Compound Heterozygosity for the C677T and A1298C Mutations of the MTHFR Gene in a Case of Hyperhomocysteinemia with Recurrent Deep Thrombosis at Young Age

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We report a case of a young woman with an extensive, recurrent deep vein thrombosis (DVT) diagnosed by CT scan and duplex ultrasound examination. All blood investigations for etiology of recurrent DVT were normal except for serum homocysteine level, which was mildly increased. No other thrombophilic factors could be found. The three main causes of hyperhomocysteinemia are genetic defects, nutritional deficiencies and insufficient elimination. In our case a genetic defect for one of the key enzymes of homocysteine metabolism was found to be the underlying cause. Oral anticoagulation and supplementation with pyridoxine, cyanocobalamine and folate was recommended. Whether therapy with B vitamins and folate can substantially reduce the recurrence of venous thromboembolic disease remains to be established.

Venous thrombosis represents a multifactorial disease. Many genetic and acquired conditions such as coagulation factors defects, trauma, surgery, birth, obesity and malignancy may predispose to venous thrombosis [1]. Although hereditary deficiencies of endogenous anticoagulants like protein C, protein S and antithrombin III or resistance to activated protein C have been well recognized as risk factors for venous thrombosis, they are uncommon even in patients with familial thrombosis. It is therefore important to reassess the risk stratification of venous thrombosis and to take into consideration that multiple factors may coexist and influence the thrombophilic status [2]. Mild to moderate hyperhomocysteinemia (HHC), meaning mildly to moderatly increased plasma homocysteine (Hcy 15–50 μmol/L), is uncommon in general population. This condition is caused by either genetic factors (mutations of homocysteine metabolism enzymes) or acquired conditions, such as deficiencies in B vitamins, renal insufficiency and some medications [1]. High homocysteine plasmatic levels have been associated with arterial and venous thrombosis. Several clinical studies support that HHC is associated with increased risk of cardiovascular diseases, atherothrombotic stroke and peripheral vascular disease. There are also consistent studies in the literature that attest the relation between HHC and venous thromboembolism [3–5].

Homocysteine is a metabolic derivative from methionine. Methylene tetrahydrofolate reductase (MTHFR) participates in regulating homocysteine metabolism, and a mutation of MTHFR may be a marker for possible elevated homocysteine levels when the serum folate level was lower than the reference range. Two common mutations involving the MTHFR gene have been identified: C677T and A1298C. The C677T mutation in MTHFR gene is one of the causes of elevated homocysteine levels in plasma and is responsible for a thermolabile variant of the enzyme, with reduced activity [6].

CASE PRESENTATION

We present the case of a 21 years-old patient admitted in our Cardiology Department with a clinical picture of proximal deep venous thrombosis (DVT) of the left inferior leg installed 12 days before admission.

Her **medical history** started three years ago when she had the first episode of deep venous thrombosis involving the right inferior leg (occurring during treatment with oral contraceptives) for which

she received oral anticoagulation which was discontinued after three months. Three months before the present admission she was admitted in another hospital for the diagnostic evaluation of a recent-onset ascites. The paraclinical tests showed that the ascites fluid was a transsudate, and were associated with signs of portal hypertension (first degree esophageal varices and splenomegaly). The work-up for viral hepatitis and systemic lupus were negative. The laboratory tests showed mildly increased hepatic enzymes (AST = 41 U/L, ALT = 37 U/L), but failed to show hypoproteinemia or coagulation disorders. The clinical picture regressed after 3 weeks of supportive treatment (with spironolactone 50 mg and vitamins), and a diagnosis of cryptogenic cirrhosis was established at that time.

Clinical exam at the present admission revealed a patient with normal body mass index, no fever, normal lung murmur, a blood pressure of 110/70mmHg, heart rate 100 bpm. The right inferior leg presented a mildly increased diameter with skin of normal color and temperature, due to post-thrombotic syndrome, while the left inferior leg showed significant edema and tumefaction both at the level of the thigh and the calf, with a circumference difference of 3 cm at the thigh level.

Electrocardiogram showed sinus rhythm, HR=100 bpm, QRS axis=+75°, with no conduction defects or ST-T changes.

Echocardiography revealed normal dimensions of cardiac chambers, normal left and right ventricular function and no signs of pulmonary hypertension.

Biological tests were within normal limits except for the homocysteine level which was mildly elevated (15.6 μmol/L; normal values 1–11 μmol/L). Urinary analysis showed proteinuria of 1.1 g/24 hours, without other changes. The search for a causative and underlying hypercoagulable state was performed. Routine coagulation test results were within normal limits. Anticardiolipin antibodies and lupus anticoagulant tests were negative. Thrombophilia tests showed that protein C, protein S and ATIII levels were within normal limits.

DNA study. Genomic DNA was extracted from the blood sample and analyzed for the mutations by real-time PCR (LightCycler) and melting curve analysis. The patient was screened for the factor V Leiden mutation, prothrombin gene mutation and MTHFR gene polymorphism. The patient was detected heterozygous for the C677T and A1298C mutations of the MTHFR gene. No 20210G/A mutation of the prothrombin gene or factor V Leiden mutation were detected.

Serum C3 complement was normal and the **tumoral markers** AFP, CEA, CA 19–9, CA 15–3, CA125 were also within normal limits. Work-up for autoimmune diseases was unremarkable.

Peripheral **venous duplex ultrasound** revealed thrombosis of the left common iliac vein with extension on the external iliac vein, common femoral vein, superficial femoral vein and popliteal vein with collateral circulation developed at the popliteal level, also confirming post-thrombotic syndrome of the right inferior limb with a residual thrombus of 3 mm at the level of the right popliteal vein.

Abdominal ultrasound confirmed the splenomegaly (long-axis =14.6cm), and found right kidney atrophy (5/2.5cm) with compensatory left kidney hypertrophy (16/6cm). Moreover, a venous dilatation in the hepatic hilum was found, followed by a hypoplastic aspect of the portal vein which had a diameter of 4 mm at the cephalic pancreatic level, these findings raising the differential diagnosis between a portal cavernoma and a pseudo-cavernoma as a result of a recanalized portal vein thrombosis (Fig. 1). We therefore performed a **CT scan** with contrast, which confirmed the portal cavernomatous aspect in the hepatic hilum (Fig. 2), showed partial thrombosis of superior mesenteric vein and portal vein with portal hypertension (esophageal and perigastric varices and collateral circulation in the abdominal wall) (Fig. 3), hepato- and splenomegaly, and segmental partial thrombosis of inferior vena cava at the infrarenal level (Fig. 4).

The severe and recurrent proximal venous thrombosis episodes, without data for a secondary cause (malignancy, autoimmune or other systemic disease, drugs, etc.), together with the laboratory evidence of hyperhomocysteinemia, called for a more detailed search for genetic causes, which revealed two heterozygote mutations for the MTHFR gene, the patient associating both C677T and A1298C polymorphism.

In the absence of any other systemic cause for proteinuria, which was repeatedly below a nephrotic level, while kidney biopsy was not possible during essential anticoagulation treatment, this finding was

interpreted as a result of hyperfiltration by a hypertrophic left kidney (compensatory to the congenital atrophy of the right kindney).

The **final diagnosis**, based on the medical history, clinical exam and laboratory tests was: *Left inferior leg deep venous thrombosis. Partial thrombosis of infrarenal inferior vena cava. Portal hypertension due to portal vein thrombosis. Post-thrombotic syndrome of right inferior leg. Compound heterozygosity for the C677T and A1298C mutations of the MTHFR and hyper-homocysteinemia.*

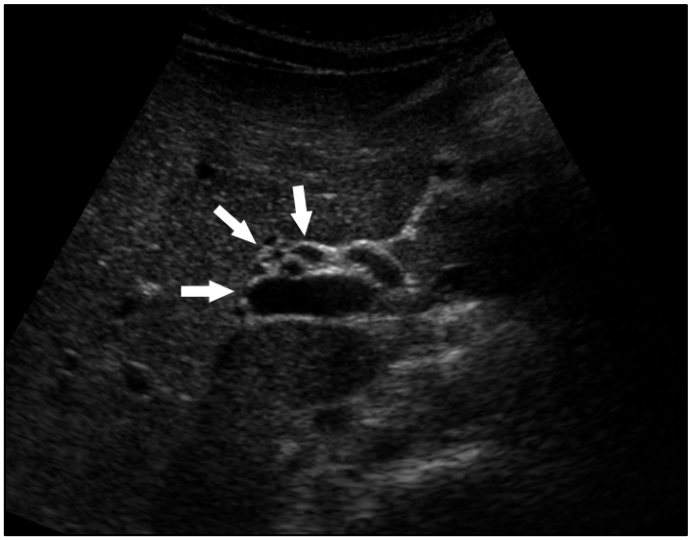

Fig. 1. –Echocardiographic B-mode view at the level of the hepatic hylum showing multiple vascular trajectories (arrows).

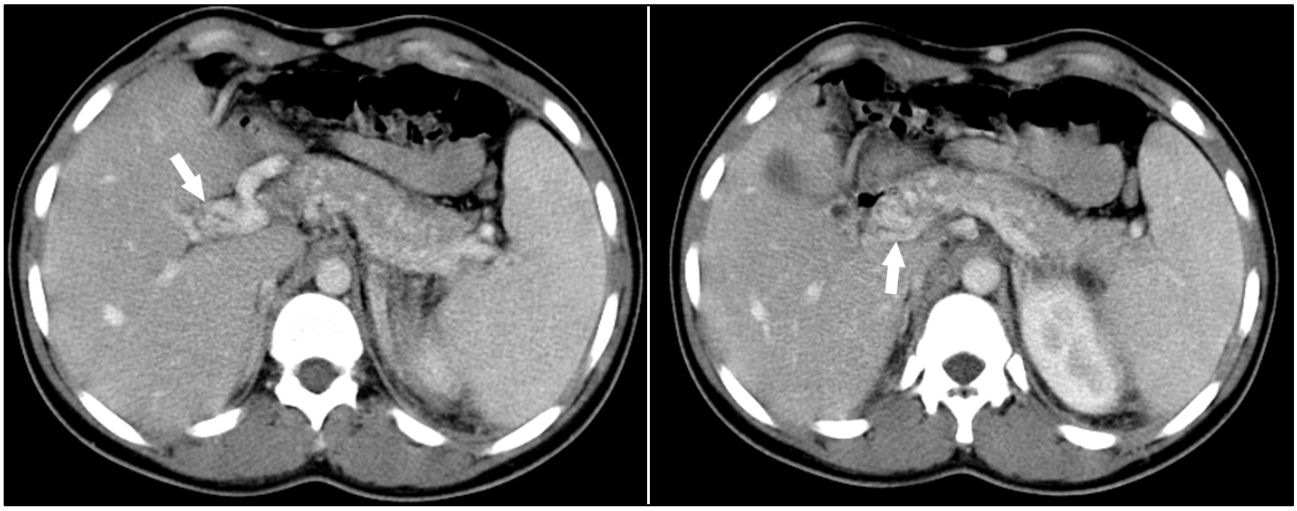

Fig. 2. – Abdominal CT slices after contrast injection showing multiple vascular trajectories at the level of the hepatic hylum (suggesting a portal pseudocavernoma) (arrows), without visualization of the initial part of the portal vein.

DISCUSSION

In this young female patient of reproductive age, identifying potentially curable causative mechanisms of the extensive recurrent venous thrombosis was very important, as well as assessing the risk for thrombosis recurrence. While the first DVT episode appeared to be triggered by oral contraceptive treatment, the 2 recurrent proximal severe DVT episodes were not associated to any trigger event.

Many case-control and cross-sectional studies revealed that HHC is prevalent in patients with first episode of peripheral deep venous thrombosis or in patients with recurrent thrombosis. At present, HHC is considered to represent a risk factor for DVT and a common risk factor for recurrent venous thrombosis [7][8]. There are many hypotheses which have been proposed to explain how HHC may lead to venous thrombosis. One hypothesis is that it has a toxic effect on the vascular endothelium and this reduces the activation of protein C, which leads to a

prothrombotic status. Another potential negative effect of HHC is the abnormal methionine metabolism which affects the methylation of DNA and cell membranes that stimulates the clotting cascade [9]. Ebbesen *et al*. revealed that the whole blood coagulation profile was influenced by high plasma HHC by (1) prolonging the initiation phase, (2) increasing the velocity of the coagulation propagation and (3) increasing the maximum clot firmness. These changes in the blood coagulation profile may contribute to the increased risk of thrombosis in hyperhomocysteinemic individuals [7][9][10].

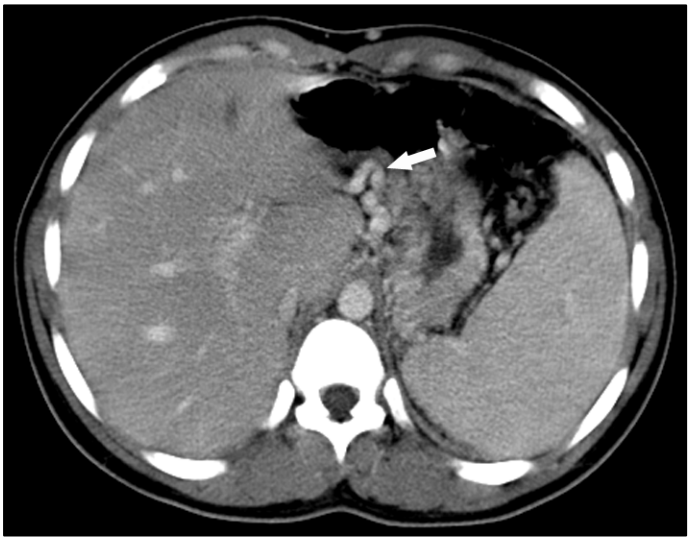

Fig. 3. – Abdominal CT showing splenomegaly and rich peri-gastric collateral circulation (arrow).

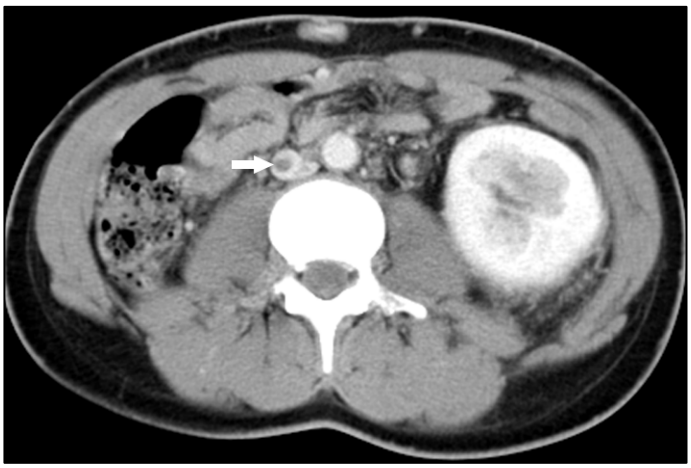

Fig. 4. – Contrast CT showing a lacunar image at the level of the distal part of the inferior vena cava, suggesting thrombosis (arrow). Collateral circulation at the level of the anterior abdominal wall.

We found a genetic cause to be the mechanism of HHC in our patient. Heterozygotic status for two polymorphisms of the MTHFR gene, the thermolabile C677T and A1298C polymorphism, were found and the presence of this combination is known to be associated with HHC [11].

For this genetic disorder there is no evidence-based treatment which could prevent the recurrence of the thrombosis [12][13] but there are studies which suggest that the vitamin supplementation with folic acid, vitamin B6 and B12 may help in lowering the homocysteine concentrations, and even in reversing endothelial dysfunction regardless of the underlying cause of HHC [7].

The patient was dismissed from the hospital with indications of long-term oral anticoagulation (aiming to an international normalized ratio between 2 and 3), as well as folic acid, vitamin B6 and vitamin B12 supplements. Counseling regarding contraceptive methods, pregnancy-related thrombotic risks and anticoagulation attitude during a possible pregnancy were explained to the patient.

The **particularities** of the present case were the recurrence of thrombotic events and the extensive proximal thrombosis in a young patient with only one identifiable cause for the thrombophilic status – hyperhomocysteinemia due to two heterozygotic mutations in the MTHFR gene.

In **conclusion**, portal and mesenteric vein thrombosis is an uncommon event, even in patients with known inherited predisposition to venous thrombosis. It seems appropriate to investigate such patients for the possible coexistence of multiple predisposing factors for thrombosis, including measurement of the serum homocysteine level, in addition to investigations for mutations of the MTHFR, the prothrombin and the factor V genes.

Prezentăm cazul unei paciente tinere diagnosticată prin examen computer tomografic şi examen ultrasonografic duplex cu tromboză venoasă profundă extensivă, recurentă. Investigaţiile biologice efectuate pentru identificarea etiologiei acestei patologii au exclus prezenţa factorilor trombofilici clasici, singura modificare patologică fiind prezenţa unui nivel uşor crescut al homocisteinei serice. Cele trei cauze principale ce pot determina hiper-homocisteinemie sunt defecte genetice, deficite nutriţionale şi, respectiv, eliminarea insuficientă. În cazul de faţă cauza hiperhomocisteinemiei s-a dovedit a fi prezenţa unui defect genetic la nivelul unei gene ce codifică o enzimă cu rol esenţial în metabolismul homocisteinei. Pacientei i s-a recomandat tratament anticoagulant oral, la care s-a adăugat şi tratament cu suplimente de vitamine B6 şi B12 şi acid folic. În prezent nu este dovedit efectul terapiei cu vitamine B şi acid folic în reducerea riscului de recurenţă a bolii tromboembolice venoase.

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