Thrombophilia/prothrombotic disorders

Silva A.S., Brazão M.L., Granito S., Escórcio S., Jardim M., Silva S., Andrade J.L., Vieira R. Teixeira C., Freitas D., Araújo J.N.

Abstract

Venous and arterial thromboembolic diseases, as a whole, constitute a major problem for public health, as their cardiovascular and cerebrovascular effects are the main cause of mortality and morbidity in Western societies, including Portugal.[1]

A better understanding of the morbidity and mortality associated with thromboembolic disease, the discovery of various hypercoagulable states, the emergence of new antithrombotic drugs, and more specific and reliable diagnostic tests, have revolutionized this area of Medicine.

In this article, the authors review the literature on thrombophilias, addressing clinical, diagnostic and therapeutic aspects.

Key words: Venous thromboembolism, low molecular weight heparin, thrombophilias.

INTRODUCTION

Venous Thromboembolism (VTE) as a prototype multifactorial disease

In the 19th century Virchow described the existence of a thrombophilic state that predisposed patients to venous thrombosis and was characterized by the existence of three assumptions, since then termed Virchow's triad: Venous stasis, vessel wall injuries and changes in blood coagulability.

Descriptions of families with increased predisposition for venous thrombotic events have been published since the beginning of the 20th century[2]. The limited knowledge of the chemical composition of the blood and the properties of fibrin clot formation regulatory systems, and the limited availability of diagnostic resources, obviously represented a major obstacle to research on cases of family thrombosis at that time. Nevertheless, it is remarkable that even in the initial descriptions, it is possible to recognize foundations that remain the most up-to-date in our understanding of the etiology of venous thrombosis: the notion that acquired risk factors (such as surgery and pregnancy) contribute to the occurrence of thrombosis, but that genetic factors (whose existence can be inferred by the family's thrombotic history) coexist, and also have an important role, determining the risk of thrombosis.[2]

Although the pathogenesis of VTE is not yet completely understood, there is much evidence that the process is influenced by a complex interaction of genetic and environmental factors, which are generically termed risk factors. The characterization of risk factors is a crucial step towards a better understanding of the pathogenesis of thrombosis. Risk factors for VTE differ from risk factors for arterial thrombosis. Hypertension, smoking, dyslipidemia and diabetes, for example, which are established risk factors for arterial thrombosis, are not risk factors for venous thrombosis.[3]

"Classic" VTE risk factors include: advanced age, prolonged immobilization, surgery, fractures, use of oral contraceptives and hormone replacement therapy, pregnancy, puerperium, malignant neoplasm, infections and antiphospholipid antibody syndrome.

Over the last few decades, significant progress has been made towards understanding the pathophysiological mechanisms of VTA. Numerous changes associated with the hyperactivity of the coagulation system and susceptibility to thrombotic events have been identified, and the description of "hypercoagulable states" has substantially changed our view of venous thrombotic disease.[3] The most significant advance was the confirmation of the concept that conditions of inherited hypercoagulability are present in a high percentage of patients with venous thrombosis and pulmonary embolism. In fact, it is estimated that over 60% of predisposition to thrombosis is attributable to genetic components.[3,4] These new concepts have led to the introduction of the term thrombophilia to describe increased thrombophilic predisposition, usually genetic, to the occurrence of VTE.

Medical Service 1 of the Hospital Central do Funchal

Received for publication on 9th August 2007
Accepted for publication on 30th September 2008

Thrombophilias are congenial situations, and in rare cases, acquired, and promote or facilitate changes in blood clotting, which results in an increased risk of thrombosis. The thrombotic phenomenon usually affects the venous territory.

Patients with thrombophilia have their first thrombotic event before the age of twenty-five, and the chances of a recurrence increase with age and association with other risk factors. In fact, evidence suggests that a greater thrombotic predisposition arises from the combination of several risk factors.[4,5]

Thrombophilias: Clinical manifestations and relevance

VTE is a very common disease, and is a major cause of morbidity and mortality throughout the world, with high incidence in various populations studied (about 7/10,000 individuals per year).[6] The most frequent clinical manifestations are DVT of the lower limbs (leg DVT and proximal VTE) and PTE. More rarely, thrombosis occurs in other sites (retinal veins, intra-abdominal veins, upper limbs, central nervous system, superficial thrombophlebitis and utero-placental venous system).[7] The clinically significant problems associated with thrombosis include morbidity related to acute event, the recurrence of thromboembolic events, post-thrombotic syndrome, and mortality from pulmonary embolism.[7] In the U.S.A., VTE accounts for 260,000 hospitalizations per year, chronic venous insufficiency affects 500,000 individuals, and pulmonary embolism is the cause of death in 50,000 to 100,000 cases annually.

Patients with genetic thrombophilia exhibit an increased predisposition to recurrence of thrombotic events, and thrombosis tends to occur at an early age (before the age of 45-50). A family history of venous thrombosis can be identified in one third of cases.[8]

Thrombophilic states that predispose to thrombosis are divided into two groups: Primary, hereditary or congenital thrombophilia and secondary or acquired thrombophilia (*Table I*).[8]

PRIMARY OR HEREDITARY THROMBOPHILIAS

Primary thrombophilias are inherited from parents or other relatives, therefore the patient is born with this condition and may not have symptoms during childhood or adolescence. In fact, the risk of thrombosis due to this condition is low before the age of fifteen, increasing by two to four percent per year from this age onwards.[9] At the age of around fifty, between 50% and 70% of patients have already had a thrombotic event (half spontaneously and the other half in the presence of one risk factor such as surgery, trauma or pregnancy).[10]

Primary thrombophilias, are due to a deficiency in certain coagulation inhibitors. Some of the most common are:

- Deficiency of natural anticoagulants:
  - Protein C deficiency;
  - Protein S deficiency;
  - Antithrombin III deficiency;
- Hyperhomocysteinemia;
- Dysfibrinogenemia;
- Resistance to activated protein C and factor V

TABLE I

Risk factors for VTE

Acquired	Inherited
Advanced age	Antithrombin deficiency
Trauma or Surgery	Protein C deficiency
Prolonged immobilization	Protein S deficiency
Malignant neoplasm	Factor V Leiden and Resistance to activated protein C
Pregnancy and puerperium	Prothrombin G20210A mutation
Oral contraceptives	Dysfibrinogenemia
Hormone replacement therapy	Hyperhomocysteinemia*
Antiphospholipid antibody syndrome*	High plasma coagulation factor VIII levels*
Hyperviscosity	
Myeloproliferative diseases	
Nephrotic syndrome	
Resistance to activated protein C not related to change in the factor V gene	
Mild to moderate hyperhomocysteinemia	
Paroxysmal nocturnal hemoglobinuria	

* May present hereditary or acquired etiological factors

Leiden:
• Prothrombine gene mutation

Natural anticoagulant deficiencies

During the activation of the coagulation system, serum proteases with procoagulant activity are generated sequentially, culminating in the formation of a stable fibrin clot. The activity of these proteases is inhibited by a group of proteins called natural anticoagulants or physiological coagulation inhibitors (*Figure 1)*. Antithrombin III (AT), protein C (PC) and protein S (PS) are crucial components of the anticoagulant system. Genetic defects in these coagulation inhibitors result in a high risk of thrombosis[11].

Antithrombine III

AT is a member of the super-family of proteins called serpins ("serine proteinase inhibitors"). AT is a natural anticoagulant, and is the main thrombin inhibitor, but it also has inhibitory effects on other coagulation factors such as factors IXa, Xa, XIa and XIIa. Additionally, AT accelerates the dissociation of the complex tissue factor VIIa, and prevents its reassociation.[11]

AT deficiency was the first hereditary change to be associated with family thrombosis. In fact, in 1965, Egeberg described a Norwegian family in which patients with reduced AT plasma levels had thrombotic phenomena and, since then, numerous studies have described similar clinical and laboratory findings, where it has been established that the AT deficiency is a genetic risk factor for thrombophilia. AT deficiency affects both sexes equally and is considered the thrombophilia with the highest risk of thrombosis, even in heterozygous state (about 70% of pregnant women with AT deficiency will present venous thrombosis during pregnancy). An homozygous state is very rare for this deficiency and incompatible with life.[11]

The AT encoding gene is located on chromosome 1 (1q23-25). It has 13.4 kb of DNA and seven exons. The first mutation linked to AT deficiency was described in 1983. Since then, the identification of a variety of mutations in the AT gene have revealed that the molecular basis of AT deficiency is highly heterogeneous.[12] the diagnosis and classification of AT deficiency can be obtained by determining the antigen activity and plasma concentrations, using functional and immunological methods, respectively.

AT deficiency is divided into Type I (quantitative deficiency, characterized by reduced antigen plasma levels and reduced AT functional activity) and type II (qualitative deficiency, characterized by the presence of AT variant in plasma, with normal antigen levels and decreased operation).[13] Type II is subdivided into defective RS ("reactive site"), defective HBS ("heparin binding site) and PE (pleiotropic, that is, with multiple effects on AT function). More than 80 different mutations causing antithrombin deficiency type I or type II have been recognized to date. Mutations in the AT gene are periodically updated and published in the form of a database, on the Internet.

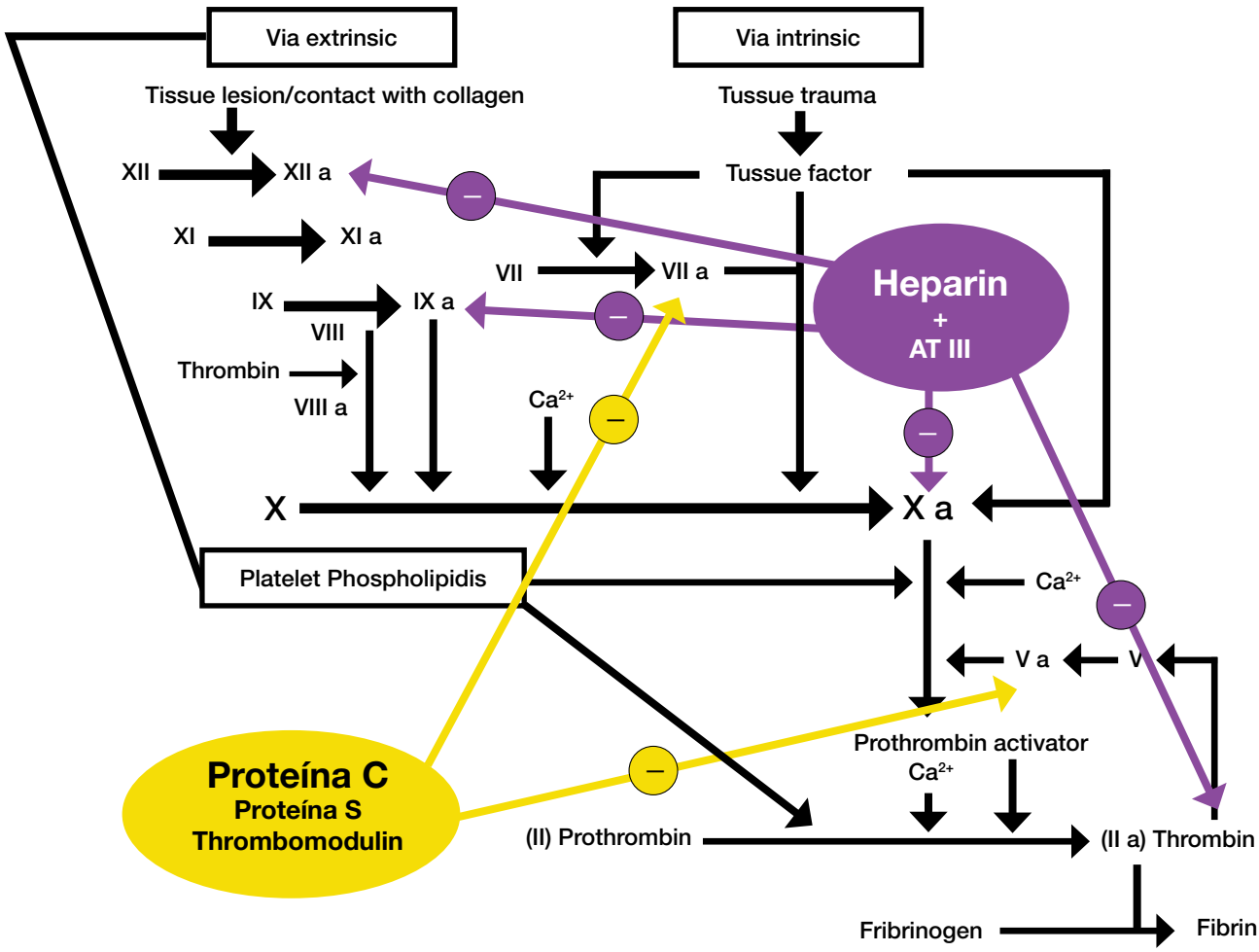
AT deficiency is considered a rare abnormality, and its prevalence among the general population ranges from 0.2/1000 to 11/1000, in different studies.[13] Estimates of thrombotic risk linked to AT deficiency and its prevalence in patients with thrombosis also vary between different studies, probably reflecting differences in study designs and patient selection. Together, the data derived from different studies show that AT deficiency is a well-established but unusual cause of thrombophilia, and it is accepted that the heterozygous state is associated with a five to ten times higher risk of thrombosis.[14]

It is interesting to highlight that the initial description of AT deficiency as a cause of VTE led, at the time, to the hypothesis that thrombophilia was a monogenic disease with incomplete penetrance.[14] This view has substantially changed in subsequent decades.

Protein C and Protein S

PC and PS deficiencies result in defects in the blood anticoagulant system, and will be discussed in this paper. PC is a vitamin K-dependent plasma protein, which is synthesized in the liver in its inactive form and activated following the binding of thrombin to its receptor (thrombomodulin) in the endothelium. Activated PC cleaves and inactivates clotting factors Va and VIIIa, thus inhibiting the formation of fibrin clot. PS acts as a non-enzymatic cofactor of activated PC, increasing the efficiency of these reactions. Bearing in mind its functions, it is likely that PC and PS deficiencies are linked to hypercoagulable states and increased risk for the occurrence of VTE. In fact, in the 1980s, genetic defects leading to PC and PS deficiency were first recognized as causes of inherited thrombophilia.[13,14]

The PC gene, located on chromosome 2 (2q13-14), is approximately 10 kb in length and has nine



Blood clotting cascade.

FIG. 1

exons. Mutations of the "loss of function" type on this gene lead to PC deficiency, which is considered a well-established cause of VTE. Like AT deficiency, molecular changes associated with PC deficiency have been identified in several families, and are highly heterogeneous.[15] The diagnosis and classification of PC deficiency can be carried out through plasma determination of the activity and concentrations of its antigen, using functional and immunological methods, respectively. PC deficiency is classified as Type I (low plasma concentrations of functional activity and of its antigen) and type II (low levels of protein functional activity with normal antigen levels). More than 160 different mutations of the PC gene are described in the database, mostly "missense" mutations.[15] Other defects described include mutations in the promoter region, abnormalities in "splice" sites, deletions, insertions and "nonsense" mutations. The most frequent clinical occurrence is recurrent DVT (63%) and pulmonary embolism (40%).[16] It can also accelerate disease of the small vessels.

The active gene responsible for the production of

PS is called PROS1. There is also a pseudo gene, called PROS2, which has high structural similarity with the PROS1 but is not transcribed. PROS1 and PROS2 were mapped on chromosome 3 (3p11.1-q11.2).[16] PROS1 has 80 kb and 15 exons. "Loss of function" mutations on the PROS1 gene lead to PS deficiency, an established inherited cause of venous thrombotic disease. The inheritance pattern of PS deficiency is usually autosomal dominant. PS circulates in free form (fraction designated free PS, corresponding to approximately 40% of the circulating protein) and is bound to protein C4b-BP (60% circulating PS). The designation total PS is used when both the free and bound forms are considered together. Based on the determination of plasma levels, PS deficiency is classified as type I (quantitative deficiency with reduction of total and free PS), type II (qualitative deficiency, characterized by decreased activity and normal antigen levels of total and free PS) and type III (normal levels of total PS and low levels of free PS). The characterization of genetic defects responsible for cases of PS deficiency showed that their molecular bases are very heterogeneous.[16, 17]

The prevalence of PC deficiency in the general population is estimated at around 1/300. Recent data on the prevalence of PS deficiency in the general population indicate frequencies of between 0.03% and 0.13%. The heterozygosis for PC and PS deficiency is associated with an increased risk of VTE in different populations. As in the case of AT deficiency, information on the prevalence and thrombotic risk for heterozygous PC and PS deficiencies vary from one study to another. In general, family studies give higher risk estimates than case-control studies. It is believed that the PC and PS deficiencies in heterozygous state are associated with similar levels of thrombotic risk, which is about ten times higher than people without these deficiencies.[17]

The homozygote for PC and PS deficiencies is associated with a severe clinical phenotype known as *purpura fulminans*, characterized by a state of massive microcirculation thrombosis, which is manifested soon after birth, although less severe forms of late onset homozygous PC deficiency have also been described.[17]

Since proteins C and S are two vitamin K-dependent, natural anticoagulants, the start of oral anticoagulation may lead to a sharp decline in these proteins, precipitating the occurrence of thrombotic events. These situations characteristically involve skin necrosism which can be prevented by heparin.

The heterogeneity of molecular defects in cases of AT, PC and PS deficiency represent a major obstacle to the use of molecular methods in the investigation of these thrombophilic states. In fact, the analysis of the AT, PC and PS genes is not used in the routine investigation of cases of VTE, and even in the near future, it is unlikely that the search for mutations on these genes will become a part of the diagnostic tools used to elucidate the etiology of cases of thrombophilia. Thus, as mentioned earlier, the diagnosis of AT, PC and PS deficiencies is established by plasma determination of the antigen activity and concentrations, using functional and immunological methods, respectively.[17]

It should be noted that although AT, PC and PS deficiencies are independent risk factors for the occurrence of VTE, together, these three abnormalities are detected in 5% to 15% of cases of VTE, and are a relatively rare, but well-established cause of venous thrombotic disease.

Hyperhomocysteinemia

Hyperhomocysteinemia (abnormally high plasma concentrations of the amino acid homocysteine) is an established risk factor for venous thrombosis, which is associated with a two to four times higher risk of thrombosis. Genetic and acquired factors interact to determine the concentrations of homocysteine in the plasma and, therefore, hyperhomocysteinemia is classified as a "mixed" risk factor for VTE. Hyperhomocysteinemia can be further classified into severe (plasma level> 100 μ mol / L), moderate (25 to 100 μ mol / L) or mild (16 to 24 μ mol / L). The mechanisms by which hyperhomocysteinemia contributes to thrombogenesis are only partially understood, and various studies point to disturbances in different components of the haemostatic system.[17]

Acquired causes of hyperhomocysteinemia include nutritional vitamin B12, vitamin B6 and folate deficiencies, advanced age, chronic renal failure, and the use of anti-folic drugs. Defects on the methylenetetrahydrofolate reductase (MTHFR) and cystathionine beta-synthase (CBS) enzymes genes involved in the intracellular metabolism of homocystein may lead to enzyme deficiency and hyperhomocysteinemia. Numerous mutations in the MTHFR and CBS have been identified, most of which are rare, and only have clinical consequences in homozygosity. When it

leads to a case of severe hyperhomocysteinemia, this condition is characterized by homocystinuria, multiple neurological deficits, psychomotor retardation, seizures, skeletal disorders, *ectopia lentis,* premature arterial disease and VTE. In contrast to the rarity of these defects, two MTHFR mutations (677 C and 1298 T → A → C) and a CBS mutation (844ins68) are prevalent, and deserve further discussion.[16,17]

The MTHFR 677 C → T mutation is a polymorphic variant with high prevalence in the general population. It is associated (in homozygous state) with reduced enzyme activity, a phenotype of enzyme thermolability, and hyperhomocysteinemia (mild to moderate), but its role as a genetic risk factor for the occurrence of VTE , or as a modifier of thrombotic risk conferred by other thrombophilic changes, is controversial.[18]

The MTHFR 1298 A → C mutation alone does not appear to be associated with hyperhomocysteinemia, but in compound heterozygosity with the MTHFR 677 T → C mutation, it can result in decreased enzyme activity and high plasma levels of homocystein. The MTHFR 1298 A → C mutation does not appear to significantly influence the risk of venous thrombosis, but further studies are needed to better define the role of this polymorphism in combination with other Prothrombotic conditions in thrombophilia.[18]

An 68-bp insertion in the CBS (844ins68) gene was recently described. This polymorphism alone does not influence levels of homocysteine or risk of deep vein thrombosis, but in combination with MTHFR 677 C → T, it can lead to an increased risk of thrombosis.[18]

Hyperhomocysteinemia is usually diagnosed by determining the levels of homocysteine in the plasma (in fasting and/or after the methionine administration test) using mass spectrometry or HPLC (high performance liquid chromatography) techniques with electrochemical or fluorescent detection. Alternative methods include immunoassays, ion exchange chromatography, gas chromatography and radioenzymatic assays. Some authors recommend studying the MTHFR 677 C → T mutation as part of the laboratory investigation of VTE etiology. However, given the fact that no genetic alteration in the enzymes involved in homocysteine metabolism was previously identified as an independent risk factor for VTE, and considering that the MTHFR 677 C → T mutation is not confirmed as a risk factor for thrombophilia in most studies, the systematic study of MTHFR and CBS mutations is not recommended as part of the routine examination of patients with VTE. Research on this variant can still be performed if the intent is to elucidate the cause of hyperhomocysteinemia that may have been detected in a patient.[17,18]

A mutation in the MTHFR (Methylenetetrahydrofolate reductase) gene is the most common cause of moderate increase of homocysteine, and can be found in 5% to 15% of the population. Mutation in homozygosity is associated with a five to six times increased risk of venous thrombosis. Homocysteine is an independent risk factor for atherosclerosis, stroke, peripheral vascular disease and heart diseases.

Dysfibrinogenemias

Dysfibrinogenemias and homocystinuria are very rare causes of thrombosis. More than fifty mutations in the fibrinogen gene have been reported around the world, associated, in some cases, with thrombotic complications, such as the Dusart and Chapel Hill III fibrogens.[18]

Hyper-fibrinogenemia is associated with increased risk of VTE, but further studies are needed to better define its exact prevalence and clinical relevance, as well as the advantages of systematic investigation in patients with thrombosis.

Activated Protein C Resistance (APCR) and factor V Leiden (FV: Q506): The main genetic abnormality involved in the etiology of thrombophilias

The coagulation factor V is a glycoprotein consisting of 2196 amino acids whose function is to stimulate the production of thrombin. Protein C, when activated, inhibits factor V, resulting in the inhibition of thrombin production.[18]

Resistance to activated protein C was first described in 1993 in the city of Leiden by Dahlback and colleagues, and is the main genetic mutation involved in thrombophilia, being found in 10% to 60% of cases of VTE. It is inherited in autosomal dominant form, with incomplete penetrance, and its frequency among the general healthy population ranges from 3% to 7%, but may be as high as 20% among individuals who have had a thromboembolic event.

The genetic mutation most often responsible for this resistance is the substitution of adenine for guanine at nucleotide 1691 of the coagulation factor V

gene, which will restrict the substitution of arginine by glutamine at residue 506 of the resulting protein (factor V). This altered protein is called factor V Leiden (FVL). The inactivation of this factor by activated protein C is much slower, which leads to the generation of an excess of thrombin, contributing to a hypercoagulable state with increased susceptibility to the occurrence of venous thromboembolic phenomena.

Other mutations in coagulation factor V (factor V Hong Kong and factor V Cambridge) have been described, which are rarer and do not, in isolation, appear to be risk factors for venous thrombosis.

The prevalence of factor V Leiden and consequently, of thromboembolic phenomena is higher in Caucasians compared with Asians or African Black people.[18]

Heterozygous individuals for this mutation have a five to ten times higher risk than the general population of suffering an episode of venous thrombosis, whereas in homozygous individuals, this risk is fifty to a hundred times higher. It is estimated that 3% to 7% of the general population is heterozygous and 1% is homozygous for this mutation.

Resistance to activated protein C should be tested routinely in the study of patients with VTE.

APCR diagnosis is established by using the modified APTT test (in the absence and presence of activated PC) and sample dilution with factor V deficient plasma, which results in more reliable discrimination between heterozygous and homozygous patients, as well as non-carriers. Alternatively, gene analysis techniques based on PCR amplification of exon 10 of factor V gene can be used to detect the FVL mutation.[18]

Finally it should be noted that the identification of FVL, as a mutation present in a large number of VTE cases, substantially changed our view of thrombosis, since it demonstrates the contribution of a genetic factor in the occurrence of this disease.

G20210A polymorphism in prothrombin gene

Prothrombin or coagulation factor II, is a vitamin k dependent protein. During clotting, prothrombin is transformed into thrombin by the prothrombinase complex (factor Xa, Va, Ca^{2+} and membrane phospholipids).[18]

In 1996, a new genetic risk factor involved in the etiology of VTE was described: a G→A transition at nucleotide 20210 position does not translate the 3' of the coagulation factor II gene (FII G20210A). The FII G20210A is associated with high plasma prothrombine levels and increased risk of VTE. This mutation is found in 1% to 3% of individuals in the general population, and in 6% to 18% of patients with VTE. These studies have established that FII G20210A, in heterozygosity, is associated with two to five times higher the risk of VTE and cerebral thrombosis. The risk is greatly increased by the use of oral contraceptives (149 times) and during pregnancy.

The mechanisms by which FII G20210A leads to an increased risk of thrombosis are not well known. In the original description of the mutation, an association of the mutant allele with hyperprothrombinemia was found. This finding was confirmed in subsequent studies, in which it was found that plasma prothrombin levels in patients with the mutation were higher than in those without the mutation. The levels of thrombin-antithrombin complexes and prothrombin fragment 1+2 were also higher in carriers of the mutation, providing evidence for the existence of an association between the mutation and increased formation of thrombin. These data point to an association of the FII mutation with excessive formation of thrombin, which may contribute to the understanding of its role in thrombotic diseases.

Controversial results have been published regarding the role of the FII G20210A mutation as a risk factor for recurrence of VTE, but this topic has yet to be clarified. The diagnosis of this change can only be established by genotyping, using gene analysis techniques. FII G20210A mutation is the second most prevalent genetic mutation linked to thrombophilia, and its description reinforced the concept of VTE as a multigenic disease.[18]

Increased plasma levels of coagulation factors (VIII, XI, IX)

Plasma concentrations of coagulation factor VIII reflect the combined influence of hereditary and acquired factors. For instance, genes encoding the ABO blood groups and the Von Willebrand factor influence the levels of factor VIII. Additionally, family aggregation of high levels of factor VIII (not linked to blood group or von Willebrand factor) was also described, pointing to the existence of unknown genetic components determining plasma concentrations of coagulation factor VIII. One of the acquired

factors that influence the factor VIII levels is inflammation, as factor VIII acts as an acute phase protein.[18]

High levels of factor VIII represent an established risk factor for VTE. In the "Leiden Thrombophilia Study", plasma levels ≥ 150 IU/dL were associated with a approximately five times higher risk of venous thrombosis. However, to date, no specific molecular abnormality has been identified in the factor VIII gene to explain the high plasma levels or increased thrombotic risk.

A recent study showed that factor XI high plasma levels (above the 90th percentile) are associated with a approximately 2.2 times higher risk of venous thrombosis. A dose-response relationship between the level of factor XI and thrombotic risk was observed, and the risk provided by factor XI levels was independent of other established genetic or acquired risk factors.[18]

It was recently reported that factor IX plasma concentrations above the 90th percentile are associated with a two to three times higher risk of deep vein thrombosis. This risk is not influenced by other factors, being higher in females (2.5 times higher risk) compared with males (1.9 times higher risk).[18]

The clinical use of systematic determination of coagulation factors levels in patients with VTE should be confirmed in future studies before the procedure is adopted as a routine investigation of thrombophilic states.

Rare genetic risk factors

Rare causes of thrombophilia include dysfibrinogenemia, plasminogen deficiencies and heparin cofactor IIa. In cases of high suspicion of thrombophilia, in which the investigation of the most common factors was negative, the three changes mentioned can be investigated through the plasma levels.

Study of new risk factors

Mutations in other genes, particularly factor XIII, tissue factor and thrombomodulin, have been described in recent years, but their association with thrombophilia is still under investigation. *Table II* shows the prevalence of genetic and "mixed" risk factors in the general population, and in patients with VTE. The idea that these different risk modifiers interact dynamically to determine the risk of thrombosis is useful for a better understanding of VTE as a multifatorial disease.[18]

TABLE II

Table II. Prevalence of genetic and "mixed" risk factors in the etiology of thrombophilias

Risk factor	General population	Patients with VTE
AT deficiency	0.02<5	1-3%
PC deficiency	0.2-0.4<5	3-5%
PS deficiency	0.03-0.13%	1-5%
Factor V Leiden	1-1.5%	10-50%
Factor II G20210A	2-5%	6-18%
Hyperhomocysteinemia*	~ 5%	~ 10%
Plasma ↑ Factor VIII*	11%	25%
Plasma ↑ Factor IX**	3%	7,6%
Plasma ↑ Factor XI#	10%	19%

*Factor VIII ≥ 150 UI/dL; **Factor IX> 150 UI/dL; #Factor XI> 120 UI/dL

SECONDARY THROMBOPHILIAS

Secondary or acquired thrombophilias arise at any time of life, including intra-uterine life, and their main cause is antiphospholipid antibody syndrome. The most frequent causes are:

- Antiphospholipid antibody syndrome;
- Prolonged immobilization;
- Trauma and surgeries;
- Advanced age;
- Malignant neoplasm;
- Paroxysmal nocturnal hemoglobinuria;
- Myeloproliferative diseases;
- Pregnancy and puerperium;
- Use of oral contraceptives or hormone replacement therapy;
- Nephrotic syndrome;
- Mild to moderate hyperhomocysteinemia;
- Hyperviscosity;
- Resistance to activated protein C is not related to alterations in the factor V gene.

Antiphospholipid antibody syndrome (APLS)

First described by Hughes et al in 1983, APLS, also known as Lupus anticoagulant (LA) syndrome and anticardiolipin syndrome, is a disorder of unknown

TABLE III

Clinical cases associated with APLS

Primary APLS Arterial thromboembolic and/or venous disease, recurrent miscarriages, thrombocytopenia, without any other defined underlying disease
Secondary APLS Associated with rheumatic or connective tissue diseases SLE, rheumatoid arteritis, systemic sclerosis, temporal arteritis, Sjogren's syndrome, psoriatic arthropathy, Behçet's disease.
Other associations Viral (HIV, hepatitis C, varicella), bacterial (syphilis), parasites (malaria) infection Lymphoproliferative disorders (lymphoma, paraproteinemias) Drugs (phenytoin, quinidine, hydralazine, procainamide) Other (ITP, autoimmune hemolytic anemia, sickle cell anemia, Guillain-Barre syndrome, livedo reticularis, toxicophilia by intravenous drug use)
APLS without clinical manifestation or identified underlying disease

Adapted from Garcia AA; Franco RF. Trombofilias Adquiridas, 2001.

cause, characterized by recurrent events of arterial or venous thrombosis, recurrent miscarriages and thrombocytopenia associated with laboratory evidence of antiphospholipid antibodies (APA).[18,19]

Since its discovery in 1983, several studies have demonstrated the presence of these antibodies in autoimmune diseases, neoplastic diseases, and those triggered by drugs (secondary forms) and in patients without underlying disease (primary form). Females are the most affected, and all age groups can be affected by this syndrome.

APA include a family of autoimmune immunoglobulins (IgG, IgM, IgA, or mixed) that recognize and bind to plasma proteins complexes associated with membrane phospholipids, in vitro laboratory tests. There is still no agreement as to whether these antibodies are involved in the pathogenesis of the syndrome or whether they are just an epiphenomenon, since they occur in about 5% of healthy individuals.

The two main plasma proteins that act as antigenic targets in complexes recognized by the APA are the ß2-glycoprotein I (ß2GPI) and prothrombin (clotting factor II). Other proteins that can bind to phospholipids and form the complex target of APA include: apolipoprotein H, protein C, protein S, annexin V, factor X, high molecular weight kininogen, factor XI and the protein component of heparan sulfate. The diversity of complex protein/phospholipid potential affects the most important feature of this syndrome, which is the heterogeneity of clinical and laboratory manifestations.[19]

In fact, thrombosis cases manifested in various ways, according to the territory involved. Approximately 70% of events occurr in the venous territory, and 30% occur in the arterial territory. The retinal vascular obstructions cause amaurosis fugax with transient visual field changes, which in elderly patients, are confused with small emboli detached from atherosclerotic plaques of carotid arteries. Neurological changes are common, and there may also be localized or focal ischemia, causing multiple cerebral infarctions with serious neurological repercussions, such as dementia. Cardiac involvement can occur in several ways, such as valve injury producing changes similar to Libman-Sacks endocarditis, and are more frequent in the aortic valve than the mitral valve. No correlation has been found between the presence of APLS and myocardial infarction in patients aged under fourty-five. Vascular changes in the skin present as fixed livedo reticularis, predominantly in the lower limbs. A more frequent pulmonary involvement is due to the occurrence of multiple microthrombi, leading to pulmonary hypertension with overload of the right chambers of the heart. Many patients develop heart failure as a result of this involvement. The liver can also be compromised, and manifestations are dependent on size of the vessel involved. Thrombi in larger vessels cause single or multiple strokes or Budd-Chiari syndrome, and in small vessels, they produce veno-occlusive disease, nodular regenerative hyperplasia or enzyme elevation due to the necrosis produced by multiple microthrombi. Finally, there is a form of APLS known as "catastrophic", with sudden, severe and fatal onset of occlusive disease. It is characterized by renal failure, retinopathy, ischemic stroke, osteonecrosis, skin necrosis, acute myocardial infarction, disseminated intravascular coagulation and immune cytopenias.[19] *Table III* shows the clinical picture associated with APLS.

Laboratory diagnosis of APLS: The test for lupus anticoagulant was the first to be developed. It is so named because it was found in the serum of a patient

TABLE IV

Criterial for Diagnosis of APLS, by the American College of Rheumathology, 2004

Clinical Criteria	Laboratory Criteria
Venous thrombosis Arterial thrombosis Obstetric Criteria*	Anticardioplin IgG or IgM in the blood** Lupus anticoagulant in the plasma

*1 or more unexplained fetal deaths of a morphologically normal fetus after 10 weeks of pregnancy, 1 or more pre-term deliveries of infants with normal morphology, before 34 weeks of pregnancy due to preeclampsia or severe placental insufficiency, 3 or more consecutive spontaneous miscarriages before 10 weeks of pregnancy, not associated with the mother's hormonal or anatomical pathology.

**Medium/high titer (ELISA for AAC anti- ß2GP)

with systemic lupus erythematosus. This antibody has the ability to prolong clotting time when added to normal plasma, characterizing its presence. It is also detected in several other diseases, as well as in patients with thrombotic events without the disease that justifies them.

The study of anticardiolipin antibody is done by radioimmunoassay (ELISA) and reveals the presence of two types of antibodies: IgG and IgM. The isotope IgG (subclasses IgG2 and IgG4) is more closely related to the formation of thrombi, while IgM is more common in infectious processes. In recent years it was discovered that these antibodies require a cofactor, beta-2-glicoprotein1, to interact.

Several studies indicate that lupus anticoagulant is less sensitive but more specific than the anticardiolipin antibody for the diagnosis of APLS.[19]

Other laboratory findings include not very accentuated thrombocytopenia, probably linked to the interaction of the antibody with platelet receptors, as well as the actual consumption of these for the formation of thrombus in the vascular endothelium.

Because it presents a broad spectrum of clinical manifestations, the American College of Rheumathology (ACR) proposed that for the diagnosis of the APLS, one clinical criterion and one laboratory criterion should be present, as shown in *Table IV.*

LABORATORY INVESTIGATION OF THROMBOPHILIAS

Table V lists the methods used in the investigation of hereditary thrombophilia. The diagnosis of AT, PC and PS deficiency is established by determining the plasma concentrations of each protein using functional and immunological methods, as mentioned earlier. Resistance to activated protein C can be diagnosed by the modified APTT method or by the identification of the FVL mutation by gene analysis techniques. The FII G20210A mutation can only be detected by gene analysis. Hyperhomocysteinemia is diagnosed by determining homocysteine plasma levels, usually by mass spectrometry or HPLC. Since no mutation linked to hyperhomocysteinemia was

TABLE V

Laboratory diagnosis of Thrombophilias

Thrombophilia	Research methods
Antithrombin deficiency	Determination of plasma AT (functional method)
Protein C deficiency	Determination of plasma PC (functional method)
Protein S deficiency	Determination of plasma free PS (immunological method)
Resistance to activated protein C	APCR Test (coagulation method)
Factor V Leiden mutation	Gene analysis
Factor II G20210A mutation	Gene analysis
Hyperhomocysteinemia	Plasma determination
Lupus anticoagulant Syndrome	Lupus anticoagulant research
Antiphospholipid antibody syndrome (APLS)	Anticardiolipin antibody igG and igM research
Dysfibrinogenemia	Determination of plasma fibrinogen by functional and immunological methods
Heparin factor II deficiency	Plasma determination (functional method)
Plasminogen deficiency	Plasma determination (functional method)

*Functional method for the determination of AT and PC, and immunological method for the determination of PS. Immunological methods can be used for further characterization of cases of AT and PC deficiency.

Source: Dr. Silvia Hadler Villela – CRM 17.601 and Dr. Rendrik Franco – CRM 79.124.

clearly linked with increased thrombotic risk, we do not recommend routine investigation of MTHFR or CBS mutations in the evaluation of thrombotic patients. The real usefulness of measuring plasma levels of coagulation factors in patients with VTE has yet to be demonstrated, thus, to date we cannot recommend its implementation in the routine investigation of thrombophilias.[19]

It should also be mentioned that the inclusion criteria for thrombophilia tests are not the same in all centers.

A realistic strategy is to mandatorily investigate all patients with objective diagnosis of venous thrombotic event when one or more of the following conditions are present: relatively young patients (<50 years), recurrent VTE, thrombosis in unusual sites (retinal veins, intra-abdominal veins, upper limbs, central nervous system, superficial thrombophlebitis) and a positive history of venous thrombotic disease. The extension of the same investigation to relatives of patients with thrombosis with a specific thrombophilic abnormality identified can, in theory, benefit asymptomatic carriers, as prophylaxis for VTE could be adopted in appropriate circumstances. This point, however, remains very controversial. The reasons for the controversy are related to the psychological pressure generated by the search for genetic mutations, the problem of "labeling" healthy individuals as having an "abnormality", and problems with health insurance and social security. In addition, there are uncertainties regarding the actual benefits of identifying inherited prothrombotic states in asymptomatic carriers. To properly examine this last point, more data are needed on the incidence and absolute risks of thrombosis in asymptomatic individuals are needed. However, although some studies have addressed this aspect among relatives of symptomatic and asymptomatic carriers of mutations linked to thrombophilia, data derived from large prospective studies are still needed to resolve this issue.

Which patients should be investigated?

Patients with objective diagnosis of DVT, PET or superficial thrombophlebitis, with at least one of the following characteristics:[19]

- Age up to 55;
- Recurrent thrombosis;
- Thrombosis in unusual sites (intra-abdominal veins, retinal veins, upper limbs, and central nervous system);
- Family history of venous thrombosis or pulmonary embolism.

Which changes should be investigated?

Genetic causes of thrombophilia, including antithrombin III deficiency, protein C and protein S deficiency, resistance to activated protein C/mutation of factor V Leiden, G20210A mutation of factor II (prothrombin) and hyperhomocysteinemia, as well as the acquired condition called antiphospholipid antibody syndrome (APLS), which is investigated through the search for lupus anticoagulant and anticardiolipin antibodies (IgG and IgM).[19]

When this investigation is negative, but there is high suspicion of thrombophilia (spontaneous thrombosis in young patients, recurrent thrombosis, recurrent thrombophlebitis, thrombosis in an unusual site), the search for rare causes of thrombophilia (dysfibrinogenemia deficiency of heparin cofactor II and plasminogen deficiency) can be considered.

Table V lists the laboratory methods used in the investigation of thrombophilias.[19]

TREATMENT

The first controlled clinical trial for the treatment of DVT was carried out in 1960. Since then several studies have been conducted.

The goals of VTE treatment are symptomatic relief, an attempt to prevent recurrence and progression to pulmonary embolism, and an attempt to reduce the incidence or at least the morbidity of post-thrombotic syndrome (PTS). PTSD is a common complication of DVT defined as a chronic venous insufficiency secondary to venous obstruction by thrombosis or secondary to injury of the venous valves at the time of the reorganization of the thrombus.[19]

Unfractionated heparin (UFH)

This is a very effective drug in the treatment of VTE in the active phase. It combines with antithrombin and catalyzes its anticoagulant activity, making it more effective in the deactivation of thrombin and the coagulation factor Xa, IXa, XIa and XIIa. It also indirectly inhibits the activation of factors V and VIII by thrombin.[20]

Once the DVT episode is confirmed, treatment should be started as soon as possible, provided the patient has no contraindications for the use of anti-

TABLE VI

Nomogram for administration of heparin* according to Cruickshank et al.[13]

aPTT	Bolus	Perfusion pause (min)	Infusio** (UI/h)	Time to aPTT repetition (horas)
<50	5000	0	+120	6
50 -59	0	0	+120	6
60 -85	0	0	0	24
86 – 95	0	0	-80	24
96 -120	0	30	-80	6
>120	0	60	-160	6

*5000 IU bolus followed by intravenous administration of 1280 IU/h first APTT after 6 hours
**Dilution of 20,000 IU of heparin in 500 ml of dextrose 5% (40 IU / ml)

coagulant. Before beginning therapy, a blood platelet tests and screening of the haemostatic system, partial thromboplastin time (aPTT), and prothrombin time (PT) with international normalized ratio (INR) must be carried out. Start with a bolus intravenous (IV) of 5,000 IU or 80 IU/kg, followed by 30,000 IU in the first 24 hours (18 IU/h), in continuous infusion. Alternatively, administer a bolus of 80 IU, iv followed by infusion at 18. IU/kg/h. Every effort should be made to achieve a therapeutic level in the first 24 hours of treatment.[20, 21]

Alternatively, heparin can be used subcutaneously for the treatment of the acute phase of VTE, provided clinically effective doses are used and they are properly monitored by aPTT. One of the recommended regimens is the use of 17,500 IU/kg every twelve hours, for the first 24 hours, with the subsequent doses adjusted to APTT. APTT should be determined at 6 hours and then follow one of the following nomograms *(Tables VI and VII)*.

By day 5, if heparin is not discontinued and replaced with another antithrombotic agent, ask for a platelet count, to eliminate heparin-induced thrombocytopenia, which occurs with thrombocytopenia and severe and recurrent thrombotic phenomena, in case heparin is not withdrawn and replaced by another antithrombotic agent. From day 5, the platelet count should be performed at least every 3 days upon discontinuation of therapy, or until day 14, after which the complication becomes less frequent.[20-21] Osteoporosis is a potential complication of prolonged use of heparin.[311-17] In case of severe bleeding, the anticoagulant effect of heparin can be completely reversed by infusion of protamine sulphate (administered 1 mg protamina/for 100 IU of heparin).[21]

A preventive dose of UFH varies from 5000 to 7500 IU, subcutaneously every 12 hours or every 8 hours.

Low molecular weight heparin (LMWH)

Comprised of smaller fractions of the heparin molecule and administered subcutaneously. Has replaced non-fractionated heparins with some advantages. Specifically inhibit the activity of coagulation factor Xa. In addition, LMWHs have a longer plasma half-life, better bioavailability after subcutaneous administration, and less variability in response to a fixed-dose offering an stable and lasting anticoagulant effect when administered once or twice daily, without the need for monitoring with laboratory tests.[20, 21]

Some studies have shown that LMWHs have the same clinical efficacy as unfractionated heparins, with fewer side effects, including lower recurrence of thrombotic events, fewer bleeding complications and a lower incidence of osteoporosis and thrombocytopenia (platelet count is carried out only on day 7 of the treatment). However, the pharmacokinetic of LMWH

TABLE VII

Nomogram for administration of intravenous heparin based on body weight

Initial dose	Bolus of 80U.I./kg, perfusion18 U.I./kg/h
APTT < 35s (< 1.2 x control)	Bolus of 80U.I./kg, perfusion 4 U.I./kg/h
APTT 35 – 45s (1.2 to 1.5 x control)	Bolus of 40U.I./kg, perfusion 2 U.I./kg/h
APTT 46 to 70s (1.5 to 2.3 x control)	Should not be changed
APTT 71 to 90s (2.3 to 3x control)	Reduce infusion by 2 U.I./kg/h
APTT > 90s (< 1.2 x control)	Interrupt for one hour and then Reduce perfusion by 3 U.I./kg/h

Adapted from Raschke and col.[13]

TABLE VIII

Examples and doses of some LMWHs used[16-17]

LMWH	Usual Therapeutic doses
Nadroparin calcium	0.1 ml/10kg every 12 hours, Sc (medium 0.6 ml) every 12 hours
Dalteparin sodium	<46 kg – 7.500 IU/day, SC 46 to 56 kg – 10,000 UI/day 57 to 68kg – 12,000 UI/Day 69 to 82 kg- 15,000 UI/day ≥ 83kg or more – 18,000 UI/day
Enoxaparin	1 mg/kg every 12 hours, SC

is not reliable when patients have creatinine clearance of below 30 ml/min, when the patient's weight exceeds 100 pounds, or when the patient is pregnant. There should not be an alternate use of pharmacologically different agents that comprise the class of LMWHs in the same treatment, because not all of these drugs have the same pharmacological profile, particularly with regard to their anti IIa and anti Xa activity.[21] Research based on evidence suggests that LMWHs have superior efficacy compared to UFH in the initial treatment of DVT of the lower limbs, particularly in reducing mortality and risk of major bleeding, and are at least as effective as UFH in the treatment of PTE. However, in cases of severe bleeding, the LMWH have the disadvantage that their effect not completely reversed by protamine sulphate, and in these situations, the following procedures should be adopted: If the LMWH was administered in the last 8 hours, administer 1 mg of protamine sulphate/100 IU of anti-factor Xa (1 mg of enoxaparin is equivalent to 100 units anti-factor Xa).

In patients with severe renal impairment, the excretion of LMWH is more difficult to predict, and it is better to use the continual intravenous UFH infusion (*Table VII*).[20, 21]

Oral anticoagulants

These are generically known as coumarin agents and make up the therapeutic arsenal used for long-term anticoagulation in patients with VTE. Of these agents, warfarin has the best profile for clinical use, with less risk of overdose and easier anticoagulation control, which has justified its increased use in clinical trials around the world. These drugs act in the liver by inhibiting δ post-ribosomal carboxylation of glutamic acid residues of the N terminal region of vitamin K dependent coagulation factors (II, VII, IX and X). In the treatment of VTE, warfarin should be started on the first day of treatment along with the start of heparin, after obtaining the baseline PT (INR) and APTT values. The currently recommended dose is one 5 mg tablet per day, with daily INR control in the first days of treatment and dose adjustment in cases of excessive INR extension.[20, 21]

Simultaneous treatment with heparin (or LMWH) and oral anticoagulants should last for at least 4 to 5 days, at which point heparin may be discontinued when it has reached INR levels of 2.0 for two consecutive days.[21]

In all cases, the target is to achieve a 2.5 INR, with values of between 2.0 and 3.0 being admitted.

The warfarin dose is variable, since its activity is influenced by several factors including: Ingestion of foods containing vitamin K, genetic polymorphisms in enzymes involved in their metabolism, and various drug interactions. In an attempt to remedy these problems, over the last decade, new anticoagulant molecules have been developed that will certainly improve the therapeutic approach to VTE in the future. In this context, new drugs have been developed directed against factor Xa (such as fondaparinux) and direct thrombin inhibitors (such as melagatran/ximelagatran). Further studies are needed for the application of these drugs in clinical practice.

Undoubtedly, the less-established side of VTE treatment is the duration of oral anticoagulation after an initial episode, and particulalry during recurrence. Many factors must be taken into account for the decision on the duration of oral anticoagulation, and several studies with this goal are currently underway. *Table IX* shows one of the strategies currently advocated.[20, 21]

Other therapeutic approaches

Elastic compression stockings: Evidence suggests that early use of elastic compression stockings, since the 1st month of diagnosis to 2 years after the thromboembolic event, reduces the incidence and severity of post-thrombotic syndrome[20]

Inferior vena cava filters In cases of proximal VTE or PTE, the placement of an inferior vena cava filter is the treatment of choice in patients with contraindications for systemic anticoagulation, or patients

TABLE IX

Duration of oral anticoagulation in patients with VTE[13-16]

VTE Episode	Environmental risk factor	Thrombophilia	Duration in months
Single	Yes	No.	3 to 6
Single	No.	No.	6
Single	Yes	Yes	6
Single	No.	Yes	12
Single	Indifferent	Mixed	Unknown
Single	Indifferent	Homozygous	Unknown
Recurrent	Indifferent	No.	12 to Undetermined
Recurrent	Indifferent	Yes	Undetermined

with recurrent episodes of PTE, despite adequate anticoagulation. However, there is little evidence to support its use.[20]

Thrombolysis Catheter-directed thrombolysis involves the direct administration of thrombolytic agents in the region of the thrombus. This approach can be effective in selected patients, but the evidence is still insufficient.

Associated therapeutic measures

At the beginning of treatment, the patient should remain at rest with the lower limbs raised or placed in the Trendelenburg position.

For pain control, in addition to rest, raising the lower limbs and the start of heparin can be used. In refractory cases, opioid analogues can be used, since the non-steroid anti-inflammatory drugs are contraindicated.[20]

Mobilization of the lower limbs should be encouraged early on, to improve venous flow, and walking should start as soon as symptoms allow, usually during the second or third day of hospitalization.

At the time of discharge, the patient should be advised to use high compression elastic stockings, first thing in the morning, to minimize the formation of edemas and control other secondary changes to chronic venous hypertension.[20]

TREATMENT OF THROMBOPHILIAS IN SPECIAL CONDITIONS

Patients with asymptomatic DVT confined only to the leg veins present a low risk of progression to PTE (<1%) and follow up can be carried out only by systematic repetition of ultrasound in the two weeks following the identification of the thrombus. If signs of an increase or extension of the thrombus occur, the patient should be anticoagulated.[20]

Symptomatic patients, even with thrombosis confined to the leg, should be treated, except for patients who can be followed up with serial imaging exams.

In pregnancy, VTE treatment should strive for the use of unfractured heparin or LMWH, since coumarins have teratogenic effects when used in the first term of pregnancy, and increase the risk of bleeding when used in the last quarter. Oral anticoagulation should be initiated in the immediate postpartum period and maintained until the fourth or sixth week after childbirth, because this is precisely the period when the patient is more susceptible to new thromboembolic events.[20]

In more severe cases with femoroiliac segment thrombosis and side effects, thrombectomy may be indicated.

Patients for whom anticoagulant treatment is not recommended should have special treatment, such as milder anticoagulation regimens or similar regimens to those used for prophylaxis.

Antithrombin Deficiency III: In the presence of an acute thrombotic event, sodium heparin and antithrombin III concentrates are recommended to maintain levels of 100%, followed by administration of sodium warfarin in the long term, while maintaining an INR of 2 to 3 as a prophylactic treatment.

Antiphospholipid antibody syndrome: As a general rule, individuals with antiphospholipid antibodies, without any manifestation arising from or attributable to these antibodies, are not treated prophylactically. However, in the presence of high titers of anticardiolipin antibodies, 100 mg of aspirin per day may be prescribed as an anti-platelet, provided there is no contraindication. For patients with repeated cases of vascular thrombosis, initial anticoagulation must be with LMWH, and subsequent maintenance should be with oral anticoagulants for an indefinite time, keeping the INR around 3.0 to 3.5.[20]

References

1. Silva F. Doenças Tromboembólicas da prevenção à terapêutica, Grupo de Consulta de Denças Tromboembólicas do Centro Hospital de Lisboa Ocidental, Hospital Egas Moniz, Serviço de Medicina II, 2006, 1ª edição.

2. Franco R.F. Trombofilias hereditárias. Medicina, Ribeirão Preto 2001;34: 248-257.

3. Rosendaal F.R. Venous thrombosis: a multicausal disease. Lancet 1999;353: 1167-1173.

4. Federman D.G. Kirsner R.S.: An Update on Hypercoagulable Disorders. Arch Intern Med 2001; 161:1051-1056.

5. Franco R.F., Reitsma P.H. Genetic risk factors of venous thrombosis. Human Genetics 2001;109: 369-384.

6. Osorio G.: Hematología: Diagnóstico y Terapéutica. Segunda Edición, 2001. Editorial Mediterráneo.

7. Lopez JA et al. Deep venous thrombosis. Hematology. Am Soc Hematol Edu Program 2004: 439-456.

8. Seligshon U., Lubetsky A. Genetic susceptibility to venous thrombosis. NEJM 2001; 344:1222-1231.

9. Laffan M., Tuddenham E. Assessing thrombotic risk. BMJ 1998;317:520-523.

10. Lane D.A., Grant P.J. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. Blood 2000;95: 1517-1532.

11. Kiekebusch G., Perucca E.P. Trombofilias hereditárias. Ver Chil Obst Ginecol 2003; 68(5): 424-429.

12. Brigden M. The hypercoagulable state. Coagulation series. Postgraduate Medicine 1997; 101:249-263.

13. Páramo J.A., Lopez Y., Muñoz C. Diagnóstico de los estados de hipercoagulabilidad. Revista Española de Cardiología 1997; 243:494-502.

14. Barger AP. Hurley R. Evaluation of the hypercoagulable state: whom to screen, how to test and treat. Postgraduate Medicine 2000:108(4).

15. Stefano V., Rossi E., Paciaroni K., Leone G. Screening for inherited thrombophilia: Indications and therapeutic implications Trends in Hematology / Oncology 2002; 87(10) :1095-1108.

16. Tripodi A., Mannucci P.M. Laboratory investigation of thrombophilia. Clinical Chemistry 2001;47:1597-1606.

17. Van Cott E.M., Laposata M. Laboratory evaluation of hypercoagulable states. Hematol Oncol Clin North Am 1998; 12:1141-1166.

18. Walker, ID, Greaves, M, Preston, FE. Investigation and management of heritable thrombophilia. Br J Haematol 2001; 114:512.

19. Jodi B. Segal M.D. Michael B et al. Management of Venous Thromboembolism: A Systematic Review for a practice Guideline, Annals of Internal Medicine 2007; 146 (3):211 -222.

20. Riszatti E.G., Franco R.F. Tratamento do tromboembolismo venoso - Simpósio Hemostasia e trombose, 2003; (Capitulo V) : 269 – 275.

21. Centro de informação do medicamento Informações sobre heparina de baixo peso molecular http:/www.ufpe.br/ufhc/heparina 1.htm.