

**„VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND
PHARMACY FROM TIMIȘOARA
FACULTY OF MEDICINE
DEPARTMENT IV - BIOCHEMISTRY AND
PHARMACOLOGY**

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PhD THESIS

Scientific Coordinator

PROF. UNIV. DR. ANGHEL ANDREI

**Timișoara
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**The significant impact of thrombophilia
biomarkers in pregnancy and delivery – a western
Romania study**

A B S T R A C T

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Hematology is the study of blood and blood-related issues. In the field of Hematology, an important chapter is dedicated to blood clotting disorders, among which is thrombophilia, an important pathology, with an incidence in the Caucasian population between 5-6%, and 8% in Romania.

All inherited and acquired disorders associated with an increased susceptibility to thrombosis are together referred to as thrombophilia. Thrombosis or bleeding (severe disorders) can be caused by an imbalance between the stimulating and inhibitory components of the hemostasis system, while thrombophilia is caused by moderately disturbed hemostasis.

Weakly activated hemostasis system is a hallmark of a pre-thrombotic condition. Thrombosis develops from a prethrombotic state when activation exceeds inhibitory component capacity.

Changes in coagulation factors, plasmatic proteins, blood flow, vascular surfaces, and cellular components—among the haemostasis components—lead to a hypercoagulable state, which ends up with thrombosis. Arterial or venous thrombosis is the outcome.

A haematological evaluation is necessary in confirming a thrombophilia diagnosis. Numerous coagulopathy types can be diagnosed with the aid of biochemical tests.

Certain biochemical alterations in the blood are typically linked to abnormal clinical situations, and these alterations can be found using particular investigations.

The first step in treating a thrombotic patient properly is to determine whether the hypercoagulable disease is acquired, genetic, or mixed.

The haemostatic system undergoes an increased level of blood clotting during pregnancy, reaching its peak at the time of delivery. Women with a history of venous thrombosis, including an episode of acute venous thrombosis, are often screened for thrombophilia, especially in the prenatal state.

The incidence of thrombophilia in pregnancy is about 0,2%. The risk of bleeding and thromboembolic events rises with pregnancy. Forming and

maintaining the circulation is necessary for fetal survival, and the utero-placental entity plays a crucial role in preserving contact between the maternal and fetal circulations.

The topic of our study corresponds with current national and global concerns about maternal health, being focused on the effects of maternal circumstances on reproduction.

The goal of our study is to investigate the impact of the significant thrombophilia biomarkers both before and after giving birth, focused on the situation in the western part of Romania.

The study is divided into two main sections: the general part and the specific part, covering 208 pages, including 257 bibliographic references, a rich iconography (26 figures, 42 tables) and annexes.

The general part has three chapters: the first chapter, entitled thrombophilia, covers a general outlook about the coagulation system, the classification of thrombophilia and the risk that comes along with this coagulopathy; the second chapter, presents a general overview of pregnancy and the risk of thrombosis in pregnancy and the mechanism of thrombogenesis; the third chapter, presents the impact of thrombophilia in pregnancy, this important aspect including information about hereditary, acquired, and mixed thrombophilia. The same chapter pointed out the fact that most pregnant women are susceptible to coagulation disorders throughout pregnancy, being vital to adapt the general therapy of the pregnant woman to the particularities caused by thrombophilia.

The specific part aims to investigate the impact of the significant thrombophilia biomarkers both before and after giving birth, focused on the situation in the western part of Romania.

After a topic has been selected, one of the first tasks in a larger written assignment is usually to complete a literature review.

Reading and critical analysis can help refine a topic and formulate research questions. Doing a literature study before starting a new investigation shows our familiarity with and understanding of recent research in each field.

To realize the proposed goal, next objectives were established, and the study was structured on the following research directions:

- 1) A comprehensive updated review of the literature evidence on the management of thrombophilic pregnant women. This review provides a thorough analysis of all clinical factors, concerning the management of thrombophilia in pregnant women, updated to reflect current technological advancements and procedural guidelines.
- 2) Evaluation of the particularities of thrombophilia correlated with the evolution of pregnant women in the western part of Romania: anthropometric parameters such as age, body mass index, gestational period, the weight of the newborn, socioeconomic factors like the living environment, lifestyle behaviors such as smoking habits, as well as the genetic indicators linked to thrombophilia. This pertinent analysis represents the base to improve the actual standard of care for the thrombophilic pregnant women.
- 3) Thrombophilia analysis as an influencing factor of the mother and the fetus: maternal features (body mass index, hemostasis measures, monitoring of the specific medication provided in the thrombophilia groups) and newborn characteristics (gestational period, birth weight, and the Apgar score) were examined.

This study is focused on the western part of Romania situation, the subjects of the study group and the care units belonging to this region.

The fundamental objective of our first research entitled “**Approach to Thrombophilia in Pregnancy—A Narrative Review**”, published in *Medicina* **2022**, 58, 692, was to obtain a comprehensive updated assessment of the literature evidence on the management of thrombophilic pregnant women.

This literature study outlines the information in the field, being the first narrative review regarding this subject.

It would be essential to take the appropriate preventative action prior to becoming pregnant. Because women with thrombophilia frequently experience coagulation issues during pregnancy, it is equally important to adjust conventional prenatal treatment to meet the specific needs of these women. During screening, the degree of genetic damage manifested as thrombotic risk is quantified. Each patient's anticoagulant and antiaggregant drug regimen is unique depending on different factors.

The high level of risk that the advancement of pregnancy provides will lead to an increase in the frequency of patient monitoring in an effort to prevent any thrombotic event that could endanger pregnancy. Last, but not least, the postpartum antithrombotic prophylactic regimen will persist, utilizing medicine customized to the degree of risk associated with the severity of thrombophilia.

It also enhances new aspects of research, the implications of miRNAs in pregnancy, non-coding molecules that contribute to the process of posttranscriptional gene expression. But still, the study of miRNAs in thrombophilia is a topic of much debate.

The microRNA profiling will be a future useful tool for diagnosis, monitoring, and prognosis, and possibly a future new treatment strategy.

Thrombophilia is a genetic predisposition to hypercoagulable states caused by acquired haemostasis conditions; pregnancy causes the haemostatic system to become hypercoagulable, which grows throughout the pregnancy and peaks around delivery. Genetic testing for thrombophilic gene mutations is evaluated using different methodologies of real-time polymerase chain reaction and DNA microarrays of specific genes. Adapting the general care of the pregnant woman to the particularities caused by thrombophilia is an important component, so screening is preferred to assess the degree of genetic damage that manifests itself as a risk of thrombosis. The major goal of this narrative review was to quantitatively evaluate the literature data on the specific care of

pregnant women with thrombophilia that are at risk of developing unplanned miscarriages.

The main objective of our study entitled **“Assessment of the Particularities of Thrombophilia in the Management of Pregnant Women in the Western Part of Romania”**, published in *Medicina* 2023, 59, 851 is based on the evaluation of the particularities of thrombophilia correlated with the evolution of pregnant women in the western part of Romania: anthropometric parameters such as age, body mass index, gestational period, the weight of the newborn, socioeconomic factors like the living environment, lifestyle behaviors such as smoking habits, as well as the genetic indicators linked to thrombophilia. This pertinent analysis represents the base to improve the actual standard of care for the thrombophilic pregnant women.

To begin our case–control study, we studied 450 patients. A cohort of 450 pregnant women with thrombophilia who were examined in routine clinical practice in the western region of Romania between 2018 and 2020 participated in the noninvasive case-control study. In 2020, the last year of our study, the number of people seeking appropriate therapy fell dramatically due to the coronavirus disease (COVID-19) pandemic. Three groups were created based on the kind of thrombophilia present in the cohort. 150 individuals with inherited thrombophilia were included in the first group. It was determined that the second group of 150 patients had acquired thrombophilia. Lastly, 150 patients with a mixed thrombophilia diagnosis made up the final group.

Caucasian women who were singleton pregnant at the time of enrollment, had available results for inherited, acquired, and mixed thrombophilia, and had a positive obstetrical history (recurrent pregnancy losses) were included in the study population. Women who were not pregnant, subjects who had twin pregnancies, and pregnant women with incomplete thrombophilia screen results were excluded.

Following the application of the inclusion and exclusion criteria, 178 patients remained: 28.65% (51 patients) had inherited thrombophilia (group 1),

28.65% (51 patients) had acquired thrombophilia (group 2), and 42.7% (76 patients) had mixed thrombophilia (inherited and acquired – group 3).

Microsoft Excel was used to compile the database. We used Microsoft Excel and JASPV16.4, two separate pieces of software, for statistical analysis. We first performed a descriptive analysis on our database, and then we made use of the Shapiro-Wilk test to determine the data distribution and the kinds of tests to run. To determine whether there were any noteworthy differences between the two groups, the Mann-Whitney U-test was employed. We employed the Friedman test to observe the evolution of the D-dimers' value during pregnancy, and the Kruskal-Wallis test was used to examine medical test results among our three groups. We performed a regression analysis and determined the correlation coefficients at the conclusion of the investigation. For the whole investigation, the significance threshold was fixed at $\alpha=0.05$.

Thrombophilia in pregnant women is a condition whose incidence is constantly increasing worldwide, and, under these conditions, the development of preventive procedures is becoming essential. In this study, we aimed to evaluate thrombophilia in pregnant women in the western part of Romania and to establish anthropometric characteristics, socioeconomic features, and genetic and risk factors. 178 pregnant women were divided into three study groups, according to the type of thrombophilia, aiming to carry out the genetic profile and the acquired one. Anthropometric measures and biological tests were performed. The mixed type of thrombophilia predominates. The particularities of pregnant women diagnosed with thrombophilia are higher age, living in an urban environment, with normal BMI, approximately 36 weeks of gestational period, and having at least one miscarriage. Regarding the most frequent thrombophilic genetic markers, we obtained the MTHFR gene mutation C677T and A1298C, followed by the PAI-1 4G/5G gene mutation. Smoking represents an aggravating factor in the evolution of this pathology, manifested through the increase of D-dimers and the decrease in antithrombin values, simultaneously with the increase in therapeutic need. The predominance of MTHFR and PAI-1 4G/5G

gene polymorphism is a particularity of pregnant women with thrombophilia from the western part of Romania. Smoking is confirmed as an important risk factor in spontaneous abortion.

Researching thrombophilic disorders will aid in the development of preventive measures. At the moment, there is no national program for thrombophilia screening.

It is necessary to undertake a wide range of coagulation and genetic testing, and clinical competence is needed to interpret the results. Women should therefore, at the absolute least, get tested genetically.

Our study targets the most prevalent mutations in the western part of Romania, which lead to spontaneous abortions, in the absence of adequate treatment. This study also signifies the genetic imprint in the management of thrombophilia in the pregnant patient in the western part of Romania.

An original contribution was the optimization of the management of pregnant women with thrombophilia in accordance with the particularities of this type of pathology in the western part of Romania.

Anticoagulant therapy is one example of management adaptation; due to the normal BMI of pregnant women with thrombophilia, their proper anticoagulant treatment will not influence the weight of the newborn at birth. It was recommended to give up smoking during pregnancy, because smoking affects both the level of DDimers and the dose of needed anticoagulant.

Although the general genetic profile of thrombophilia is milder compared to the standard profile at international level, a fact which reduces the risk of thrombosis, the drug protocol was maintained, which is certified, and the effectiveness of this therapeutic attitude was validated by the 100% success on the antepartum and postpartum evolution of the pregnant women and their newborns.

Due to the current demographic changes, namely, the ageing of the population, it is normal for most of the population of childbearing age to be found in urban areas, which is also revealed by the 78,86% percentage from our study.

Research on thrombophilia in both the conception and the pregnancy period will help develop preventive measures. This could be a new field of research for the western part of Romania, focusing on women known with miscarriages, and it also involves a potential national screening program.

The purpose of our study entitled **“Maternal and Newborn Characteristics—A Comparison between Healthy and Thrombophilic Pregnancy”**, published in *Life* **2023**, *13*, 2082, was to perform a thrombophilia analysis as an influencing factor of the mother and the fetus: maternal features (body mass index, hemostasis measures, monitoring of the specific medication provided in the thrombophilia groups) and newborn characteristics (gestational period, birth weight, and the Apgar score) were examined. This study is focused on the western part of Romania situation, the subjects of the study group and the care units belonging to this region.

This five-year follow-up study, which runs from 2018 to 2022, focuses on a cohort of 500 women who were hospitalized for birth in the western part of Romania.

The participants from this study were Caucasian women who were pregnant with a singleton pregnancy at the time of registration, had available results for inherited, acquired, and mixed thrombophilia, had a positive obstetrical history (recurrent pregnancy losses), and received LMWH during and after delivery.

The thrombophilia screen included the following components: the FVL gene mutation, Factor II gene mutation, MTHFR gene mutation, PAI-1 gene mutation, the pathological mutation of factor XIII Val34Leu, AT, PC, PS, Lupus Anticoagulant, anticardiolipin antibodies, antiphospholipid antibodies, and homocysteine levels. Exclusion criteria included pregnant women who had not had their regular checks, nonpregnant women, subjects with twin pregnancies, and pregnant women with inadequate thrombophilia screen results.

Following the application of the inclusion and exclusion criteria, the number was reduced to 350 women, who were divided into four groups: 60

patients with hereditary thrombophilia, 60 patients with acquired thrombophilia, 80 patients with mixed (hereditary and acquired) thrombophilia, and 150 healthy pregnant women (the control group).

Descriptive statistics were used to calculate central tendency and dispersion parameters for the numerical variables in the investigation. For ordinal, nominal, and dichotomous data, frequency tables were created, and key percentages were extracted. The Shapiro-Wilk test was used to examine data distribution. The Mann-Whitney test was used to compare two different samples, while the Kruskal-Wallis test was used for more than two different samples. When examining the evolution of specific medications, the Friedman test was utilized, and the Wilcoxon Signed Rank test was used to compare differences between two time points. The investigation culminated with a linear regression analysis, which included the computation of Pearson and determination coefficients. The significance level was established at $\alpha = 0.05$. Microsoft Excel was used to create the database. Statistical analysis was carried out using two independent programs: JASPV17.3 and Microsoft Excel

In addition to documenting the basic features of the patients and their newborn babies, we looked into the potential influence of thrombophilia on the baby's development and pregnancy management. Several characteristics were measured for the patients in the study, including BMI in the first and third trimesters, GP, birth weight, and the Apgar score.

It is the first study of this kind in Romania in which the impact of thrombophilia on the correlation between the therapeutic situation with thrombophilic markers and the parameters of the newborn is evaluated.

The following findings about the effects of thrombophilia on mothers and their newborns are drawn from this study: the most prevalent type of thrombophilia is the mixed one (40%); the newborn is influenced by the mother's features - the Kruskal Wallis test revealed that the newborn weight in the control group is considerably higher ($p < 0.05$) than in the thrombophilia groups; the higher the mother's weight, the higher the likelihood that the fetus would be

underweight at birth; by increasing the dose of LMWH, it enhances the possibility that the fetus will be delivered with a lower birth weight ($p < 0.05$).

As a result, in the same management optimization note, the risk factor of the weight of the newborn, will have to be taken into account in the management of the birth process, and it will be considered in the management of the newborn with low birth weight.

Statistical differences were found after examining the specifics of the homeostatic parameters amongst the three thrombophilia groups. The newborn's characteristics are also influenced by the integrity of the pregnant woman's homeostatic system; our data indicate that the weight of the newborn will be lower ($p < 0.05$) in the case of women diagnosed with hereditary and mixed thrombophilia, with decreased values for PT and APTT; additionally, significant correlations were found between the level of maternal fibrinogen and the impact on the newborn's development ($p < 0.05$) in all thrombophilia groups.

The main contribution of our study is the fact that if a pregnant woman diagnosed with thrombophilia receives the proper care, maintains a normal weight, and does not exhibit any additional risk factors mentioned above, her child will have traits comparable to a healthy pregnant woman.

A thrombophilic woman is more likely to experience difficulties during pregnancy, difficulties that will also affect the development of the newborn. This study aims to compare maternal and newborn characteristics between healthy and thrombophilic pregnancy. The following characteristics were analysed: maternal characteristics (BMI- body mass index, haemostasis parameters, thrombophilia-specific treatment) and newborn characteristics (gestational period, birth weight, the Apgar score). This follow-up study spanning five years, from 2018 to 2022, focuses on a cohort of 500 women who underwent delivery hospitalization in the western region of Romania. The maternal characteristics influence the newborn: the greater the weight of the mother with thrombophilia, the more the chances that the fetus will have a lower birth weight; increasing the dose of LMWH (low molecular weight heparin), connected with the necessity to

control the homeostasis parameters, the more likely the fetus will be born with a lower birth weight. A pregnant woman with thrombophilia, treated appropriately, having a normal weight, and not presenting other risk factors independent of thrombophilia, will have a newborn with characteristics similar to a healthy pregnant woman.

Our study is part of the modern trends of personalized medicine; after conducting a pertinent and exhaustive analysis of thrombophilic biomarkers in pregnant women in the western part of Romania, the study elaborates recommendations adapted to this particular profile of thrombophilia, recommendations which will increase the quality and effectiveness of the medical act in the management of pregnant women with thrombophilia in the western part of Romania.

In a world where the trend is to evaluate each biomarker on existing pathologies, with no translational benefits in the short and medium term, the optimization of the whole existing disease biomarkers panel in correlation with psychosomatic, anthropometric factors must be a permanent concern in the process of improving current treatment protocols.

This research was carried out with the support of the Doctoral School and the departments of the Faculty of Medicine, from “Victor Babes” University of Medicine and Pharmacy Timisoara, through whose interdisciplinary collaboration I managed to obtain data, information, expertise, procedures, and the necessary equipment for the deployment and completion of this study.