinical severity, and diagnostic approach, while also highlighting the potential pros and cons of inherited thrombophilia testing (Table 1).

Which tests?

The most common inherited thrombophilic risk factors include deficiency of natural anticoagulants such as antithrombin, protein-C, protein-S, or genetic mutations that alter the function of some clotting factors such as factor V Leiden or mutations in the promoter of the prothrombin gene (i.e., G20210A) [7]. There is an almost inverse relationship between the severity of the condition and its prevalence in the general population, as more severe conditions – such as antithrombin deficiency – are understandably associated with a significantly higher risk of thrombosis, leading to an early occurrence of thrombotic events, and which provide a reasonable basis for a kind of natural selection [7]. On the other hand, milder risk factors are compatible with a virtually longer thrombosis-free life, and would hence be more common in the general population. Regarding the overall severity of heterozygous conditions, antithrombin deficiency tops the list with a relative risk of thrombosis between 10 and 20, followed by protein C and protein S deficiency with a relative risk of around 5 to 10, followed by milder risk factors such as factor V Leiden (which leads to APC) and G20210A prothrombin gene mutations, displaying a relative risk between 2 and 8 (Table 1). Of course, the homozygous conditions of this defects are associated with much higher risk, with complete deficiencies of antithrombin, protein C, and S being virtually incompatible with birth or early life [7].

In addition, there are a number of other less common causes of inherited thrombophilia, whose incidence is so low that they rarely require laboratory investigation, except in selected cases when all other conditions have been excluded. These include dysfibrinogenemia, plasminogen activator inhibitor-1 (PAI-1) deficiency, heterogeneous deletion of antithrombin-converting enzyme (ACE 1/D), apolipoprotein E2/3 polymorphism, thrombomodulin deficiency, heparin cofactor II deficiency, tissue factor pathway inhibitor (TFPI) deficiency, genetically-determined Lipoprotein(a) excess, and sticky platelet syndrome [8].

A potential thrombophilia panel would therefore include the so-called first-line laboratory tests, which would have to consider most of the common inherited disorders, i.e., assessment of antithrombin, protein C and S, factor V Leiden and/or APC, G20210A prothrombin gene mutation, and some common acquired thrombophilic disorders such as lupus anticoagulants and anticardiolipin antibodies [9]. The panel of second-line tests could instead include evaluation of fibrinogen with functional and antigenic assays, homocysteine (both phenotypic and genetic testing), the assessment of the concentration of some procoagulant factors, especially factors VIII, IX, XI, and XII, the concentration of plasminogen with its activator (tPA) and inhibitor (PAI-1), thrombomodulin, heparin cofactor II, and other rarer genetic conditions such as those involving factor XIII or platelet glycoproteins [9].

<table>
<thead>
<tr>
<th>Condition</th>
<th>Prevalence in the general population</th>
<th>Relative risk for thrombosis</th>
<th>First-line assay</th>
<th>Second-line assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin</td>
<td>0.02-0.17%</td>
<td>10-20</td>
<td>Chromogenic assay (anti-II or anti-X)</td>
<td>Antigenic (immunometric)</td>
</tr>
<tr>
<td>Protein C</td>
<td>0.14-0.50%</td>
<td>7-10</td>
<td>Chromogenic assay</td>
<td>Antigenic (immunometric) and clotting</td>
</tr>
<tr>
<td>Protein S</td>
<td>0.10-1.0%</td>
<td>5-10</td>
<td>Immunometric (free protein S)</td>
<td>Antigenic (immunometric), chromogenic or clotting (total protein S)</td>
</tr>
<tr>
<td>APC/Factor V Leiden</td>
<td>3.0-5.0%</td>
<td>3-8</td>
<td>APTT-based with FVDP</td>
<td>Genetic analysis</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>1.0-3.0%</td>
<td>2-3</td>
<td>APTT-based without FVDP</td>
<td></td>
</tr>
</tbody>
</table>

APC, activated protein C resistance; APTT, activated partial thromboplastin time; Ag, antigen; FVDP Factor V plasma depleted

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When?
The second important aspect to consider is when to screen for thrombophilia. There are a variety of guidelines or recommendations on this aspect [10]. The basic assumption is that inherited thrombophilia should be investigated when thrombosis occurs at an early age (e.g., before 50 years of age), especially if it is not triggered by a clear risk factor or is associated with mild thrombophilic conditions (such as minor surgery, use of oral contraceptives, immobility, etc.). A second paradigm that may prompt investigation is a positive family history of venous thrombosis, which is highly suggestive of a condition that has been inherited. Inherited thrombophilia should then be investigated when recurrent venous thrombotic events occur, especially developing at a young age or when the thrombosis involves unusual venous sites such as abdominal or cerebral veins [10].

Importantly, according to recent guidelines from the British Society for Hematology (BSH) [6], routine (generalized) thrombophilia screening is discouraged, whether by phenotypic or genetic testing. As emphasized by the BSH, testing should be performed only in circumstances when identification of a potential abnormality would influence clinical decision making, such as the determination and/or duration of anticoagulant therapy [6]. Because thrombophilia testing does not require urgent decision making in any circumstance and the early antithrombotic or anticoagulant treatment may strongly bias the results of some tests, thrombophilia testing must not be conducted earlier than 3 months after acute thrombosis [6]. This aspect, which is very often neglected by clinicians, is of key importance and has been reiterated in several guidelines and recommendations [11].

How?
Early guidance on this topic was developed more than 20 years ago and have not changed significantly over time [12]. With regard to the most common thrombophilic conditions, two decades ago it was already in place the recommendation to measure antithrombin with an anti-X assay, protein C with an amidolytic assay using snake venom as activator, protein S with an immunnoassay to quantify the free fraction, and APCr with an activated partial thromboplastin time (APTT)-based clotting assay without factor V-deficient plasma [12]. It was also indicated to determine antiphospholipid antibodies with phospholipid-dependent assays such as Kaolin Clotting Time (KCT) or Diluted Russell Viper Venom Time (dRVVT), while performing also an immunnoassay for anticoagulant antibodies. Prothrombin genotyping has also been advocated to detect the G20210A polymorphism in the prothrombin gene, along with immunometric or chromatographic determination of homocysteine and measurement of coagulation factors with clotting assays [12].

Regarding antithrombin, the ISTH (International Society of Thrombosis and Hemostasis) now recommends the use of an amidolytic activity assay for initial testing, using either a thrombin- or Xa-based chromogenic method, and then, for classification of deficiency, an immunnoassay to quantify the antigen value and calculate the activity-to-antigen ratio [13]. It should be noted that some important aspects may confuse antithrombin assay results. The most common of these include physiological reduction in neonates, late pregnancy and early postpartum, liver disease, disseminated intravascular coagulation (DIC), nephrotic syndrome, severe sepsis, recent thrombosis, treatment with heparin or L-asparaginase. Artefactual increases can be observed in patients taking direct oral anticoagulants (DOACs) or heparin when a clotting-based assay is used [13].

Evaluation of inherited disorders of protein S essentially involves measurement of the free fraction of the protein, using specific immunnoassays. Total protein S immunnoassays or even clotting or chromogenic assays can then be used in patients with low free antigen levels to more accurately classify the nature of the deficiency, although such tests can be only performed by specialized laboratories [14]. The most important variables to consider when interpreting test results include physiologic reduction in newborns, pregnancy and puerperium, oral contraceptive pill or hormone therapy, physiologic or pharmacologic vitamin K deficiency (e.g., vitamin K antagonists such as warfarin), liver disease, nephrotic syndrome, DIC, severe sepsis, recent thrombosis, or acute-phase reaction. An artifactual increase may be observed in patients using DOACs or heparin when a clotting-based test is used [14].

The chromogenic protein C activity assay is recommended for screening for potential deficiencies because is highly specific, although it is unlikely to accurately detect the rare type Iib defect [15]. In particular, if severe congenital protein C deficiency is clinically suspected, a clotting-based assay in combination with an immunometric test for antigen detection may be advisable. Test results must be interpreted accurately in light of potential confounding factors such as physiologic reduction in neonates and children, physiologic or pharmacologic vitamin K deficiency (e.g., vitamin K antagonists such as warfarin), liver disease, DIC, or severe sepsis. In patients taking DOACs or heparin, artifactual elevations may be observed when performing clotting-based tests [15].

Uncertainty remains concerning the methods used for measuring APCR. Most guidelines now uniformly recommend the use of an APTT-based assay with factor V-deficient plasma predilution because of its extremely high sensitivity for detecting an underlying factor V Leiden condition (i.e., up to 100%) [16]. On the other hand, the use of an APTT-based test without factor V deficient plasma predilution would not allow detection of other nongenetic causes of APCR, such as elevated levels of factor VIII [16]. In this way, a balance could be struck in the field between the advantages and limitations of these two approaches. Of course, a positive result of an APTT-based test without factor V-deficiency plasma predilution would then require additional genetic analysis to distinguish the possible presence of factor V Leiden from other nongenetic causes of prolongation [16]. Since there is no reliable phenotypic test for screening for prothrombin gene polymorphism G20210A, a genetic assay must necessarily be used for this purpose [6].

Final considerations
As mentioned earlier, there is an open debate about the potential benefits and limitations of testing for inherited thrombophilia, as excessive or inappropriate tests may prove more harmful than beneficial. It is now clear that generalized testing must be discouraged for a variety of reasons, including high cost, potentially low yield, and, most importantly, the significant risk of obtaining false positive test results. For example, it has been estimated that in the best possible scenario, in the absence of clinical or analytical caveats, the false-positive rate may be around 5% [17]. However, this false positive rate can increase to as high as 50% if something goes wrong throughout the testing process (from test ordering to result interpretation) [17], thus generating artifactual data that may ultimately compromise clinical decision making and patient health. A paradigmatic example of the predictable cost of a complete thrombophilia test panel is that of the American Society of Hematology (ASH), which estimates that the cost of such a panel can exceed US$400, but can also vary widely from one country to another and, in particular, from the type of local health expenditure coverage [18].

There are a number of positive and negative factors that may convince a person with or without a thrombotic episode to undergo testing for inherited thrombophilia, as summarized in Table 2 [17]. The positive aspects include the fact that the identification of one or more abnormalities may contribute to justify the onset of thrombosis, to classify the family member as at potentially higher risk of venous thrombosis, to guide thromboprophylaxis during exposure to other prothrombotic risk factors, but may also influence the clinical management, wherein more severe thrombophilic conditions may need reinforced and/or longer anticoagulation. On the other hand, the drawbacks include a high risk of false-positive test results, lack of guarantee that a negative test will ultimately be associated with zero risk of thrombosis, excessive anxiety about enduring a potentially...
Table 2 Pros and cons of testing for inherited thrombophilic conditions

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombosis explanation</td>
<td>High risk of false positives</td>
</tr>
<tr>
<td>Other family members potentially affected</td>
<td>No absolute guarantee of being at low risk of thrombosis</td>
</tr>
<tr>
<td>Inform thromboprophylaxis</td>
<td>Anxiety</td>
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<tr>
<td>Clinical management</td>
<td>Change of lifestyle</td>
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<tr>
<td></td>
<td>Unjustified overtreatment</td>
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<td></td>
<td>Incremental healthcare expenditure</td>
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<tr>
<td></td>
<td>Laboratory overload</td>
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</table>

life-threatening condition, important lifestyle changes due to avoidance of exposure to other prothrombotic risk factors (e.g., long-haul flights), potential overtreatment, and additional health care expenditures because no cost-effectiveness analysis has yet been performed on the many and varied possible scenarios. Finally, excess testing will also contribute to laboratory overload.

In general, what must be ultimately considered is that a negative result of a test for inherited thrombophilia does not guarantee safety, whereas a positive result is not necessarily associated with a future thrombotic episode.

References


