Clinical Reflections on He

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To be effective and safe, the coagulation process should induce the formation of a blood clot in the right amount and at the right time. Haemostasis, is the result of interplay between damaged blood vessels, platelets and coagulation factors. The coagulation pathway is a proteolytic cascade, with each enzyme of the pathway present in the plasma as a zymogen (an inactive form), which on activation undergoes proteolytic cleavage to release the active factor from the precursor molecule. The initiation of the coagulation cascade can arise from two major pathways, the Intrinsic and Extrinsic pathways, that finally merge into one common pathway, with thrombin as its final product. Thrombin converts soluble fibrinogen to insoluble fibrin that is essential for clot formation. In similar fashion to other physiological processes, the coagulation cascade involves a number of positive and negative feedback mechanisms that ultimately produces a fine balance between thrombophilic and thrombolytic processes. Any variation in the protein structure of any of the components of the coagulation system can give rise to coagulation disorders.

Whilst the Hereditary Haemophilias (Classic Haemophilia – factor VIII deficiency – and Christmas Disease – factor IX deficiency) are serious conditions, potentially causing severe morbidity and an increase in mortality, their prevalence in the general population is relatively low. The reverse side of the coin, i.e. hereditary thrombophilias (an increased tendency for thrombosis), whilst not rare are often overlooked. The most common proteins involved in hereditary thrombophilias are Protein C, Protein S, prothrombin, Factor V and methylene tetrahydrofolate reductase.

Protein C is synthesized in the liver as an inactive protein, which circulates in the blood. Activation of the protein occurs on cell surfaces by the action of thrombin. Activated Protein C inhibits thrombin formation by inactivation of key cofactors (FVa and FVIIIa) required in procoagulant enzymatic complexes. Hereditary Protein C deficiency is inherited as an autosomal recessive condition with a prevalence of around 1 in 200 to 300. Protein S is a vitamin K–dependent anticoagulant protein which acts as a cofactor to activated protein C. Hereditary Protein S deficiency is an autosomal dominant disease. The prevalence of hereditary Protein S deficiency is estimated to be around 1 in 700.

Prothrombin (Factor II) is the thrombin precursor protein produced by the liver and, similar to the other coagulation factors, present within the plasma protein component. Prothrombin is converted into thrombin as part of the coagulation cascade and is pivotal in clot formation. Blood prothrombin levels have a direct affect on the efficiency of the coagulation process. A single point mutation in the untranslated, 3' region of the prothrombin gene (G20210A) causes elevated plasma prothrombin levels, which in turn leads to increased rates of thrombin generation, and an increase in the risk for a thrombotic event due to the potential for excessive growth of fibrin clots. The presence of this allele increases the risk of deep vein thrombosis (DVT) by 2-3 times.[1,2] It is estimated that around 2.7%[3] of the Maltese population carries this allele.

Activated Factor V (FVa), together with activated Factor X (FXa), induce the conversion of Prothrombin to Thrombin. The gene for this factor is located on chromosome 1 and is about 70Kb (kilobases) in length (approximately equal in length to the whole of the beta globin gene locus) and is made up of 25 exons. In contrast to most of the other coagulation factors, FVa acts as a cofactor and is essential for the conversion of prothrombin. It is thus also the prime site for the negative feedback mechanism that limits thrombin formation. This occurs through the inactivation of FVa by activated Protein C. Certain mutations within the Factor V gene, produce a protein that shows a resistance to Protein C degradation, with the resultant increased activity of Factor V. The most common mutation within this group is the replacement of an arginine residue with glutamine at amino acid position 506 (R506Q), commonly known as Factor V Leiden. It is estimated that the prevalence of Factor V Leiden amongst the Maltese population is of 2.3%[3] and the presence of this allele, increases the risk of DVT by 3-8 times.[1,4]

Methylenetetrahydrofolate reductase (MTHFR) is a cytoplasmic enzyme that irreversibly reduces[5],10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which in turn is used to convert homocysteine (a potentially toxic amino acid) to methionine by the enzyme methionine synthase. Two DNA sequence variants (polymorphisms) in the MTHFR gene at basepair 677 (a change from a C to a T) and at basepair 1298 (a change from an A to a C), reduce its enzymatic activity, by 50% and 34% respectively. Homozygosity of either polymorphism or compound heterozygosity increases the risk of DVT.[5]

Juvenile Venous Thromboembolism
Recurrent Venous Thromboembolism
Family history of Venous Thromboembolism
Thrombosis in unusual sites (portal or mesenteric veins, cerebral sinus)
Recurrent fetal loss
Pre-eclampsia, HELLP-syndrome
Skin necrosis induced by coumarins (deficiency of Protein C or S, prothrombin mutation)
Neonatal Purpura Fulminans (homozygous Protein C or homozygous Protein S deficiency)
Heparin resistance (severe antithrombin deficiency)

Table 1: *Clinical presentations of the various inherited thrombophilic conditions.*

ereditary Thrombophilia

Thrombophilic Status	Relative Risk of Venous Thrombosis
Normal	1
Oral contraceptive (OC) use	4
Factor V Leiden, heterozygous	5 to 7
Factor V Leiden, heterozygous + OC	30 to 35
Factor V Leiden, homozygous	80
Factor V Leiden, homozygous + OC	>100
Prothrombin Gene Mutation, heterozygous	3
Prothrombin Gene Mutation, homozygous	Also possible risk of arterial thrombosis
Prothrombin Gene Mutation, heterozygous + OC	16
Protein C deficiency, heterozygous	7
Protein C deficiency, homozygous	Severe thrombosis at birth
Protein S deficiency, heterozygous	6
Protein S deficiency, homozygous	Severe thrombosis at birth
Antithrombin deficiency, heterozygous	5
Antithrombin deficiency, homozygous	Thought to be lethal prior to birth
MTHFR Deficiency, homozygous	2 to 4
MTHFR Deficiency, homozygous combined with Factor V Leiden, heterozygous	20

Table 2: *The relative risk of venous thromobosis amongst carriers, homozygotes and heterozygotes of the various inherited thrombophilias.*

Considering the high prevalence of these variants in the Maltese population and considering the potentially serious conditions they are associated with, who should be tested and how? Though the easiest answer to this question would be a whole population screening, this cost (both of the actual tests as well as in counseling time) to benefit ratio of such an approach is high and it would result in a high degree of undue anxiety and stress to the individual being tested. A more reasonable approach would be that of targeted testing. The recommended testing protocol would include:

1. PATIENTS WITH VENOUS THROMBOEMBOLISM

- Patients with a first episode of venous thromboembolism in young patients (<50 years of age)
- Patients with a first episode of venous thromboembolism at >50 years with a positive family history for thrombotic phenomena
- Patients presenting with recurrent episodes at any age without the presence of any other predisposing condition.
- Venous thrombosis in unusual sites (such as hepatic, mesenteric and cerebral veins).

2. PATIENTS WITH ARTERIAL THROMBOSIS

- Young patients who develop acute arterial thrombosis in the absence of other traditional risk factors
- Myocardial infarction in female smokers under 50 years of age
- Female patients receiving hormonal replacement therapy
- Patients with early saphenous vein graft failure

3. ASYMPTOMATIC WOMEN

- Asymptomatic women with a positive family history for venous thromboembolism before use of oral contraceptives or hormone replacement therapy
- Women with recurrent pregnancy loss or unexplained intrauterine fetal growth retardation or stillbirth
- Women with severe pre-eclampsia

4. OTHER ASYMPTOMATIC SUBJECTS

- Asymptomatic relatives of patients with known inherited thrombophilia

Considering the relative high risk of venous thrombosis in individuals heterozygous for Factor V Leiden or heterozygous prothrombin deficiency and oral contraceptive therapy (OCT), it is arguable whether testing for these thrombophilia conditions should be carried out prior to starting OCT. It is generally considered that the cost to benefit value of screening for thrombophilia prior to OCT is low. On the other hand it is advisable to take a detailed personal and family history of deep vein thrombosis prior to the prescription of OCT or at a first antenatal visit. This should detect those individuals that are at risk of having one of the inheritable thrombophilias and in whom testing would be worthwhile.[6]

DNA tests for mutations causing inheritable thrombophilic disorders are available from the Laboratory of Molecular Genetics at the Pathology Department and requires a blood sample in an EDTA vial. Other biochemical tests are also available at the Pathology Department and require a citrated blood sample. Those individuals with a positive result should be given adequate counselling (can be referred to the Molecular Genetics Clinic, Speciality Clinics at Mater Dei Hospital) as well as proper advice (as required) regarding anticoagulant treatment, oral contraceptive and hormone replacement therapy, folic acid supplementation as well as proper hydration and exercise in cases of prolonged bed rest and long haul flights.

References

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