The procoagulant status. Hypercoagulability as a risk factor of primary and secondary infertility

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Abstract
Thrombophilia is a disorder that makes patients susceptible to intravascular thrombosis that may increase the risk of developing a pregnancy on a known pathology. The female patient diagnosed with hypoplastic uterus and hereditary thrombophilia had a favorable evolution under properly administered anticoagulant treatment. The homozygous status for the C677T mutation may lead to an increase in plasma homocysteine levels, especially in pregnant women, being an associated risk factor for thrombosis. The risk of developing intravascular thrombosis requires primary prevention measures by adding D-dimers in the early diagnostic algorithm, being the most accurate marker of hypercoagulability and endogenous fibrinolysis. The corroborating of the hypercoagulability status with the results of genotyping, the frequencies of the minor/major alleles studied, single mononucleotide polymorphisms (SNPs) and the establishment of preventive therapy, aims to prevent intravascular thrombosis and thromboembolic phenomena.

Keywords: D-dimers, hypercoagulability, thrombosis.

Introduction
Today, infertility and recurrent miscarriage are serious problems with growing clinical concern. The efforts of modern medicine are concerted to reduce the level of infertility worldwide. It is estimated that approximately a third of the causes of infertility are due to female factors [1].

The presence of a hereditary abnormality in the coagulation–fibrinolysis system associated with an additional risk factor such as pregnancy, surgery, trauma, smoking, venous stasis, atherosclerosis, or consumption of contraceptive pills predisposes to the onset of the thrombotic process [2–4].

In recent decades, we have found many data on the association between a hypercoagulable condition and its causes and one of the adverse outcomes of pregnancy – recurrent pregnancy loss (RPL).

Early studies focused on the association between thrombophilia and RPL have highlighted the role of reduced clotting inhibitors in RPL. Subsequently, studies have highlighted a pathogenic role of the gene variant associated with hypercoagulable status in the occurrence of RPL.

During pregnancy, there are various changes in the hemostatic balance of women with a tendency to thrombophilia, a necessary change for the hemostatic challenge of childbirth [5].

Thrombophilia is a disease caused by various defects, in which the combination of procoagulant defects or acquired factors play an important role [6].

Inherited thrombophilia is a genetic condition to suffer various clinical thrombotic events, with frequent recurrences without apparent cause. This entity is defined as a primary risk factor for abnormal pregnancy due to different genes: G1619A (Factor V Leiden), R2 H1299R (Factor V Leiden polymorphism), A1298C [Methylenetetrahydrofolate reductase (MTHFR) enzyme mutation], C677T (MTHFR polymorphism), V34L (Factor XIII polymorphism), G20210A (mutation of the prothrombin gene), a/b L33P (ribosomal polymorphism of MTHFR enzyme) and 4G/5G [plasminogen activator inhibitor-1 (PAI-1)] [7].

Although many studies have shown an association between thrombophilia and embryonic/fetal loss, there are several other studies that have not shown such an association [6].

On the other hand, acquired thrombophilic abnormalities as acquired C protein, S protein, antiphospholipid syndrome, antithrombin III deficiency, drugs induced thrombophilia are a well-known cause of RPL. All should be considered for a screening.

These data should be considered, as recent studies...
have suggested the role of extensive thromboprophylaxis in females with RPL, which should only be addressed in case of increased risk of venous thromboembolism and known thrombophilia. However, the increase in D-dimer has often been associated with subclinical thrombophilia, due to its high predictive negative value in case of suspected thrombosis [8].

Aim

This presentation aims to educate patients in preventing the thrombosis process stirred by cause of stasis, venous parietal lesions, and hypercoagulability phenomena in the deep and placental venous system, as well as in the prevention of embolic potential, which can endanger the evolution of the pregnancy due to an existing pathology. In this case, our patient diagnosed with uterine hypoplasia, having three previous miscarriages, manages to bring to maturity the fourth pregnancy after performing the thrombophilia profile and following appropriate anti-coagulant treatment throughout the pregnancy.

Patient, Materials and Methods

We present the case of the 37-year-old female patient, referred to as ‘TG’, from an urban area, 40 kg, in the medical records of the Filantropia Municipal Hospital, Craiova, Romania, diagnosed with uterine hypoplasia, having a history of three miscarriages. The patient underwent clinical evaluations and paraclinical explorations: molecular techniques [real-time polymerase chain reaction (PCR) sequencing], hematological, biochemical, immunological, bacteriological tests, the biological material studied was blood collected in containers with anticoagulant [Ethylene-diaminetetraacetic acid (EDTA), respectively 3.8% Sodium Citrate], as well as without anticoagulant and endocervical secretion.

The hybridization reactions used to identify nucleic acids in the biological product were performed in two steps: denaturation of the nucleic acid in the research sample followed by hybridization of the chains obtained with known nucleic acid sequences.

Southern blot, the deoxyribonucleic acid (DNA) visualization method was performed after its separation into fragments with restriction enzymes, followed by polyacrylamide gel electrophoresis, transfer to the nitrocellulose membrane, and hybridization with labeled probe and autoradiography detection.

PCR, the highly sensitive cyclic method of amplifying specific DNA sequences, required a set of primers (short oligonucleotide sequence) and a heat-resistant DNA polymerase. Each cycle comprised three stages: denaturation of the DNA in the target sequence, hybridization of complementary primers, and extension of the primers using polymerase.

The obtained DNA sequences (amplicons) were then identified by hybridization with labeled probes or by restriction fragment length polymorphism analysis (RFLP). RFLP was based on electrophoretic analysis of nucleic acid fragments obtained by cleavage using restriction enzymes.

Hematological, biochemical, immunological, and bacteriological investigations were performed according to the working techniques corresponding to the reagent of the manufacturing company.

The pregnant woman was constantly monitored through medical imaging techniques, and at birth the placenta was sent to the Department of Pathological Anatomy for the histopathological (HP) examination.

For HP examination, classical Hematoxylin–Eosin (HE) staining was used to identify placental elements, Periodic Acid–Schiff (PAS)–Hematoxylin (PAS-H) staining to highlight basement membranes and massive fibrinogen deposits and special immunohistochemical staining with the anti-cluster of differentiation 34 (CD34) antibody was used to see the infarcted areas without vascularization (Dako, monoclonal mouse anti-human CD34 Class II, clone QBE10, antigenic exposure 1:50 Citrate buffer, 1:50 dilution, labeling neoformed blood vessels).

Case presentation

The results of the paraclinical investigations indicate the homozygous genotype for the C677T mutation and the PAI-1 gene (675 4G/4G polymorphism), and the coagulation tests confirm hypercoagulability (Table 1).

| Investigation type | Investigation | Mean ± SD | Positive results |
|-------------------|--------------|-----------|-----------------|
| Antiphospholipid syndrome profile | Anticardiolipin antibodies IgG [GPL/mL] | 3.6±0.08 | – |
| | Anti-beta2 glycoprotein 1 antibodies IgG [U/mL] | 2.4±0.07 | – |
| | Anti-beta2 glycoprotein 1 antibodies IgM [U/mL] | 1.2±0.02 | – |
| | C protein [%] | 108±1.77 | – |
| | C protein [%] | 110±1.91 | – |
| | S protein [%] | 103±1.32 | – |
| | Factor V Leiden mutation | Negative | – |
| Hereditary thrombophilia profile | MTHFR gene | C677T mutation | Homozygous genotype |
| | A1298C mutation | Negative | – |
| | PAI-1 gene | 67S 4G/5G polymorphism | Risk of thrombophilia |
| | | 67S 4G/4G polymorphism | Homozygous genotype |
| Bacteriology | Chlamydia, Mycoplasma hominis. Ureaplasma urealyticum | Negative | – |

GPL: IgG phospholipid units (1 GPL unit = 1 μg of IgG antibody); IgG: Immunoglobulin G; IgM: Immunoglobulin M; MTHFR: Methylene-tetrahydrofolate reductase; PAI-1: Plasminogen activator inhibitor-1; SD: Standard deviation.
The patient is a homozygous carrier of deletion in the PAI-1 gene promoter. The genetic analysis report identifies in the mother an elongated secondary constriction on the long arm of chromosome 9, normal karyotype with 46, XX,9q+, while the paternal chromosomal map does not indicate anomalies in number or structure, 46,XY karyotype.

The evolution during the last pregnancy was spectacular by supplementing the medication with anticoagulant (Clexane 0.4 UI/mL), along with antiplatelet therapy (Aspenter 75 mg), tocolytic therapy (Magnesium Sulfate) and progesterone therapy (Arefam 200 mg). Clexane’s interference with thrombin is insignificant, as evidenced by Quick time (QT), international normalized ratio (INR) values before and after administration. The effective antithrombotic action was due to the overpowering inhibition of activated Factor X and the activation of the circulatory and parietal fibrinolytic system, complemented by the antiplatelet mechanism of Aspenter.

Following anticoagulant therapy, laboratory tests showed that the value of D-dimers decreased from 4.3±0.05 μg/mL to 0.48±0.01 μg/mL, QT increased from 11.0±0.15 s to 12.3±0.31 s, INR increased from 0.77±0.09 to 0.92±0.12, the sideremia increased from 116.0±3.38 μg/dL to 121.1±3.42 μg/dL, and the serum ferritin value increased from 36.34±0.55 ng/mL to 40.3±0.65 ng/mL, thus observing the importance of anticoagulant, vitamin, and antiplatelet treatment (Table 2).

| Variable (mean ± SD) | Before medication | After medication: Clexane, vitamin B1, B6, Aspenter, vitamin C |
|----------------------|------------------|-------------------------------------------------------------|
| D-dimers [μg/mL]    | 4.3±0.05         | 0.48±0.01                                                   |
| QT [s]              | 11.0±0.15        | 12.3±0.31                                                   |
| INR                 | 0.77±0.09        | 0.92±0.12                                                   |
| Sideremia [μg/dL]   | 116.0±3.38       | 121.1±3.42                                                  |
| Serum ferritin [ng/dL] | 36.34±0.55 | 40.3±0.65                                                   |

INR: International normalized ratio; QT: Quick time; SD: Standard deviation.

All biological parameters before medication signal a state of hypercoagulability.

After birth, the HP examination showed at the level of the placenta frequent stem villosities of variable sizes with fibrous stroma, sometimes zonal or circumferential fibrinoid necrosis and massive calcification zone (Figure 1, A and B), frequent mature intermediate villosities and of terminal type with angiomatous appearance with stasis, rare syncytial buds, dilated umbilical vein, thrombosis, lesions of endothelial discontinuity, middle tunic dissociated from interstitial edema (Figure 2B), areas of hemorrhagic necrosis that alternate with areas with intervillositary blood infiltrates (Figure 3A) and areas of placental infarction, fibrosed, with lack of immunolabeling of vascular endothelial cells are observed (Figure 3B).

**Discussions**

Thrombophilia includes several states of hypercoagulability, which can lead to intravascular thrombosis. The causes of thrombophilia can be inherited (hyperactivity of coagulation, deficiency of the anticoagulant system) or acquired, which carry out a procoagulant status [9–11].

Antiphospholipid syndrome is one of the causes of thrombophilia. Among the manifestations of this syndrome, increased titers of antibodies against associated plasma proteins and anionic membrane phospholipids, cause venous or arterial thrombosis or other complications that can lead to pregnancy termination [12, 13].

In the case of our patient, the paraclinical examinations refuted the antiphospholipid syndrome profile, so it is excluded as the cause of thrombophilia, antibodies specific to the investigation of the antiphospholipid syndrome profile [anticardiolipin antibodies immunoglobulin G (IgG), anti-beta2 glycoprotein 1 antibodies IgG, anti-beta2 glycoprotein 1 antibodies immunoglobulin M (IgM)], being within normal limits.

In an individual suffering from hereditary trauma, even surgery constitutes an associated risk factor for thrombosis. D-dimer dosing has a negative predictive value for deep vein thrombosis of the lower limbs and pulmonary thromboembolism in the sense that obtaining a negative result in patients suspected of these diseases excludes their presence in a percentage of over 90%.
There are several conditions in which it has been observed an increase in D-dimer without ongoing thrombosis as well as infections, chronic inflammation, malignancy, necrosis, acute coronary syndromes. We performed this analysis starting from the role of dimer d in the diagnostic screening of women who associated thrombophilia and sterility [8, 14].

Ever since Brenner et al. (1999) mentioned that over 40% of women with RPL have the main cause of thrombophilia, the medical world was concerned about the pathogenetic role of thrombophilia inherited in this category of women [15].

Five years ago, it was mentioned that inherited thrombophilia presents different patterns in different ethnic groups, suggesting that there is no difference between patients with two abortions and patients with three or more abortions [16]. Our patient diagnosed with inherited thrombophilia had three abortions.

One of the risk factors for RPL is thrombophilia gene polymorphism, and this theory is proved in our patient. Probably, Caucasian race females with RPL risk have this thromophilic genes polymorphism, as it is mentioned in a few studies.

The Barut et al. study was conducted between 2012 and 2016 to decipher, in Turkish women, the importance of mutations in genetic polymorphisms for the impact of thrombophilia (homozygous/heterozygous) on RPL. In the peripheral blood samples of these patients, several genetic mutations were found – of prothrombin G20210A, Factor V Leiden H1299R, MTHFR A1298C, MTHFR C677T, PAI-1 4G/4G and PAI-1 4G/5G [17]. Factor V Leiden was not found in the patient studied, although it is thought to be responsible for more than three-quarters of the inherited activated C protein resistance, more precisely it is the most common inherited thrombotic risk factor associated with RPL [17].

A study published in 2018 concluded that both MTHFR C677T and Factor V Leiden polymorphisms were significantly associated with RPL, in contrast to the lack of significant association between PAI-1 4G/5G polymorphisms and prothrombin G20210A in Bosnian women [18].

Similar results were mentioned by other authors who stated that prothrombin A20210G and/or Factor V Leiden are two genetic variants commonly associated with RPL [4, 19]. These gene cues were not highlighted in our patient.

The C677T homozygous mutation of the MTHFR gene may promote thrombosis [20]. Thromboembolism can
generate cardio-respiratory failure in an organism, with a
genetic pathology and an increased risk of thrombosis,
which is why anticoagulant treatment and monitoring of the
patient using coagulation tests have been recommended
[21].

The homozygous status for the C677T mutation may lead
to increases in plasma homocysteine levels, especially
in patients with low folate and B vitamins. Hyper-
homocysteinemia (HHCY) is a risk factor for arterial,
venous thrombosis and miscarriage, hence balancing the
homocysteine levels through adequate vitamin replacement
and regular control of homocysteinemia is recommended.
Associated risk factors, such as immobilization, trauma,
surgery, pregnancy, smoking, obesity, use of oral contra-
ceptive pills may lead to a marked predisposition to thrombosis.
The role of this mutation as a risk factor for coronary heart
disease, myocardial infarction and preeclampsia is
obvious [22].

The pathogenetic role of HHCY in RPL has been
studied worldwide. The results published in the literature
on the relationship between HHCY, MTHFR C677T gene
polymorphism and RPL are not in fact unambiguous, the
possible explanation being due to the ethnicity of the
patients [15, 23].

Thus, recently developed techniques in genetic screening,
such as next-generation sequencing, are a better choice to
detect variants of genetic risk for thrombosis in ethnic
groups [24].

Not to be overlooked is the fact that folic acid
supplementation during pregnancy may lead to a transient
reduction in homocysteine concentration [15].

Sterility conditioning by MTHFR gene polymorphism
and homocysteine-associated metabolism it is also possible
through a vicious lifestyle, with a diet low in vitamin B12
and folic acid [8]. The role of MTHFR C677T polymorphism
in pregnancy complications as preeclampsia, placental
infarcts, fetal growth restriction was confirmed in several
other studies [25].

The homozygous status for PAI-1 gene promoter deletion
(4G/4G mutant status) promotes preeclampsia in pregnant
women. Deletion of guanosine in position 675 of the PAI-1
gene leads to an increase in the level of PAI-1 in the
blood, being a risk factor for thromboembolic disorder.

The carrier status of the 4G allele is an element of risk
for thromboembolic disorder and is also correlated with
a high blood pressure and increased risk of myocardial
infarction, and the transmission of the genetic defect is
autosomal recessive, so a family check-up is recommended.
Congenital diseases, in general, aggravate the clinical-
biological evolution of the pregnant woman and the
embryo [26].

Provisional to the studies cited in medical literature,
there is no direct correlation between gene polymorphisms
involved in folate metabolism, the process of fibrinolysis
and the risk of miscarriage [27, 28].

Although it is certainly established that the women’s
predisposition to RPL correlates with the presence of
thrombophilic polymorphisms, future research is needed
to determine the timing of anticoagulant treatment in people
with these mutations [18], as we demonstrated in our
patient management – the clinical result can be improved
by applying an antithrombotic treatment during pregnancy.

Anticoagulant treatment is indicated when there is
a history of at least two miscarriages/two pregnancies
stopped evolving without another determining cause or
when associative thrombotic phenomena occur [29]. The
patient diagnosed with uterine hypoplasia and thrombo-
ophilia, under correctly administered anticoagulant treatment
and monitoring had a favorable evolution and gave birth
to a male fetus at 38–39 weeks of gestation. Learning the
habits of maintaining good health, by avoiding additional
risk factors, is an important element in maintaining general
health.

Conclusions

The severity of the consequences induced by the presence
of intravascular thrombosis, justifies primary prevention
measures, corroborated with the need to add D-dimers in
the early diagnostic algorithm, being considered the most
accurate marker of hypercoagulability and endogenous
fibrinolysis. The correlation of the hypercoagulability status
highlighted by coagulation tests with the results of geno-
typing, the frequencies of minor/major alleles studied,
single mononucleotide polymorphisms (SNPs) and the
application of preventive therapy, aim to prevent intra-
vascular thrombosis with very serious consequences. D-
dimer dosing should be included in screening tests for
women of childbearing potential to diagnose subclinical
thrombophilia and reduce the risk of unexplained primary
or secondary infertility. The recommendation of the extended
thrombophilic profile in the preconceptional period aims
at decreasing fetal and perinatal mortality and morbidity
as well as increasing the probability of obtaining, at the
end of pregnancy, a full-term, eutrophic newborn, with
possibilities for normal, physical, and mental development.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors’ contribution

Simona-Daniela Neamțu and Anca-Maria Istrate-Offte
equally contributed to this article.

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