

GENETIC AND BIOCHEMICAL THROMBOSIS RISK MARKERS IN PREGNANCY. I. COAGULATION PATHWAYS

Received for publication, June 17, 2014

Accepted, November 20, 2014

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Abstract

Thrombophilia is a health state manifested by hypercoagulability or prothrombotic state. This is characterized by abnormal blood coagulation leading to increased risk of clots formation in blood vessels, a pathological event named thrombosis affecting endothelial cells of vascular system. It is a critical state for pregnant women and knowledge of its hereditary and environmental factors enable medical services to be optimized for maintenance of normal pregnancy and avoidance of adverse events such as miscarriages. Genetic and biochemical estimation of thrombosis risk may decrease the frequency of such events, providing a correct interpretation of the results and relevant risk stratification. We present a diagnosis algorithm based on the discussion of the biological and clinical significance of two major metabolic pathways involving central factors of: I. coagulation and II. homocysteine as the one-carbon metabolism. Additional genes and metabolites are introduced in this complex algorithm in order to correctly evaluate the thrombosis risk and to establish efficient personalized treatment or prophylaxis scheme.

Keywords: thrombophilia, genetic risk, coagulation factors, homocysteine, pregnancy adverse events

1. Introduction

Thrombophilia is a health state manifested by hypercoagulability or prothrombotic state that is characterized by deficient blood coagulation leading to blood clots formation or thrombosis. It can be congenital or acquired and therefore implies multiple genetic and environmental factors influencing the coagulation process. The risk of venous thrombosis in women with a deficiency of natural anticoagulants such as antithrombin, protein C, or protein S is substantially increased during pregnancy and the puerperium. Thrombophilia has been linked to numerous adverse pregnancy events. Inherited thrombophilias increase the risk of maternal thromboembolism and adverse pregnancy outcomes, with the greatest risk occurring during the puerperium.

Genetic and biochemical markers of thrombophilia evaluation have been developed based on the molecular mechanism of the coagulation processes. Virchow, in the 19th century,

defined three mechanisms of thrombophilia: vessel-wall injury, stasis, and “changes in the composition of blood” (hypercoagulability), the last two predominating in the pathological venous thrombosis [1]. Hypercoagulability has a strong genetic base, but it can be also acquired, through specific metabolic pathways that may be altered by environmental factors, such as nutrients, lifestyle (smoking, air traveling) and medications and through specific developmental life events such as pregnancy, diseases (cancer). The inherited thrombophilia is suspected when a patient has recurrent or life-threatening venous thromboembolism, has a family history of venous thrombosis, at an age younger than 45 years, or has no apparent acquired risk factors, or if the patient is a woman who has a history of multiple abortions, stillbirth, or both. Acquired and genetic causes interact, which makes it difficult to decide which patients should be tested for inherited thrombophilia, what biochemical or genetic tests to perform and when to order them, whether the results of the tests will affect the anticoagulant therapy, and whether to examine and correctly inform family members.

Hypercoagulability biochemistry. The human hemostatic system can be defined as consisting of multiple independent but correlated cellular and protein components, acting to maintain *blood fluidity* under normal conditions and also, at sites of vascular injury, to promote localized, *temporary thrombus formation*. Six major components have this hemostatic system: vascular endothelium, platelets, plasma coagulation proteins or “factors,” natural anticoagulant proteins, fibrinolytic and antifibrinolytic proteins [1].

In the presence of an intact endothelium, even though a low, basal, physiologic level of coagulation factor activation is occurring continuously, there is *no clot formation* should occur inside the blood vessels. This highly regulated hemostatic system maintains a delicate balance between a *prohemorrhagic* and *prothrombotic* state, which is maintained by the two sides of the hemostatic processes: concomitant actions of platelets, coagulation factors, and fibrinolytic inhibitors and of natural anticoagulants and fibrinolytic proteins. Therefore, coagulation processes are strictly coordinated by two major factors: coagulation ones and natural anticoagulant ones, in order to maintain the blood fluidity.

Hypercoagulable states have been described that represent generically **thrombophilia**; they are leading to pathologic thrombosis. Such state is defined by altered mechanisms that increase susceptibility to venous thrombosis: accentuated platelet aggregation, increased activity levels of coagulation factors, and excess plasma levels of fibrinolytic inhibitors. Consequently, quantitative or qualitative deficiencies of a natural anticoagulant, coagulation factor resistance to inactivation by a natural anticoagulant, and a deficiency of a fibrinolytic protein, may be associated with a state of hypercoagulability [1,3]. The general deficiency in unbalanced coagulation control is represented in Fig.1 by deficient generation of thrombin, protein C pathway and by deficient antithrombin activity in neutralization of thrombin, processes that are usually impaired through specific gene mutations. Recently, another important blood process, fibrinolysis, was considered in order to prevent excess clotting by breaking down the fibrin clot.

Hypercoagulability genetic factors match the above mentioned blood proteic markers. The control of thrombin generation may be compromised by mutations in the genes regulating the above described processes, the most important ones being *factor V* and *factor II (prothrombin) genes* [2-4].

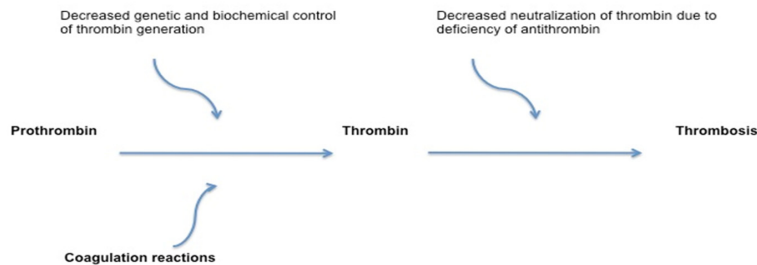


Fig. 1. Thrombosis mechanism is dependent on blood composition and the activity of the factors controlling the coagulation process.

The Arg506Gln substitution in **factor V gene (Leiden)** involves the first of three sites of factor Va; these ones are cleaved by activated protein C. The proteolytic inactivation of factor Va is slowed down by mutation, leading to the augmented thrombin generation. Therefore, the inactivation of factor VIIIa by activated protein C involves a diminished cofactor activity of the mutant factor V. Both these abnormalities in factor V are currently exploited as biochemical and genetic markers of thrombosis risk evaluation. The basic concept is referred to the fact that it causes the *in vitro* phenomenon of resistance to activated protein C, resulting in the *failure of activated protein C prolongation of the activated partial-thromboplastin time*. It was identified in 1993 as the major cause of activated protein C resistance, and is the most common inherited thrombophilia, being carried by 5% of Caucasians [5]. Heterozygotes have a three to eight fold increased risk of venous thromboembolism; homozygotes are more severely affected, with a 50- to 80- fold increase in risk [2-4].

Literature indicates also that the G20210A point mutation in the 3' untranslated region of the **prothrombin (Factor II) gene** is associated with an increased level of plasma prothrombin, promoting the generation of thrombin and impairing the inactivation of factor Va by activated protein C. This mutation is found almost exclusively in Caucasians and in 6% of patients with venous thromboembolism [3,4]. Literature indicates that heterozygosity for a mutation in the promoter of the **factor II gene**, present in 2–3% of white European populations, leads to increased (150–200%) circulating levels of prothrombin and an increased risk of thromboembolism. This mutation determines a frequency of 17% of thromboembolism events in pregnancy; however, the actual risk of clotting in an asymptomatic pregnant carrier of this mutation is only 0.5%. Also, adverse events in pregnancy were associated with this mutation: fetal loss, abruption, severe preeclampsia. Homozygosity for the prothrombin mutation confers a risk of thrombosis, equivalent to that of factor V Leiden homozygosity [4,6]. Therefore, persons with factor V Leiden or the prothrombin G20210A mutation have a higher thrombotic risk and the predisposition is greater in homozygotes than in heterozygotes.

The **PAI-1 gene's** promoter region contains at least two alleles producing either a 4G or 5G base-pair region. The normal 5G allele permits the binding of transcription factor inhibitors that suppress gene transcription, while the 4G allele is too small to bind these gene repressors. Consequently, individuals homozygous for the 4G/4G allele have a three to five-fold higher level of circulating PAI-1 compared with those bearing the 5G/5G or 5G/4G alleles. Homozygosity for the 4G/4G allele is relatively common and causes a modestly in-

creased risk of thromboembolism, fetal loss, IUGR, preeclampsia, and preterm delivery [7].

Factor XIII is a coagulation factor acting for stabilizing the fibrin clot. It is represented by a human protein, encoded by the F13A1 gene, named coagulation factor XIII A subunit, an enzyme acting as a transglutaminase to catalyze the formation of gamma-glutamyl-epsilon-lysine, able to crosslink between fibrin molecules and alpha-2-plasmin inhibitor, or fibronectin, to the alpha chains of fibrin. Therefore, defects in its gene can result in a lifelong bleeding tendency, defective wound healing, and habitual abortion, especially when associated to PAI-1 mutations [8].

Other coagulation pathways, involving the **endothelial protein C receptor (EPCR)** may modify the risk of thrombosis in connection with the above genotypes. EPCR enhances the rate of protein C activation, thus contributing to the regulation of thrombin formation by the protein C anti-coagulant pathway [9]. The rare allele of 4600A/G (haplotype A3) is associated with increased plasma levels of soluble EPCR (sEPCR), however, its association with risk of thrombosis is controversial [10,11]. This retains its ability to bind both protein C and activated protein C (APC), and blocks APC anticoagulant activity [12,13]. Another rare allele of 4678G/C (haplotype A1) was associated with increased levels of APC and reduced risk of thrombosis [14,15] and the more rare allele of 3811 G/A (haplotype A4), with a slight increase in the risk of thrombosis [11].

Hyperhomocysteinemia is also a central marker of the thrombosis risk that interacts with factor II and V Leiden mutations and their combination significantly increases the risk of venous thrombosis [16]. It is also determined by genetic and environmental complex factors and it is described in a subsequent (Part II) article on thrombophilia risk evaluation.

Acquired thrombosis during pregnancy, for patients with no genetic risk, may be explained by pregnancy-associated changes in hemostasis and fibrinolysis. It is proved that clotting and hemorrhage in the placenta are part of the normal processes resulting from a delicate balance. The hereditary thrombophilia adds more risks to thrombosis in pregnancy and complicates this reproductive period with adverse events (miscarriages, pre-eclampsia, growth restriction etc). The effect of pregnancy-associated changes in hemostatic, anticoagulant, and fibrinolytic proteins is to enhance the risk of thromboembolism. Thus pregnancy exacerbates the clinical effects of the inherited thrombophilias by naturally promoting clot formation and stabilizing it. Therefore, a 20–200% increase in levels of fibrinogen and factors II, VII, VIII, X, and XII, was detected in pregnancy, whereas concentrations of factors V and IX are unchanged. Compared to the age-matched individuals, the risk of thrombosis is 5-fold higher in pregnant women. Other natural state of coagulability factors in pregnancy are worth to be managed: endogenous anticoagulant levels increase minimally (tissue factor pathway inhibitor), remain constant (antithrombin and Protein C), or significantly decrease (Protein S); levels of immunoreactive and functionally active PAI-1 increase up to three-fold. If superposed to an eventual hereditary thrombophilia this hypercoagulability state should be carefully evaluated by the physicians in order to correctly manage the pregnancy evolution and hence to avoid adverse events by personalized treatment or prophylactic schemes even for the postpartum period, when the risk of thrombosis is even 20-fold higher [17,18].

Antithrombin-III (AT-III), protein C, and protein S are natural inhibitors of coagulation, and deficiencies of these result in an increased risk of venous thromboembolism. Protein C (PC) is a glycoprotein synthesized in the liver and is activated by the binding of the thrombin to the endothelial receptors. Activated PC inhibits the clot formation by binding to the factors Va and VIIIa and inactivating them [19]. Protein S (PS) is a glycoprotein synthesized vitamin K-dependently and activates PC as a cofactor [3]. These deficiencies are not common as

causes of thrombosis, however they should be considered in patients with a strong family history of clots while they test negative for factor II and V Leiden mutations. Heterozygotes for these three conditions have a 10-fold increased risk of venous thromboembolism, whereas most homozygotes were registered with severe thrombotic events even in early infancy [20].

Antiphospholipid syndrome is another **acquired** thrombophilia. Antiphospholipid syndrome (APS) characterized by arterial and venous thrombosis predisposition, repeated miscarriage and the presence of antiphospholipid antibodies is one of the leading causes of **acquired** thrombophilia. The negatively charged phospholipids and the phospholipid antibodies developing against the phospholipid protein complexes (lupus anticoagulant-LA and anticardiolipin antibodies-AKLA) are believed to be responsible for clinical manifestations, however the pathogenesis of the syndrome is not elucidated clearly. Lupus anticoagulant is the more relevant test because it detects β 2-glycoprotein-1 antibodies, highly correlated with thromboembolic complications and pregnancy morbidity [21,22].

Other acquired thrombophilia factors are linked with events determining conditions that may lead to hemoconcentration, such as dehydration, lower oxygen pressure and foot swelling that are believed to induce venous stasis, like those notice during air travel [23]. Immobilization may also result in blood accumulates backwards; thus, among the activated platelets and clotting factors, thrombin in particular accumulates locally, leading to the formation of thrombus [24]. Smoking might be another risk associated with higher plasma concentration of fibrinogen, reduced fibrinolysis [25], inflammation and increased blood viscosity [26,27].

This work aimed at documenting the biological basic mechanisms underlying the principal processes controlled by the genes and metabolites frequently tested for thrombophilia. It envisaged to stratify hereditary thrombophilia criteria combined with acquired thrombosis risk in pregnant women. A combination of genotypes and coagulation biochemical markers was considered thus for elaboration of a complete relevant diagnosis algorithm. The final goal is to estimate the extend to which genetic and biochemical testing would lead to improved health outcomes for individuals with thrombophilia or high risk of thrombotic events, especially in pregnancy, by specific recommendations.

The management strategies to minimize both familial and thrombotic events in individualized manner are explained based on the evaluation of specific prophylactic treatment outcomes (e.g. avoidance of recurrent thrombotic events) in adults with personal history of primary thrombotic events and of improved outcomes (e.g. avoidance of an initial or primary thrombotic event) in adult family members presenting mutations. Therefore, an estimation of the usefulness of genetic testing in medical, personal and public health should be carefully explained to patients.

2. Materials and methods

Patient group: A group of 41 pregnant women presented for monitoring pregnancy events at Generys Medical Center during January 2013-January 2014. They were selected based on their previously performed genetic tests during and after their former pregnancy events. After their consent for genetic and biochemical tests and for their pregnancy management was given, their blood was collected on heparin and NaEDTA, and assayed for biochemical and genetic factors involved in coagulation processes.

Biochemical techniques were based on ELISA, chromogenic (Siemens), coagulometric (Siemens) methods and performed at Bioclinica Medical Center on Sysmex CS-2000i

equipment. They comprised the following metabolites: Proteins C and S activity, antitrombin III, Lupus anticoagulant, anticardiolipin antibodies.

Genotyping technique implied DNA amplification and reverse hybridization on strips. Qiagen minispin blood kit was used for rapid DNA extraction; a ViennaLab primer kit for PCR amplification in multiplex system was used, followed by using the same company strip system for the realization of the RFLP reverse blotting of the amplicons, their fixation and color revealing after labeling with avidin probes provided by the same kit. A diagram (*Fig. 2*) on a strip with bands corresponding to each allele form was used for comparison of the colored strips and recording the allele variation results (*Table 2*).

3. Results and discussions

This paper attempted to present the routine laboratory workup for a hypercoagulable state in pregnant women used to include assay of antithrombin III, protein C, and protein S as biochemical markers and FV, FV Leiden, FXIII, EPCR as genetic markers. Our conclusion is referred however that the critical coagulation proteins should not be measured routinely because the results may be misleading and because deficiencies of antithrombin III, protein C, and protein S rarely occur.

Thrombophilia screening for the acquired anomalies and for the levels of the natural anticoagulants is shown in *Table 1*. The genotypes blotted on strips for genes controlling the coagulation pathways are represented in *Table 2*. A diagram on strips obtained by reverse blotting is represented in *Fig. 2*. A group of 41 pregnant women were investigated for genotypes and biochemical markers for thrombophilia. They were selected so that 32 out of 41(78%) presented with previous pregnancies adverse events and infertility records. The rest of the group were asymptomatic for thrombophilia and presented no mutation in critical genes associated with thrombophilia, nor altered biochemical aspects. The results showed an interesting combination of genotypes and biochemical factors demonstrating the possibility to evaluate more realistic the risk of thrombosis in pregnancy based on the complex genetic and environmental aspects.

Table 1. Biochemical parameters of the thrombosis risk assay

	IgG Anticardiolipin Ab	IgM Anticardiolipin Ab	Protein C	Protein S	Antithrombin III	Lupus Ab
Variation	Min 1.4.....1/16...6%	Min 0.4- 0.7.....4/16.....25 %	Min 83.3- 88.....3/16.... 19%	Min 44- 52.....3/16...1 9%	Min 81- 85.....3/16....19 %	absent
	Max 5.5- 8.2.....2/16...12.5 %	Max 0.9- 2.3.....2/16....1 2,5%	Max 125- 137.8.....5/16.... 31%	Max 111- 119..2/16...12 .5%	Max 90- 113.1.....8/16... 49%	
	Median 2- 2.7...7/16....44%	Median 0.9- 1.7..4/16...25%	Median 101- 104.3..4/16...25 %	Median 63- 98..9/16..56.2 %	Median 86.8- 89.7...3/16...19 %	

Protein C (PC) and Protein S (PS) and antithrombin (AT) deficiencies. Our investigation recorded PS deficit of cca 34-54% in 7 out of 41 cases, but these states were not linked with Factor V Leiden (FVL) mutation, which was very rare (almost 2%), as expected, because pregnancy-induced reduction in PS would enhance FVL prothrombotic effects. Our results did not show both PC and PS deficits frequently, the pregnancy and puerperal risk of

thromboembolism with these states appearing modest. Different mutations have highly variable phenotypes making it extremely difficult to predict which patients with PC or PS deficiencies will develop thromboembolism. According to literature, antithrombin deficiency is the most thrombogenic of the inherited thrombophilias with up to 90% lifetime risk of thromboembolism. In our study, no AT deficiency was registered a result in accordance with literature, which reports that because the prevalence of AT deficiency is low, one in 1000 to one in 5000, it is only present in 1% of patients with thromboembolism.

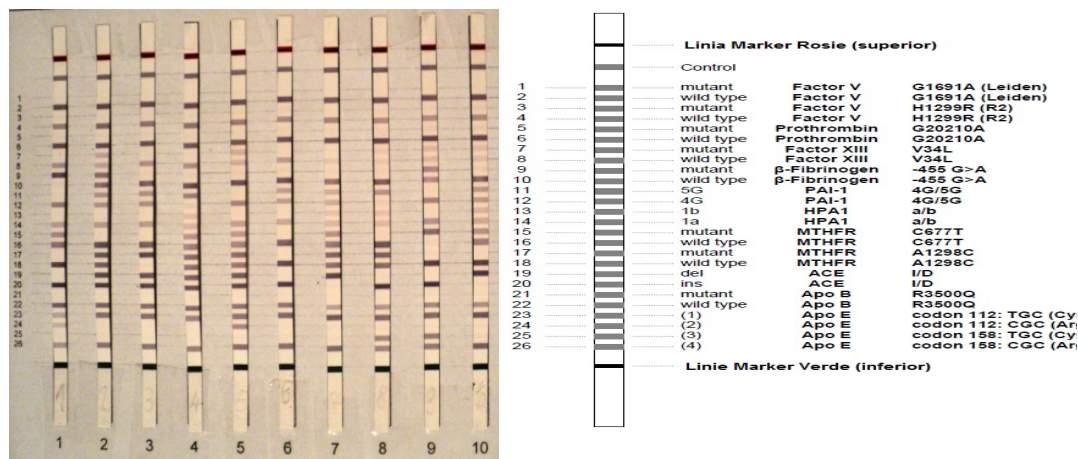


Fig.2. Example of banding patterns on strips developed by reverse blotting with PCR amplicons obtained separately for each gene for 10 individuals. The comparative diagram on the right represents bands corresponding to each gene allele.

The lupus anticoagulant is also a relevant marker, because if it is present, particularly intensive anticoagulation measures may be required. The absence of this anticoagulant in our recordings suggests no strong thrombophilic tendency in our group due to this factor (only 1 out of 41 cases).

Table 2. Polymorphic genotypes distribution in the studied groups

	Factor V Leiden	Factor V R2	Factor II	Factor XIII	PAI-1	EPCR
	MM	MM ..	MM	MM ..0	MM	A1/A3 ..2/16..12,5%
	1/16..6%	MW	MW	MW	..4/16..25%	A3/A3 ..1/16..6,25%
Variation	MW	3/16.....19%	WW	..5/16..31.2%	MW	A1/A1..1/16..6,25%
	WW	WW	100%	WW	..10/16..62.5%	A2/A2 W
	15/16..94%	13/16.....81.2%		11/16....68.75%	WW	3/16....18.75%
					2/16....12.2%	A1/A2 4/16..25%
						A2/A3
						3/16...18.75%

MM- mut homozygote; MW- heterozygote; WW- wilde type homozygote

Factor V Leiden mutation homozygosity that confers a far higher (more than 100-fold) risk of thromboembolism was present in a very small proportion (one out of 41 cases, respectively, 2,5%) homozygote and 5% heterozygote state, thus suggesting low thrombophilia risk due to this strong factor in our study group. **Prothrombin (Factor II)**

Gene (G20210A) mutation was not present in our investigated group. **4G/4G PAI-1 Mutation** showed homozygosity in 4 out of 16 individuals (25%) and the majority, 10 out of 16 (62.5%), presented the heterozygote state. **Factor XIII** shows mutations that may be associated with habitual abortion, especially when combined with PAI-1 mutations. No mutant homozygote, but 5 out of 16 individuals showed heterozygote state. **The endothelial cell protein C (PC) receptor (EPCR)** was tested in 16 cases of the total of 41. The wild type state was present in 3 out of 16 women (19%), and the other variants were represented as cca 25 and 19% respectively for heterozygote forms A1/A2 and A2/A3. The mutant variants were low represented: 12%: A1/A3, and 6% for both A3/A3 and A1/A1 rare forms.

Interpretation of the results and corresponding medical decisions. The role of thrombophilias in adverse outcome of pregnancy is still under debate, but there is a consistent correlation between thrombophilia and several adverse obstetrical conditions, such as preeclampsia, intrauterine growth restriction or placental abruption [28]. Nevertheless, thrombophilia is statistically associated with *thrombotic* events in pregnancy. As thrombotic events are usually identified in patients with thrombophilic profile, these sustain the importance of a detailed obstetric history and a comprehensive analysis in order to assess the risk profile of the gravidae [29,30].

Recently, it was showed that Factor V Leiden or prothrombin 20210 gene mutation are correlated with recurrent abortions and increased risk for miscarriage, in the first but also in the second trimester of pregnancy [31,32]. On the other hand, there are studies that point out that there is no correlation between thrombophilia and early miscarriage [33]. The same study even suggested that maternal thrombophilia (presence of factor V Leiden) even confers protection towards first trimester pregnancy loss, but was documented a possible poor pregnancy outcome after 14 week of pregnancy in association with a thrombophilic profile.

An important issue linked with thrombophilia is the management of patients to whom inherited thrombophilias are identified, regarding the indication for anticoagulation prophylaxis. Heparin constituted the cornerstone of management. Its role is to accelerate the action of antithrombin III, thereby preventing an additional thrombus from forming and permitting endogenous fibrinolysis to dissolve some of the clot. When taking in consideration the use of anticoagulant therapy during pregnancy, there is a need for a very careful assessment of risks versus benefits, regarding the bleeding or thrombotic events risk versus the possible effect of those conditions during pregnancy [34]. It is a real challenge the use of anticoagulants during pregnancy, especially due to the lack of controlled clinical trials that could assess the possible correlations between the use of anticoagulants and pregnancy outcome [35,36].

The issue of whether prophylactic heparin therapy should be offered to all pregnant patients with a history of prior venous thrombotic events has recently been examined. With the exception of pregnant patients with AT deficiency or those who are homozygotes or compound heterozygotes for the factor V Leiden or prothrombin (G20210A) mutations, there does not appear to be any justification for antenatal heparin treatment in asymptomatic patients incidentally found to have an inherited thrombophilia but who are without a history of prior venous thrombosis or characteristic adverse pregnancy outcomes. Their risks of thromboembolism and/or adverse pregnancy outcomes are likely less than 1%. However, postpartum prophylaxis appears warranted among such patients if they have an affected first-degree relative or other risk factors for thrombosis (example, cesarean delivery).

It was suggested that for gravidae experiencing thrombotic events, the same therapeutically management should be used regardless the cause of this adverse events. [28].

For pregnant women diagnosed with thrombophilia, anticoagulant therapy is not recommended in absence of acute clot identification [28].

There is a study recommending the use of different levels of anticoagulants based on a clinical score of each patient (such as age, body mass index, personal history of thrombotic events, type of thrombophilia, parity) [37]. There were delineated three risk categories: low risk gravidae who didn't have any treatment with anticoagulants before delivery, the second category included intermediate risk gravidae for whom anticoagulant therapy was indicated, using low molecular weight heparin (LMWH) and the third category, the high risk patients for whom anticoagulant therapy was indicated at the time of the enrollment in the study. The results of the trial were good, sustaining the importance of risk calculation for each pregnant patient in order to establish the best therapeutic management.

The stratification of patients as regard of the risk for adverse events during pregnancy due to their thrombophilic profile as well as the appropriate management was proposed by Battinelli group [28] (who adapted the guidelines proposed by Fogerty and Connors, 2009 [38]) and is presented in the Table 3. As for the genetic platform, the main relevant factor was considered the factor V Leiden mutation, because it is responsible for the most common hypercoagulable state. The optimal strategy is an integrated diagnostic approach that includes a methodical history taking and physical examination, supplemented by selective testing when appropriate. We suggest that details should be sought regarding the patient's history and a family history of venous thrombosis, as well as coexisting conditions, environmental risk factors, and hormonal influences.

Table 3 Risk assessment and patients' management for inherited thrombophilia

	High risk patients	Intermediate risk patients	Low risk patients
Patients' profile	Factor V Leiden homozygous, prothrombin gene homozygous, compound heterozygous, Antithrombin deficiency, any thrombophilia and history of thrombotic events	Low-risk thrombophilia with a strong family history of thrombotic events	Factor V Leiden heterozygous, prothrombin gene mutation heterozygous, protein C or S deficiency, lack of personal/family history of thrombotic events
Management	LMWH antepartum and for 4–6 weeks postpartum	Prophylactic LMWH antepartum and 4–6 weeks postpartum	Clinical surveillance antepartum and anticoagulation for 4–6 weeks postpartum

The management of pregnant patients with thrombophilia is controversial, anticoagulants being efficient for prophylaxis and as treatment thrombotic events in specific cases. It is essential to adapt the therapy management for each case after a comprehensive investigation of the patient.

4. Conclusions

Recently a better understanding of environmental and inherited risk factors for venous thromboembolism has been gained for the management of pregnancy. In clinical practice it is well established that the diagnosis of an inherited thrombophilia in a person without a history of thrombosis does not predict a thrombotic event with certainty and such a mutation indicates only an increased risk of thrombosis. A thrombophilic mutation rarely acts alone and contribute to clot formation. The genetic and biochemical approach of coagulation process

requires a correct interpretation of the results in order to stratify the thrombosis risk and to correctly apply a personalized treatment or prophylaxis. It is suggested also that the history of former thrombotic events for the pregnant women is extremely important. Also, a further approach of the homocysteine metabolism by new genes and new biochemical biomarkers, such as the homocysteine precursors, S-adenosylmethionine and S-adenosylhomocysteine is envisaged to be included in the complex management algorithm.

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