Thrombophilia PCR (polymerase chain reaction) testing

What is thrombophilia?
Thrombophilia is a condition, which is associated with an increased risk of thrombosis – individuals with thrombophilia have a higher risk of venous thromboembolism. Venous thromboembolism may manifest itself as deep vein thrombosis or pulmonary embolism.

In some cases, thrombophilia could be associated with pregnancy loss [1-3].

In general, factors contributing to venous thromboembolism might be inherited and/or acquired. Usually, venous thromboembolism is not a result of just a single risk factor, but rather, it can be due to a combination of various risk factors [4].

Thrombophilia itself can be acquired or hereditary. An example of acquired thrombophilia is antiphospholipid syndrome [3]. On the other hand, hereditary thrombophilia is a result of genetic mutations affecting the quantity and/or function of the coagulation system proteins.

It must be noted, that not all individuals with hereditary thrombophilia develop venous thromboembolism [5]. However, the risk of venous thromboembolism is increased when in addition to genetic factors there are other risk factors as well, for example, obesity, smoking, cancer, surgery, prolonged immobilisation, pregnancy, etc. Individuals with hereditary thrombophilia may experience thrombosis at an earlier age.

Generally, hereditary thrombophilia is associated with gene mutations that either increase the activity of procoagulant factors or decrease the activity of natural anticoagulants.

The risk of thrombosis is obviously higher when an individual has several mutations associated with thrombophilia.
Besides genetic factors, the role of other factors and their combinations need to be considered as well. For example, the risk of thrombosis is increased if oral contraceptives (containing estrogen) are used in addition to having genetic mutations associated with thrombophilia.

The most common genetic risk factors associated with thrombophilia are factor V Leiden (G1691A) and prothrombin (G20210A) mutations. Other genetic mutations are relatively rare. In any case, a person may be homozygous or heterozygous with regard to the mutated alleles. The risk of venous thromboembolism is higher among homozygous individuals and in those, who have several gene mutations associated with thrombophilia.
Protein C, protein S and antithrombin deficiencies can significantly increase the risk of thrombosis [6].

Overview of the roles of various genetic factors and mutations associated with them

The genes associated with thrombophilia have different roles.
For example, the MTHFR gene codes the MTHFR (methylenetetrahydrofolate reductase) enzyme. This enzyme converts 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate and the mutations of the MTHFR gene may decrease the function of MTHFR. This may lead to an increased risk of thrombosis.

As already noted above, factor V Leiden (G1691A) and prothrombin (G20210A) mutations are the most common ones associated with inherited thrombophilia. As a result of the factor V Leiden mutation, an activated factor V is more resistant to inactivation. This, in turn, increases thrombin

production and contributes to a thrombophilic state [7].

As far as the prothrombin gene mutation is concerned, it can lead to elevated levels of prothrombin and this may contribute to an increased risk of venous thromboembolism.

Mutations of the genes coding for protein C, protein S and antithrombin may result in deficiency of these anticlotting proteins and because of this, these mutations can lead to thrombophilia [1,7].

Antithrombin is a natural anticoagulant produced in the liver [8]. Antithrombin deficiency may be quantitative or qualitative. With quantitative antithrombin deficiency, the quantity of normal antithrombin is decreased. On the other hand, qualitative deficiency means that there is a defect of the antithrombin molecule itself and it cannot perform its function normally.

Protein C regulates coagulation through inactivation of factor Va and factor VIIIa. Protein S also plays an important role in the coagulation system alongside protein C and it stimulates fibrinolysis. With protein C and protein S deficiencies, inactivation of coagulation factor Va and factor VIIIa is impaired, which leads to thrombophilia [7].

Thrombophilia tests

It must be emphasized that routine screening of thrombophilia is not recommended and testing might be useful only for a subset of patients. So it is essential to consider other factors as well and to make sure that the decision to perform thrombophilia testing is justified [9,10].
This is especially important considering the fact that, regardless of thrombophilia test results, often the clinical management of the patients remains unchanged.

PCR testing of thrombophilia may include testing for the following factors:

1. Factor II (prothrombin) G20210A
2. Factor V G1691A (Leiden)
3. Factor V H1299R (haplotype HR2)
4. MTHFR A1298C
5. MTHFR C677T
6. PAI-1 -675 4G/5G

There is a brief description of these tests below.

Factor II (prothrombin) G20210A

This test is used to detect the G20210A mutation of the gene coding for coagulation factor II. Prothrombin (factor II) is a precursor of thrombin. The G20210A mutation of its coding gene is associated with increased prothrombin levels and as a result, with a higher risk of venous thromboembolism.

Factor V G1691A (Leiden)

This test is used to detect the G1691A (Leiden) mutation of the gene coding for coagulation factor V.
Factor V plays an important role in the coagulation system. Normally, it gets inactivated by the activated protein C (APC). However the Leiden mutation makes factor V more resistant to inactivation by the APC. This increases the risk of thrombosis. The risk of venous thromboembolism is even higher for homozygous carriers of this mutation.

Factor V H1299R (haplotype HR2)

This test is used to detect the H1299R (haplotype HR2) mutation of the gene coding for coagulation

factor V.
This mutation is associated with an increased risk of thrombosis, especially among the individuals who have the factor V Leiden mutation as well.

Methylenetetrahydrofolate reductase (MTHFR) A1298C and Methylenetetrahydrofolate reductase (MTHFR) C677T
MTHFR A1298C test is used to detect the A1298C mutation of the gene coding for methylenetetrahydrofolate reductase (MTHFR) and MTHFR C677T test is used to detect the C677T mutation of the same gene.

MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Both C677T and A1298C mutations are associated with decreased function of MTHFR. As a result, homocysteine level in the blood is increased. This, in turn, might be contributing to an increased risk of vascular diseases.

Plasminogen activator inhibitor type 1 (PAI-1) -675 4G/5G
This test is used for genotyping of the -675 4G/5G polymorphism in the gene coding for PAI-1. PAI-1 is an inhibitor of fibrinolysis and its increased level is associated with a higher risk of thrombosis.

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