**UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA**

**DOCTORAL SCHOOL**

# CONTRIBUTIONS TO THE STUDY OF THROMBOPHILIA INVOLVEMENT IN MATERNAL-FETAL MORBIDITY

## PhD THESIS

## *ABSTRACT*

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## GENERAL PART

### 1. GENERAL CONSIDERATIONS REGARDING PHYSIOLOGICAL HEMOSTASIS

Normal pregnancy represents a state of physiological hypercoagulation where there are involved complex changes of the blood fluid-coagulant balance, from primary hemostasis to fibrinolysis and natural anticoagulant mechanisms [1].

Pregnancy is associated to significant changes of coagulation factor values. The fibrinogen concentration doubles and there were observed increases of 20-1000% of factors VII, VIII, IX, X, XII, von Willebrand, with maximum values recorded at delivery time[2].

The coagulation activation markers in normal pregnancy are highlighted by the high activity of thrombin, increase of soluble fibrin values and high values of D fibrin [3].

In each of the three trimesters of pregnancy, there occur changes within the haemostatic system of the pregnant woman [4].

The formation of thrombocyte blood clots involves three stages, namely adhesion, activation and aggregation of thrombocytes. Secondary to the vascular lesion, thrombocytes adhere to the subendothelial collagen, through the von Willebrand factor bridges. This is connected at one end to the subendothelial collagen, and to the other end to the thrombocyte receptors GPIb/IX/V [5] .

### 2. HEREDITARY THROMBOPHILIAS AND GESTATION STAGE

Hereditary thrombophilias are genetic abnormalities associated with haemostasis disorders that induce a high probability of thrombic, venous and arterial events, which together with the procoagulant status during pregnancy increase the risk for thrombosis in these patients [6].

The most frequent types of hereditary thrombophilia are: mutation of V Leiden factor [7], mutation of antithrombin III gene, genetic deficits of protein C, S and antithrombin III [8]. Over 50 % of the maternal venous thrombic events are caused by these genetic abnormalities, in other words half of the thrombotic events that occur during pregnancy have as a determining or adjuvant factor the presence of thrombophilia [8].

### 3. ACQUIRED THROMBOPHILIA AND PREGNANCY

The most known acquired thrombophilia, with high importance for pregnant women, is the antiphospholipid syndrome [9, 10].

The antiphospholipid syndrome directly affects the trophoblast from the first trimester of pregnancy, due to some precoagulant factors. These inhibit beta-2-glycoprotein, protein C and antithrombin, favour beta-2-glycoprotein I and prothrombin in cellular membranes [11].

## 4. THROMBOPHILIC PATHOLOGY ASSOCIATED TO PREGNANCY

During gestation, the thrombogenic potential of inherited or acquired conditions, is due to the hypercoagulation induced by he physiological changes during pregnancy [12].

Recurrent miscarriages refer to three or more miscarriages with a gestational age under 20 weeks and with a conception product weight under 500 grams [13].

Numerous studies evaluated the relation between the heterozygot V Leiden factor and severe preeclampsia. The V Leiden factor was identified in 4.5-26% of patients with severe preeclampsia, eclampsia or HELLP syndrome [15-17].

In a recent systematic study, the V Leiden factor and mutation of prothrombin gene were associated with a high risk for RCIU [9].

Fetal mortality associated to thrombophilia represents the challenge of modern obstetrics and also an infinite source of medical notions that need to be compared and standardized.

## 5. NORMAL AND PATHOLOGICAL MORPHOLOGICAL ASPECTS OF THE MATERNAL-FETAL INTERFERENCE

Various guides recommend that every placenta should be examined postpartum by the obstetrician. There are situations when there is necessary that the placenta should be examined by the anatomopathologist, but there are no recommendations that all the placentas should be sent for examination by this specialty [17, 18]. The macroscopic examination techniques of the placemta include systematic analysis of fetal and maternal placental surfaces, of the placental disk and vilositary tissue, of extra placental membranes and umbilical cord [18, 19].

The macroscopic examination of the placenta could be done immediately after expulsion, after its refrigeration or after formalin fixation.

The microscopic examination of the placenta is performed after fixation, sectioning and staining [18, 19].

## 6. ULTRASOUND ASPECTS IN MATERNAL-FETAL COMPLICATIONS MEDIATED BY THE PLACENTA IN THE CONTEXT OF THROMPBOPHILIA

An accurate prenatal dating of all pregnancies represents a key process in detecting future fetuses with intrauterine growth restriction. Thus, the ultrasound evaluation requires three subsequent stages: pregnancy dating, fetal biometric evaluation and normal or abnormal growth evaluation [20].

The changes in the umbilical cord blood flow, with a decrease of dyastolic speed and increase of pulsation and resistance indexes, is due to third vilosities vasoconstriction. The diastolic speed becomes absent or even reversed in advanced stages of the functional and histological placental lesions [21, 22].

ACM is considered the standard for the evaluation of blood flow in fetuses with placental failure. The pulsation index (IP) in the ACM decreases constantly in fetuses with placental failure and prematurity, thus suggesting a progressive redistribution of the fetal blood to the vital organs [23].

## SPECIAL PART

## 1. INTRODUCTION

The PhD Thesis proposes to evaluate the role played by thrombophilia in modernobstetrics, the involvement of this deviation of coagulation status in the maternal-fetal morbidity, having as main research directions the perinatal complications mediated by the placenta in the context of thrombophilia.

In the light of the maternal-fetal role of an interface, the placenta is undoubtedly involved, both morphologically and functionally, in the pathophysiology of recurrent miscarriages, preeclampsia-eclampsia, RCIU, *in utero* fetal death or elective premature delivery.

What determined me to choose this perspective of research was, on the one hand, the frequency of thrombophilia occurrence in obstetrics, and, on the other hand, the maternal-fetal complications, sometimes catastrophic ones, generated by this pathology. The PhD Tesis acquires an interdisciplinary aspect, the study requiring a collaboration between the Obstetrician, a modern laboratory of medical, the Hematologist, as well as other physicians from other specialties, like Internal Medicine, Imagistics, cardiology, neurology or Anatomic Pathology.

### *1.1. OBJECTIVES OF THE STUDY*

During the doctoral research, I proposed to study, on the one hand, the necessity for hereditary or acquired thrompbophilias detected early during pregnancy or during the preconception check-up in patients with related significant medical history, and, on the other hand, the way in which there may be performed an early detection of changes that may lead to recurrent miscarriages, preeclampsia-eclampsia, RCIU, in utero fetal death or imminence of elective premature delivery.

Another main objective of my study was represented by the analysis of maternal-fetal management options in obstetrics thrombophilias, the impact of this pathology of the placental structure and the possible correlations with pregnancy complications mediated by the placenta.

Also, I proposed in this paper to elaborate, in the first part, a clinical study in order to search for correlations regarding the influence of thrompbophilia pathology on pregnancy, in general, and upon the trophoblast or placental structure, in particular. In the second part of my research, my main objective was to study the impact of associated clinical conditions on the placenta and fetal appendixes, through the study and its correlation to the ultrasound, morphological, microscopic and immunohistochemical studies of the placental structure. In the last part, I proposed an integrate approach of these objectives, in order to have an extended perspective regarding the maternal-fetal management within the context of perinatal complications associated to thrombophilia and mediated by the placenta.

## 2. MATERIAL AND METHOD

The retrospective and prospective, observational-descriptive doctoral study was performed between January 2014 - July 2019, on a group of 473 selected pregnant patients, diagnosed with thrombophilia (GT). Also, there was made a second group of patients, including 317 non-thombophilia pregnant patients (nGT) with or without any complications mediated by the placenta. All the patients included in the study were tested for thrombophilia, either randomly or taking into considerations their obstetrical history.

The patients included in the study group were Caucasian, aged between 17 and 43 years old, with an average of 30 years old.

After apllying the inclusion/ exclusion criteria, in the GT group there were maintained 439 patients, with 34 being excluded.

### *2.1. CLINICAL STUDY*

According to the clinical study, there were taken into consideration the following variables, presented in **Table 1**.

**Table 1.** GT and nGT clinical features

| **Clinical features and associated conditions** | **GT - n (%)** | **nGT - n (%)** |
|---|---|---|
| Age | 30 (± 13) | 31 (± 13) |
| Single pregnancy | 432 (98.4) | 307 (96.84) |
| Multiple pregnancy | 7 (1.59) | 10 (3.15) |
| Hypertension | 28 (6.37) | 36 (11.35) |
| Preeclampsia* | 96 (21.96) | 49 (15.45) |
| Diabetes** | 2 (0.45) | 2 (0.63) |
| Obesity | 144 (32.8) | 139 (43.84) |
| Smokers | 21 (4.78) | 28 (8.83) |
| Miscarriages ±recurrent | 139 (31.66) | 55 (17.35) |
| *One miscarriage* | 64 (14.57) | 29 (9.14) |
| *Two miscarriages* | 39 (8.88) | 18 (5.67) |
| *Three miscarriages* | 25 (5.69) | 8 (2.52) |
| *> 3 miscarriages* | 11 (2.5) | - |
| Pregnancy morbidity (Emryo-fetal loss syndrome) | 3 (0.68) | 2 (0.63) |
| Intrauterine death (± history) | 19 (4.32) | 2 (0.63) |
| Infertility history | 116 (26.42) | 72 (22.71) |
| Premature delivery | 102 (23.23) | 58 (18.29) |
| RCIU | 107 (24.37) | 27 (8.51) |
| Thrombosis history | 19 (4.32) | - |
| Ype of delivery | | |
| *Vaginal delivery* | 211 (48.06) | 183 (57.72) |
| *C-section* | 228 (51.93) | 134 (42.27) |
| Gestational age of miscarriage | | |
| *≤ 9gw(+6 days)* | 41 (9.33) | 21 (6.62) |
| *10-14 gw* | 98 (22.32) | 34 (10.72) |
| Birth weight | 2615 (± 1005) | 2985 (± 935) |
| Uterine associated malformations | 41 (9.33) | 24 (7.57) |

| | | |
|---|---|---|
| *Arched uterus* | 19 (4.32) | 17 (5.36) |
| *Partially septic uterus* | 6 (1.36) | 1 (0.31) |
| *Bicornuate uterus* | 4 (0.91) | - |
| *Uterus didelphys* | 5 (1.13) | - |
| *Uncornuate uterus* | 7 (1.59) | 6 (1.89) |
| n - number of cases; GT - group of patients with thrombophilia; nTG - group of non-thrombophilia patients<br>* Preeclampsia - blood pressure < 140/90 mmHg at first prenatal check-up (1st trimester), hypertension and proteinuria (≥ 0.3 g proteins/24h) after 20 gestational weeks;<br>** Type 1 or 2 gestational diabetes. | | |

The tests for thrombophilia used in the study groups are presented in **Table 2**.

**Table 2.** Tests for thrombophilia used according to the study group

| **Tests and markers for thrombophilia** | **GT (n/ %)** | **nGT (n/ %)** |
|---|---|---|
| ***Thrombophilia screening*** *(Protein C, Protein S, Lupus Anticoagulant, Antithrombin III, Anticardiolipin Antibodies IgG, IgM, anti-beta 2 glycoproteia 1 antibodies – IgA, IgG, IgM)* | 439 (100) | 317 (100) |
| ***Hereditary thrombophilia*** *(Antithrombin III, Protein C, Protein S, Mutation of V Leiden factor, mutation of Factor II, Gene MTHFR)* | 391 (89.06) | 27 (8.51) |
| ***SAFLO*** *(Anticardiolipin Antibodies, Lupus Anticoagulant, Anti-β2-glycoprotein I antibodies, Ig A, Ig G, IgM)* | 82 (18.67) | 39 (12.3) |
| ***Mutation of factor XIII*** | 34 (7.74) | 13 (4.1) |
| ***Plasminogen Activator Inhibitor Gene PAI*** | 58 (13.21) | 5 (1.57) |
| ***Endothelial receptor of Protein C*** | 198 (45.1) | 29 (9.14) |
| n - number of cases; GT - group of patients with thrombophilia; nGT - group of non-thrombophilia patients; SAFLO - Obstetrical Antiphospholipid Syndrome. | | |

### *2.2. ULTRASOUND STUDY*

The US evaluation of the study group was performed both through conventional 2D techniques, as well as through 3D or ultrasound CT, and through the spectral Doppler investigation, color or power Doppler (Voluson 730 Pro, Voluson E6, Logiq, equipped with RAB4-8L, RAB4-8D and RIC5-9-D, GE Healthcare and the equipment US Samsung H60 with a transductor CV1-8AD, Samsung Medison).

The US obstetrical evaluation included fetal morphology and biometry, as well as the investigation of the placenta, umbilical cord and amniotic liquid, the maternal-fetal Doppler profile and, in the case of multiple pregnancies, the diagnosis of chorionicity and amnionicity **(Table 3)**.

**Table 3.** Obstetric ultrasound study

| **US examination in the 1st trimester** | |
|---|---|
| ***Confirmation of living fetus*** | ▪ Presence of heart beats; |
| ***Multiple pregnancy*** | ▪ Evaluation of chorionicity and amnionicity. |
| ***VG determination*** | ▪ CRL. |
| ***Fetal anatomy*** | ▪ Fetal head;<br>▪ Fetal face;<br>▪ Fetal neck - NT;<br>▪ Fetal thorax;<br>▪ Fetal heart;<br>▪ Abdominal wall;<br>▪ Spine;<br>▪ Limbs;<br>▪ Placenta;<br>▪ Umbilical cord;<br>▪ Cervix - length of cervix canal. |
| **US examination in the 2nd trimester** | |
| ***Confirmation of living fetus*** | ▪ Presence of heart beats. |
| ***Multiple pregnancy*** | ▪ Evaluation of chorionicity and amnionicity |
| ***Fetal anatomy*** | ▪ Fetal head;<br>▪ Fetal neck;<br>▪ Spine;<br>▪ Fetal thorax;<br>▪ Fetal heart;<br>▪ Fetal abdomen - AC;<br>▪ Limbs – FL;<br>▪ External Genitals;<br>▪ Fetal appendixes:<br>○ Placenta;<br>○ Umbilical cord;<br>○ LA - AFI. |

## *2.3. MORPHOLOGICAL STUDY OF THE PLACENTA AND UMBILICAL CORD*

### 2.3.1. Morphological and macroscopic study of the placenta and the umbilical cord

The placental surfaces, maternal and fetal were systematically studied after a previous gentle washing with sterile water, in order to remove possible deposits. The macroscopic study was performed on fresh samples, immediately after harvesting and washed in sterile water, or after their refrigeration for 24-48 hours.

The macroscopic changes searched were represented by placental infarction, fibrin perivilous deposit, intervilous thrombosis, umbilical cord thrombosis, number of vessels in the umbilical cord, hyperwinding of the umbilical cord, real cord notch, lack of umbilical cord winding or its excessive length.

### 2.3.2. Microscopic morphological study of the placenta

During the microscopic morphological study, there were analyzed the tissue samples from longitudinal placental sections of 3-5 cm thickness, which were

previously fixed in 10% neutral formaldehyde solution, at room temperature, subsequently included in paraffin, according to the histopathological protocol, in 2/2 cm sections.

### 2.3.3. Immunohistochemical study

The range of used antibodies is presented in **Table 4**.

**Table 4.** Used antibodies for the immunohistochemical technique.

| Antibody | Provider | Clone | Antigen demasking | Secondary antibody | Dilution | Marking |
|---|---|---|---|---|---|---|
| ***Anti-CD34*** | Dako | QBEnd 10 | Cytrate, pH 6 | Monoclonal mouse anti-human CD34 Class II | 1:50 | *Endothelial cells of neoangiogenesis vessels* |
| ***Anti-αSMA*** | Dako | 1A4 | Cytrate, pH 6 | Monoclonal Mouse Anti-Human Smooth Muscle Actin | 1:100 | *Alfa actin of smooth muscle fibroblasts* |
| ***Anti-Ki67*** | Dako | MIB-1 | EDTA, pH 9 | Monoclonal Mouse Anti-Human Ki67 | 1:50 | *Cells found in division* |
| ***Anti-Colagen IV*** | Dako | CIV22 | Cytrate, pH 6 | Monoclonal Mouse Anti-Human Colagen IV | 1:50 | *Basal membranes* |

## 3. RESULTS

The results of the biological tests performed showed that of a total number of 439 cases included in GT, 357 (81.32%) cases represented hereditary thrombophilias, 79 (17.99%) of them were acquired thrombophilias, and 3 cases (0.68%) had an unclear result (hereditary/ acquired), in the presence of homocystein **(Table 5)**.

**Table 5.** Types of thrombophilia and specific markers

| Hereditary thrombophilias | GT (n = 357) |
|---|---|
| Antithrombin III - deficit | 41 (9.33) |
| Protein C - deficit | 14 (3.18) |
| Protein S - deficit | 123 (28.01) |
| Mutation EPCR - heterozygot | 212 (48.29) |
| Mutation EPCR - homozygot | 21 (4.78) |
| Factor V Leiden - heterozygot | 27 (6.15) |
| Factor V Leiden - homozygot | 7 (1.59) |
| Prothrombin 20210A - heterozygot | 20 (4.55) |
| Prothrombina 20210A - homozygot | 6 (1.36) |

| | |
|---|---|
| MTHFR C677T - heterozygot | 130 (29.61) |
| MTHFR C677T - homozygot | 40 (9.11) |
| MTHFR A1298C - heterozygot | 144 (32.8) |
| MTHFR A1298C - homozigot | 19 (4.32) |
| Polymorphism of gene PAI-1 4G/5G - heterozygot | 199 (45.33) |
| Poyimorphsm of gene PAI-1 4G/5G - homozygot | 14 (3.18) |
| Mutation of factor XIII - heterozygot | 75 (17.08) |
| Mutation of factor XIII - homozygot | 20 (4.55) |
| **Acquired thrombophilia** | **GT (n = 79)** |
| Lupus Anticoagulant | 68 (15.48) |
| ACA | 55 (12.52) |
| Anti-β2-glycoprotein I antibodies | 62 (14.12) |
| **Hereditary/ acquired thrombophilias** | **GT (n = 3)** |
| Homocystein | 3 (0.68) |

The macroscopic study of the placentas and umbilical cords showed that most of the morphological changes are more common in the GT group, thus suggesting the involvement of thrombophilia in the pathogenesis of pregnancy complications mediated by the placenta **(Table 6)**.

**Table 6.** Macroscopic study of the placenta and umbilical cord

| Macroscopic study of the placenta and umbilical cord | GT (n = 439) | nGT (n = 317) |
|---|---|---|
| Placental infarction n (%) | 129 (29.38) | 78 (24.6) |
| Fibrin perivilous deposit n (%) | 109 (24.82) | 54 (17.03) |
| Intervilous thrombosis/ thrombi n (%) | 63 (14.35) | 21 (6.62) |
| Bivascular umbilical cord n (%) | 3 (0.68) | 2 (0.63) |
| Thrombosis of the umbilical cord n (%) | 39 (8.88) | 7 (2.2) |
| Hyperwinding cordon n (%) | 41 (9.33) | 36 (11.35) |
| Reduced/ absent winding cordon n (%) | 2 (0.45) | 6 (1.89) |
| Real cord notch n (%) | 3 (0.68) | 8 (2.52) |
| Normal length cord n (%) | 254 (57.85) | 211 (66.56) |
| Excessive length cord n (%) | 101 (23) | 62 (19.55) |
| Short cord n (%) | 84 (19.13) | 44 (13.88) |

The results of the present study, through the light of the histological and immunohistochemical changes, reflect in a range of nine microscopic findings, described in **Table 7**.

**Table 7.** Placental morphological findings

| Microscopic study of the placenta. Placental morphological findings. | TG (n = 439) | nTG (n = 317) |
|---|---|---|
| Fibrinoid necrosis n (%) | 329 (74.94) | 193 (60.88) |
| Placental infarction n (%) | 130 (29.61) | 57 (17.98) |
| Placental thrombosis n (%) | 175 (39.86) | 65 (20.5) |
| Intravilous fibrosis n (%) | 219 (49.88) | 119 (37.53) |

| | | |
|---|---|---|
| Intervilous fibrosis n (%) | 312 (71.07) | 196 (61.82) |
| Hyalin focal degenerescence n (%) | 241 (54.89) | 98 (30.91) |
| Placental calcifications n (%) | 139 (31.66) | 59 (18.61) |
| Decidual vasculopathy n (%) | 39 (8.88) | 16 (5.04) |
| Synctyal notches n (%) | 30 (6.83) | 9 (2.83) |

Specific treatment of obstetrical thrombophilia included in my study comprised, under various forms, the treatment with acetylsalicylic acid, low-molecular weight heparines, folic acid and Vitamins B6 and B12 supplements **(Table 8)**.

**Table 8.** Specific treatment of obstetrical thrombophilia

| | |
|---|---|
| **Acetylsalicylic acid (75-100 mg once a day)** | |
| Preconception | 110 (25.05) |
| < 24 gestational weeks | 321 (73.12) |
| < 36 gestational weeks | 107 (24.37) |
| > 36 gestational weeks | 75 (17.08) |
| **Folic acid (1-5 mg once a day)** | |
| Preconception | 127 (28.92) |
| 1st trimester | 409 (93.16) |
| > 1st trimester | 212 (48.29) |
| **Vitamin B6 (supplements)** | 268 (61.04) |
| **Vitamin B12 (suppliments)** | 268 (61.04) |
| **Low-molecular weight heparine (LMWH)** | |
| Enoxaparin 40 mg (0.4 ml) subcutanoeus once a day *(CLEXANE®)* | 132 (30.06) |
| Enoxaparin 20 mg (0.2 ml) subcutanoeus once a day*(CLEXANE®)* | 51 (11.61) |
| Dalteparin 5000 UI subcutanoeus once a day *(FRAGMIN®)* | 62 (14.12) |
| Nadroparin 3800 UI (0.4 ml) subcutanoeus once a day *(FRAXIPARINE®)* | 44 (10.02) |
| Tinzaparin 5000 UI subcutanoeus once a day *(INNOHEP®)* | 35 (7.97) |

## 4. DISCUSSION

Pregnancy itself represents a state of acquired hypercoagulation, with an increase of the coagulation factors and a decrease of fibrinolysis activity, this occurring in a natural way and predisposing to deep venous thrombosis, an extra reason being also brought by the fact that the venous return slows down, through the pressure of expanded uterus, thus favoring the stasis [24].

Maternal thrombophilias, both genetic and acquired ones, are associated with various adverse perinatal results, including here maternal deep thrombosis, placental premature take off, intrauterine growth restriction, early and late miscarriage or preeclampsia [25].

Regarding these aspects, in this study we observed an incidence of 81% of hereditary thrombophilia, in comparison to 18% acquired thrombophilia, namely almost 1% unclear thrombophilia. By comparison to the published study on an intermediary group [26], significantly more reduced than the group studied in this doctoral paper, the difference is relatively significant only in the case of thrombophilia that cannot be actually included as being strictly hereditary or acquired, respectively.

Thus, the results presented in the respective article [26] presents an incidence higher than 79.68% hereditary thrombophilia vs. 81% in the present study, namely

17.18% vs. 18% acquired thrombophilia, the difference being significant in the case of unclear thrombophilia, namely 3.12% vs. 0.68% in the final stage.

These data suggest that, in the case of obstetrical thrombophilia, the main number is represented by hereditary thrombophilia.

The screening of thrombophilia during pregnancy is a controversed matter, as the association between thrombophilia and perinatal complications mediated by the placenta has not been clearly established [27].

On the other hand, though, the gestational complications mediated by the placenta, such as abruptio placentae, preeclampsia, intrauterine growth restriction, recurrent miscarriage or intrauterine fetal death, are significant causes for maternal and fetal morbidity [28].

*Gils și colab.* [27] consider that these complications partially come from the thrombosis in the placental blood flow, which could be caused by thrombophilia, thus increasing the risk for thrombosis [27].

Regarding the association between thrombophilia and gestational complications mediated by the placenta, in this study we observed a relative increase of preeclampsia incidence (TG vs. nTG) (21.96% vs. 15.45%) or of premature delivery (23.23% vs. 18.29%), as well as a significant increase of (24.37% vs. 8.51%), recurrent miscarriages (31.66% vs. 17.35%) or intrauterine fetal death (4.32% vs. 0.63%), in the group of patients associating another condition.

Fetal weight at birth was relatively lower in the GT group than in the nGT group, namely 2615 g (±1005) vs. 2985 g (± 935), the same aspect being also observed in the case of gestational age at birth, namely 33.5 sg (± 5.5) vs. 35 sg (± 6).

By comparison to these data, G*ils și colab.* [27] report an incidence of 28% of preeclampsia in patients diagnosed with thrombophilia, 24% RCIU or 16% in the case of intrauterine fetal death.

It is considered that placental pathology comes from the dysfunction of placental development in a two-stage sequence, which implies the failure of trophoblast invasion, followed by the systemic endothelial dysfunction, therefore, in the context of gestational complications mediated by the placenta, the superficial trophoblast invasion is clear, thus resulting a higher placental vascular resistance, decrease of perfusion and placental ischemia [29, 30].

In the case of gestational complications mediated by the placenta, associated to maternal thrombophilias, these aspects of the placental dysfunction imply morphological changes occurring at this level.

From the macroscopic study of the placenta, in my study, we observed a relatively high incidence (GT vs. nGT) of placental infarction (29.38% vs. 24.6%), of fibrin perivilous deposit (24.82% vs. 17.03%) or of an excessive length of the umbilical cord (23% vs. 19.55%), but also significantly high values in the GT group, like in the case of intervilous thrombosis (14.35% vs. 6.62%) or umbilical cord thrombosis (8.88% vs. 2.2%).

Related to the study performed by *Franco și colab.* [31], this consists in the fact that 13% of the patients with documented infarction are associated with maternal thrombophilia. In contrast, 62.3% of the studied placentas presented features of abnormal placentation, either in their development, differentiation or both, while 78.7% presented the mark of maternal vascular subperfusion, without any connection to infarction itself [31].

On the other hand, through, *Gogia și colab.* [25] present the placental infarction in 40% of thrombophilia cases and perivilous fibrin deposit in 23% of them.

These data suggest that the present study shows results in accordance with the ones of the previously mentioned authors.

In this study, low-molecular weight heparin was used in 324 (73.8%) of the GT cases, the choice of the active substance being based on the Obstetrician's experience, clinical practice and hematologic examination, thus being able to state that the obstetrical result is an encouraging one.

Acetylsalycilic acid in low doses, together with low-molecular weight heparin or by itself, used in the preconception period, in the first period of pregnancy or until its end, brings benefits to the maternal-fetal prognosis in thrombophilia, in general, and in the obstetrical antiphospholipid syndrome, in particular.

More than that, multiple studies up to this moment, stated that the present standard of treatment in the obstetrical antiphospholipid syndrome is based mainly on the antothrombic and antiaggregant treatment, while the association between acetylsalycilic acid in low doses and low-molecular weight heparin led to a rate of living fetus delivery up to 70–80% [32-34].

The present study has, on the one hand, some important points, but also some possible limits. The strong points of the study are represented by the complex analysis and correlations obtained in thrombophilia associated or not to gestational complications mediated by the placenta, thus integrating in a multi-disciplinary research relevant data regarding the screening and testing for thrombophilia, the treatment management, placental morphology and pregnancy result. Being a retrospective and prospective, observational and descriptive study, it is limited by certain discrepancies in collecting the data, between groups GT and nGT, and this fact may have the potential to affect the statistical value of our study.

## 5. CONCLUSIONS

This study suggests that gestational morbidity mediated by the placenta occurs with a relatively higher incidence in thrombophilia than in non-thrombophilia cases.

The data in this study support the hypothesis according to which, along the maternal thrombic possibility, obstetrical thrombophilias are associated to a high risk for prenatal complications, namely intrauterine growth restriction, recurrent miscarriages, preeeclampsia or premature delivery.

The systematic fetal evaluation should monitor the maternal and maternal-fetal biometric and hemodynamic profile in order to identify a possible onset of gestational complications mediated by the placenta.

Ultrasound abnormalities of the fetal and maternal-fetal Doppler hemodynamic profile, suggestive or relevant for the onset or progression od gestational complications mediated by the placenta, have the background in the morphological changes that involve the alteration of the placental angioarchitecure.

The thrombophilic status impacts the placental structure, mainly in its angioarchitecure.

The morphological microscopic findings in the placental structure and the statistical data from this study suggest that the maternal thrombophilic pathology associates various changes that generate placental ischemia through the alteration of the maternal endothelium, thus increasing the risk for thrombosis.

In the context of thrombophilia, associated or not to gestationla complications mediated by the placenta, there is more a combination of morphological changes and less specific findings.

By integratively analyzing the clinical, Doppler ultrasound observations and especially the morphological and immunohistochemical ones, there may be released a placental morphological model of the thrombophilic type.

The range of placental morphological changes is dependent on the type of thrombophilia, its diagnosis time and start of specific treatment, but also on the presence of gestational complications mediated by the placenta, gestational age at which these are identified or their management.

The association of obstetrical thrombophilia with uterine malformations, as an originality element in this study, indicates that, in the case of these patients, the risk for perinatal morbidity is relatively higher, on the one hand through the intereference of the trophoblast invasion in the malformation range, and on the other hand through the hematological changes caused by thrombophilia.

The antithrombic and/ or anticoagulant treatment should be administered regularly, either in the preconception period, or from the gestation onset and subsequently, also differentiated, until the second trimester or even until the end of pregnancy, these aspects being dictated mainly by the exact diagnosis of the thrombophilia type.

The integrate approach of obstetrical thrombophilia and its association to gestational complications mediated by the placenta, within this clinical, ultrasound, morphological and immunohistochemical research upon the entire gestational transitory biological system, may bring contributions both from the screening point of view and also regarding diagnosis, management and prognosis of these high-risk pregnancies.

## 6. REFERENCES


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