PHENOTYPIC PRESENTATION OF THROMBOPHILIA IN DOUBLE HETEROZYGOTE FOR FACTOR V LEIDEN AND PROTHROMBIN 20210 G>A MUTATIONS – CASE REPORT

Ljudmila NAGORNI-OBRADOVIĆ1,2, Nela MAKSIMOVIĆ1, Predrag MILIJIĆ1,3, Dragana CVETKOVIĆ1, Ruža STEVIĆ1,5, Dragica PEŠUT1,2

1 Faculty of Medicine, University of Belgrade, Serbia; 2 Clinic for Pulmology, Clinical Centre of Serbia, Belgrade, Serbia; 3 Clinic for Hematology, Clinical Centre of Serbia, Belgrade, Serbia; 4 Faculty of Biology, University of Belgrade, Serbia; 5 Clinic for Radiology, Clinical Centre of Serbia, Belgrade, Serbia


Physicians usually do not suspect pulmonary thromboembolism in younger patients except in those who have thrombophilia. In those latter patients some special conditions such as trauma or surgery may provoke the disease. In some adult persons, thrombophilia may still remain unrecognized, until appearance of additional conditions influence development of thrombosis. A 55-year-old Caucasian female, non-smoker, experienced sudden chest pain and hemoptysis without chest trauma. History taking revealed type 2 diabetes mellitus and hypothyroidism. She was overweight with body mass index 29.0. The review of the family history revealed that her father and mother died of brain infarction, while her 22-year-old son and 24-year-old daughter were healthy. Due to suspicion for thrombosis, multi-slice computerized tomography thorax scan was done and pulmonary embolism was diagnosed. Although without clear risk factor for thrombosis in our patient, we performed laboratory investigation for congenital thrombophilia. Genetic analysis showed double heterozygous for factor V Leiden and prothrombin 20210 G>A mutations. Congenital thrombophilia was risk factor for thrombosis in our patient but haemostatic imbalance was not previously clinically recognized. She had two pregnancies without complications. Appearance of other associative factors such as endocrine disorders - hypothyroidism and metabolic syndrome with diabetes type 2, and overweigh were additional potential triggers for clinical manifestation of pulmonary thromboembolism in her adult age. Her children
underwent genetic analysis, too. The son was also double heterozygous for factor V Leiden and prothrombin 20210 G>A mutations, while daughter was heterozygous for factor V Leiden, and none had clinical signs of thrombosis.

Key words: pulmonary thromboembolism, thrombophilia, risk factors, genetic.

INTRODUCTION

Thrombophilia is defined as an increased tendency to develop thrombosis, which is familial, recurrent, and present at unusual site or manifested in young age. Typical clinical manifestation of thrombophilia is venous thromboembolism (VTE), including deep venous thrombosis (DVT) and pulmonary thromboembolism (PTE). Thrombophilia is a good model of polygenic trait with clearly associated genetic risk factors.

Resistance to activated protein C (APC) is the most common inherited hypercoagulable state found to be associated with venous thrombosis (ROSENDAAL and REITSMA, 2009). It is caused by a single point mutation in the factor V gene known as factor V Leiden (FVL). FVL mutation (1691 G>A) predicts the substitution of arginine (Arg) with a glutamine (Gln) at position 506. Arg 506 is one of three APC-cleavage sites and the mutation results in the loss of this site; as a consequence coagulation cascade continues unchecked. FVL is a common unique gain-of-function mutation, with a prevalence of carriers among Caucasians of approximately 5%. Among patients with venous thrombosis it is found in 20%, and in approximately 50% of patients with familial thrombophilia. The risk of thrombosis is 5-fold increased in heterozygotes, and fiftyfold in homozygotes (ROSENDAAL and REITSMA, 2009).

The substitution guanine to adenine (G>A) in prothrombin gene at position 20210 is the second most common inherited risk factor for VTE (ONER et al., 2003; MARGETIĆ, 2014). It is a variant located in the 3’ untranslated region (UTR) of gene, supposed to have a regulatory role. Mutation predisposes humans towards forming blood clots in the veins and it confers an about 2- to 3-fold higher risk of thrombosis (ROSENDAAL and REITSMA, 2009). About 2 to 3% of Caucasians carry the variant, and it is present in about 6% of all individuals that have venous thrombosis (ROSENDAAL, 2005). The carriers of two mutations in factor V and prothrombin genes (homozygous or double heterozygous) have as much as 20 times higher risk (ROSENDAAL and REITSMA, 2009).

We here report on a 55-year-old female with previously unrecognized congenital thrombophilia until additional triggers for coagulation abnormalities have been associated. The publication of the case is approved by the Ethical committee of the Clinical Center of Serbia in Belgrade (14/4-2014).

CASE REPORT

A 55-year-old female, non-smoker, has been hospitalized for sudden strong thoracal pain and hemoptysis. From personal history: she takes oral therapy for diabetes type 2, as well as hormonal substitution therapy for hypothyreosis. She had two pregnancies without complications and had no miscarriages. Body mass index (BMI) was 29.0. She denies any previous injuries or thrombosis. The review of the family history revealed that her father and mother died of brain infarction.

She was febrile (37.5°C), cardially compensated, with heart rate 70 beats min⁻¹, blood pressure 120/80 mmHg and respiratory rate 18 breaths min⁻¹. Pulmonary auscultatory
examination was normal. Electrocardiogram, routine laboratory findings and arterial blood-gas analysis gave normal results. Blood sedimentation rate was 70 mm h$^{-1}$, C reactive protein was 21 mg/L (reference range 0-8 mg/L), and specific degradation products of cross-linked fibrin (D-dimer) was 4.3 mg/L (reference range 0-0.55 mg/L). Antinuclear antibodies, antineutrophil cytoplasmic antibodies, and antiphospholipid antibodies were not present such as serum tumor markers.

Clinical prediction for PTE ussing revised Geneva score showed low possibility (LEGAL et al., 2006). Color duplex scan of the veins on both extremities showed no signs of thrombosis of deep and superficial veins. But, as she had family history for thrombosis, multislice computed tomography (MSCT) of the lung was performed and it showed thrombosis of lower lobar branch of the right pulmonary artery (Fig.1,2) and occasional thrombosis of segmental branches for upper right and left lobus. In right lower lobe posteriorly and lateraly, subpleural a focal irregular opacities were showed (Fig.2). There were no signs of effusion in pleural, pericardial, intra and retroperitoneal spaces.

Figure 1. Axial thoracic CT scan in mediastinal window shows thrombosis of lower lobar branch of the right pulmonary artery (arrow)
Figure 2. Coronal thoracic CT scan in mediastinal window shows thrombosis of lower lobar branch of the right pulmonary artery (arrow) and subpleural irregular opacitis in right lower lobe (arrowhead).

Therapy started with intravenous heparin, followed by warfarin, keeping the international rate (INR) of prothrombin time at 2-3 range. The patient's clinical state has been gradually improved and over 2 years of follow-up she had no new thrombotic episodes.

Concerning the fact that her parents had brain infarctions, additional genetic examination of hypercoagulable state was done and findings showed that the patient was double heterozygous for FVL and prothrombin 20210G>A mutations. Methylene-tetrahydrofolate-reductase genotype (MTHFR 677 C>T) was wild type homozygous. Analyzes were also performed in her children who had no clinical signs of thrombosis. Her 24-year-old daughter, was heterozygous for FVL mutation, and her 22-year-old son, was double heterozygous for FVL and prothrombin 20210G>A mutations.

Genetic methodology description

Genomic DNA for molecular genetic analyzes was extracted from 5ml of peripheral blood by salting – out method. Detection of factor V Leiden and prothrombin 20210 G>A mutations was done using polymerase chain reaction/ restriction fragments length polymorphism (PCR/RFLPs) method; amplification of the selected gene region by PCR is followed with digestion of PCR products with corresponding restriction endonuclease enzyme in order to observe RFLPs connected to the mutation. For detection of factor V Leiden and prothrombin
20210 G>A mutations restriction endonucleases MnlI and HindIII were used, respectively, and PCR/RFLPs protocols were as described previously (BERTINA, 1994; POORT, 1996; PECHENIUK, 2000). After electrophoresis of restriction fragments on 8% polyacrylamide (PAA) gel genotypes were detected according to the observed patterns.

**DISCUSSION**

PTE is one of the most common undiagnosed conditions affecting hospitalized patients (SOO HOO, 2013). Diagnostic procedure can be challenging with a diverse range of clinical presentations from asymptomatic to death. Various resources are available, such as clinical scoring systems, laboratory data, and imaging studies which help guide clinicians in their workup of PTE. Prompt recognition and treatment are essential for minimizing the mortality and morbidity associated with PTE (TARBOX and SWAROOP, 2013). DVT and PTE are distinct but related aspects of the same dynamic disease process known as VTE. However, clinical history and examination can be notoriously misleading in reaching a diagnosis. A number of acquired etiologic risk factors are associated with a tendency to develop VTE. These include increasing age, immobilization, surgery, trauma, including invasive diagnostic procedures (NAGORNI-OBRADOVIĆ et al., 2009; PEŠUT et al., 2011), hospital or nursing home confinement, malignancy, neurologic disease with extremity paresis, as well as certain types of oral contraception and hormone replacement therapy. In addition, a variety of genetic risk factors, such as FVL and prothrombin mutations, protein S or C deficiency have also been identified (NAGORNI-OBRADOVIĆ et al., 1997).

Genetic testing in thrombophilia is a good example of the power, specificity and sensitivity of molecular genetic testing in disease management (NOVAKOVIĆ et al., 2014). A positive family history is an independent risk factor for VTE that may reflect the presence of a hereditary thrombophilic disorder. However, the predictive value of a positive family history for detection of known heritable causes of VTE is low, suggesting that there are as-yet undiscovered genetic or environmental risk factors that account for the familial clustering of this disorder (TARBOX and SWAROOP, 2013; EIKELBOOM and WEITZ, 2011; BEZEMER, 2009).

Presented female Caucasian, did not have clear risk factors for thrombosis such as posttraumatic state, recent immobilization or others. She could be misdiagnosed for PTE but she had positive family history for thrombosis, and it was a reason for doing laboratory investigation for thrombophilia. Today, thrombophilia testing is most commonly performed in young patients with VTE, patients with recurrent episodes of VTE or with thrombosis at unusual sites and in persons with positive family history (MITIĆ, 2014). Molecular genetic testing is today part of modern medical practice. In our patient genetic analysis showed double heterozygous for FVL and 20210G>A, a mutation in prothrombin. Positive family history for VTE in a first-degree relative increases the risk for VTE occurrence by 2-fold, regardless of the presence of inherited thrombophilia (MITIĆ, 2014). Patients in whom thrombophilia testing is still debated include those with unprovoked VTE over 50 years of age. Although age over 50 years is considered to be exclusion risk factor for testing, it is described that relatively weak hereditary risk factors such as heterozygosity for FVL and prothrombin 20210G>A mutations may results in a first VTE also in subjects older than 50 years (MITIĆ, 2014; DE STEFANO, 2003). On the other hand, testing of the patients aged 50 years or more might be important from clinicians point of view in terms of therapy duration (PEŠUT et al., 2011). Family history is a potentially useful genetic
surrogate marker for clinical VTE risk assessment, even in second- and third degree-relatives (Zöller, 2013).

One of the laboratory useful markers for PTE is plasma D-dimer level (Kline, 2008; Goldhaber and Bounameaux, 2012) which is usually elevated as in our patient. But normal plasma D-dimer level do not exclude PTE such as described in patient with atypical chest bilateral nodular infiltrates and prothrombin 20210G>A mutation (Nagorni-Obradović et al., 2009).

Why has not our patient developed PTE at younger age? Although VTE at a young age is an important feature of thrombophilia, the first thrombotic episode may happen later in life (Margetić, 2014). Some persons with thrombophilia do not experience a thrombotic event if additional triggering transient risk factors are not present. Interactions between hereditary or acquired thrombophilic defects and transient risk factors further increase the risk of VTE as a result of their synergistic interactions (Nagorni-Obradović et al., 2000). In presented person with previously unknown hereditary risk for thrombosis, additional triggers appeared and influenced her coagulation state. Patients with clinically hypothyroidism appear to have an increased risk of bleeding, whereas those with hyperthyroidism are more likely to be prone to thrombosis. At present, very little information is still available on haemostatic abnormalities in patients with subclinical hypo- or hyperthyroidism (Franchini et al., 2010; Squizzato et al., 2007; Franchini et al., 2009). Future clinical trials on larger series of patients are undoubtedly required to better clarify the haemostatic abnormalities in patients with thyroid dysfunctions. It is suggested that the influence of thyroid dysfunction on coagulation and fibrinolysis mainly depends on the type of thyroid disorder.

As our patient had diabetes mellitus type 2 and higher BMI, there were another potential risks factors for hypercoagulable state. Metabolic syndrome is frequently associated with a hypercoagulable condition, in that the coagulation system is switched toward a prothrombotic state, involving increased plasmatic coagulation, reduced fibrinolysis, decreased endothelial thromboresistance, and predominantly platelet hyperactivity (Franchini et al., 2010; Cornier et al., 2008). For a long time, obesity has been regarded as a risk factor for VTE (Braekkan et al., 2013). The risk positively correlates with BMI.

After the age of 50, the other factors for VTE appear to play a greater role than hereditariness. Epidemiologic studies showed that the highest risk occurs if both parents had clinical manifestation of VTE. For that reason family history of VTE is an important risk factor for thrombosis and this data should be included in the clinical medical history and further examinations for thrombophilia (Soo Hoo, 2013; Eikelboom and Weitz, 2011; Zöller, 2013; Pešut et al., 2014).

Although her children did not have clinical findings of thrombosis, we conducted genetic testing for thrombophilia in them. The results showed heterozygous for FVL mutation in daughter and son was double heterozygous for prothrombin 20210 G>A mutation and FVL.

Like their mother, children may not develop thrombosis at their young age, but it can be useful for them to know data about hemostasis abnormalities. Trauma and surgery can provoke thrombotic event in both daughter and son with defined hereditary thrombophilia, but the risk might be higher in son who carries two mutations. In addition, physicians should pay attention on women in fertile age with positive thrombosis family history and presence of thrombophilia. The condition may benefit from thromboprophylaxis implementation during pregnancy. The
women should be advised not to use oral contraceptives to prevent thrombotic event (MARGETIĆ, 2014; MITIĆ, 2014).

CONCLUSION

Thrombophilia testing should be considered in patients with family history of VTE. Presented patient with hypothyroidism, diabetes mellitus type 2 and overweight had transient risk factors which may influence haemostatic abnormalities and PTE in previously undetected thrombophilia with double heterozygous for FVL and prothrombin 20210G>A mutations. Physicians should consider potential associated factors for thrombosis much more, which in one moment could be the triggers for clinical manifestation of PTE in adults due to congenital

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education and Science of Serbia, contact ON 175081, 2011–2014 and ON 175091, 2011–2014

Received April 28th, 2014
Accepted July 28th, 2014

REFERENCES


Ljudmila NAGORN-I-OBRADOVIĆ1,2, Nela MAKSIMOVIĆ1, Predrag MILIĆ1,3, Dragana CVETKOVIĆ1, Raža STEVIĆ1,5, Dragica PEŠUT1,2

1 Medicinski fakultet Univerziteta u Beogradu, Beograd, Srbija; 2 Klinika za pulmologiju, Klinički centar Srbije, Beograd, Srbija; 3 Klinika za hematologiju, Klinički centar Srbije, Beograd, Srbija; 4 Biološki fakultet Univerziteta u Beogradu, Beograd, Srbija; 5 Klinika za radiologiju, Klinički centar Srbije, Beograd, Srbija

Izvod


Odobreno 22. VII. 2014.