UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
GRIGORE T. POPA IAȘI

The implication of oxidative stress in intrauterine growth restriction

PhD Thesis Summary

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Key words: oxidative stress, intrauterine growth restriction, preeclampsia, total antioxidant capacity, markers of angiogenesis.

The doctoral thesis includes:

- A general part containing 5 chapters on 47 pages.
- A personal part on 117 pages.
  - The personal part contains 2 chapters respecting the IMRAD structure, presenting a total number of 4 studies (one retrospective study - one chapter, 3 prospective studies - one chapter).
- A section of perspectives and final conclusions on 3 pages; 14 conclusions.
- The references contain 256 listed titles.
- An „Appendix" section containing one (1) annex.
- There have been inserted
  - 115 figures (19 in the general part and 96 in the personal part);
  - 118 tables (7 in the general part and 111 in the personal part).

Note to the reader: The present abstract selectively reproduces the bibliography and iconography in the text, following the numeration and the content of the thesis.

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INTRODUCTION

Intrauterine growth restriction (IUGR) is a complex pathology with a multifactorial etiology, presenting many challenges in terms of diagnosis and treatment.

In the management of IUGR, we discuss screening, diagnosis and follow-up of pregnancy and the fetus. There is the question of the usefulness of performing the tests necessary for screening, given the additional costs, but also the invasiveness of some of them on the mother and fetus.

IUGR is a major cause of prenatal and neonatal morbidity and mortality. Also, according to the theory known as the "Barker hypothesis", the influence of defective intrauterine development has an impact on adult life, through the appearance of cardiovascular, metabolic or endocrine adaptations that may permanently change the structure and function of the body, increasing the risk of heart disease, diabetes type 2, insulin resistance and hyperlipidemia [65–68].

Therefore, IUGR is an important pathology of the fetus, which has serious consequences not only in the perinatal period, but throughout the life of the individual. Thus, from a disease that is the prerogative of the gynecologist, initially and the neonatologist, later, it becomes an important public health problem, which influences the subsequent evolution of many diseases, thus becoming an important target for public health services. In recent decades, knowledge of the short-term and long-term consequences of IUGR has grown rapidly and has involved the discovery of many clinical aspects.

I chose to study a series of clinical-epidemiological elements that characterize RCIU based on data collected from a level III maternity hospital that serves the North-East area of Romania. In addition to these aspects, I aimed to evaluate the total antioxidant capacity and variations of placental angiogenic factors in correlation with characteristic elements of IUGR, trying to identify a series of biomarkers with predictive value that could contribute to a comprehensive approach to ICD diagnosis and treatment.

GENERAL PART

The general part is structured in 5 chapters, presenting general data related to the approached pathology and related topics.

The first chapter focuses on a number of aspects of embryology, in an attempt to individualize the most important moments of intrauterine development, with the corresponding effects on postpartum life. In addition, this aspect outlines the effects of inappropriate intrauterine development, which can have an impact on the whole life, from the first moments of evolution of the newborn, to adult life.

The second chapter addresses issues related to IUGR, in an attempt to capture as comprehensively as possible all the characteristic elements of this pathology, reviewing notions of etiopathogenesis and risk factors, screening and diagnosis, pregnancy management with IUGR, postnatal confirmation of this pathology and short-term and long-term side effects.

The third chapter addresses notions related to oxidative stress and its involvement in pregnancy. Despite its essential physiological role, molecular oxygen is a potential toxic in the body, so a deeper understanding of the mechanisms governing oxidative stress in both the placenta and the maternal vascular endothelium may provide new opportunities for the development of innovative therapeutic approaches to prevent IUGR, thus improving the future quality of life of newborns.

The fourth chapter captures a pathology closely related to IUGR and oxidative stress, namely preeclampsia, a pathology of major importance in pregnancy. An emphasis is placed, throughout this chapter, on defective angiogenesis, the imbalances between the regulatory factors of this process, which most likely cause this condition, and the connections with IUGR.

The last chapter of the general part addresses a cutting-edge topic in medicine, namely proteomics. The study of protein structures with specific properties has acquired a special interest in the last decade. These could be benchmarks for anticipating possible

identifiable changes at an early stage of triggering pathological situations with serious consequences. Proteomics is defined as the large-scale study of a set of proteins produced in an organism and is considered the most innovative way to identify and characterize proteins; the proteome represents the totality of the characteristics of the proteins contained at a certain moment at the cell level (location, interactions, post-translational changes, turnover) [160]. Along with genomics and metabolomics, proteomics seems to be a promising tool in neonatology and pediatrics. In the future, the combination of these biological levels could yield promising results in identifying biomarkers.

PERSONAL PART

The part that presents the personal contributions in the field of doctoral research is structured in two chapters that present four studies, one of which is retrospective, observational, two are prospective, observational, and one is a pilot study. In these studies I aimed to identify, on the one hand, the clinical-pathological aspects of IUGR in a group of patients from the Clinical Hospital of Obstetrics and Gynecology "Cuza Voda" Iasi, and, on the other hand, I sought to identify biomarkers with diagnostic and prognostic value in relation to oxidative stress and IUGR.

CHAPTER VI
OBSTETRICAL DIAGNOSIS OF INTRAUTERINAL GROWTH RESTRICTION: EARLY COMPLICATIONS AND RISK FACTORS INVOLVED IN INTRAUTERINAL GROWTH RESTRICTION - A RETROSPECTIVE STUDY

VI.1. Introduction

RCIU is a major cause of prenatal morbidity and mortality, having, consequently, a significant impact on public health. Clinical evidence suggests that small fetuses with placental insufficiency are associated with a weaker perinatal outcome, while the group without placental involvement has an almost normal course of life [181].

Thus, it is important to differentiate between SGA and RCIU, as these pathologies have different evolution, and, consequently, the management will have to be adapted. Doppler ultrasound is essential to achieve this goal.

The importance of diagnosing and managing intrauterine growth restriction remains a challenge in current obstetric practice. This statement remains valid in variable diagnostic criteria, in non-standardized monitoring methods and in the absence of therapeutic solutions with proven efficacy. Morbidity and mortality are increased by iatrogenic prematurity, the gestational age not contributing in such a high percentage in the neonatal prognosis [182,183].

Despite the importance of the problem, the detection rate is still low. This is due to the existence of a large number of unfollowed pregnancies, especially those without risk factors, are not properly monitored [184]. The result is reflected in the rate of uterine mortality and perinatal complications requiring intensive care.

VI.2. Motivation

The review of the literature reveals the existence of multiple studies focused on the analysis of prevalence, or identification and classification of risk factors for IUGR - thus reflecting the interest of obstetrics specialists in the diagnosis, monitoring and treatment of this pathological entity. IUGR is a pathology that requires an early and correct diagnosis, due to the evolutionary consequences both in the short and long term. From this point of view, knowing the risk factors that negatively influence the normal growth and development of the fetus is of major importance, in order to be able to quickly identify patients at risk and follow them properly during pregnancy. Last but not least, complications are a dreaded consequence of IUGR. Their early identification is the main goal of the team of obstetricians and neonatologists, to substantially increase the chances of survival, requiring an energetic and appropriate treatment to reduce morbidity and mortality.

VI.3. Purpose and objectives

The main purpose of the study was to analyze the clinical and epidemiological elements that characterize IUGR newborns, systematizing and integrating the defining aspects to define a profile that can contribute to the differentiation of IUGR newborns from SGA newborns.

In addition, the study aimed to identify risk factors that significantly influence the occurrence of IUGR.

The first objective of the study was to assess the incidence of RCIU, properly diagnosed, using pre- and postnatal diagnostic methods - consequently, evaluated in the mother and subsequently in the newborn - in order to assess the current frequency of this pathology in the area of Moldova. Achieving this goal required clear

differentiation between newborns with IUGR and SGA - the two diagnostic entities, between which there are imprecise boundaries, being frequently reported in the literature as the same condition. The differentiated diagnostic framework of cases was based on the identification of subtle differences that may be important, and that may justify a clear separation between pregnancies and newborns with IUGR and SGA.

The second objective of the study was to characterize the studied group according to a series of task-specific parameters, parameters applicable in differentiating IUGR faces from SGA faces. In parallel, we followed the analysis of the evolution and complications of RCIU and SGA newborns, including in terms of the correlation between the complication rate and the age at which the birth took place.

The third objective of the study was to analyze the risk factors associated with pregnancies with RCIU and SGA, aiming to identify those risk factors with a significant impact on the evolution of the newborn.

In summary, the objectives of the study aimed at defining clinical elements for this pathology and its complications and risk factors for the occurrence of IUGR, in an integrative approach based on experience in diagnosing and monitoring IUGR in a level 3 maternity ward, which serves the area of northeastern Romania.

VI.4. Material and methods

The study is a retrospective, observational study, developed over a period of 5 years (January 2013 - December 2017), which analyzed data from observation sheets of newborns diagnosed with IUGR and their mothers - patients being hospitalized in the „Cuza Voda" Clinical Hospital of Obstetrics and Gynecology Iasi, respectively in the University Clinics and in the Regional Center for Neonatal Intensive Care (CRTIN) of the hospital. In order to highlight the risk factors involved in IUGR, we included in the study a control group of newborns cared for in CRTIN, a group consisting of both full-term AGA newborns and premature newborns without IUGR.

Out of a total of 31683 newborns during this period, a number of 618 made up the study group (of which 498 SGA and 120 RCIU), and 129 made up the control group, with newborns with normal weight and development.

The risk factors analyzed specifically in this study were:

- maternal factors: socio-economic status; hypertension; preeclampsia; anemia; maternal infection (other than TORCH); TORCH infections; the extreme age of the mother; multiparity or nulliparity; other maternal diseases;
- fetal factors: twin pregnancy; genetic disorders;
- placental factors.

VI.5.Results

VI.5.1. IUGR and SGA incidence and prevalence

In the period between 2013-2017, the incidence of newborns diagnosed with RCIU or SGA was 2.04%, representing 11.61% of all newborns cared for in CRTIN.

VI.5.2. Characteristics of newborns in the analyzed groups - Comparative study: newborns with IUGR/SGA vs. AGA

A. Gestational age

The gestational age at which the birth occurred in the studied groups showed an average value of 35.7 ± 3.21SD, with minimum values of 25 weeks and maximum of 41 weeks.

The mean values of gestational age were significantly lower (35.56 ± 3.25 weeks vs. 36.46 ± 2.89) in newborns with IUGR ($p = .003$).

B. Prematurity

The frequency of premature babies in the study group was 49.35%, almost half of the newborns with IUGR being premature.

Statistical analysis did not demonstrate a significant association between IUGR and prematurity ($p = .2855$), although we

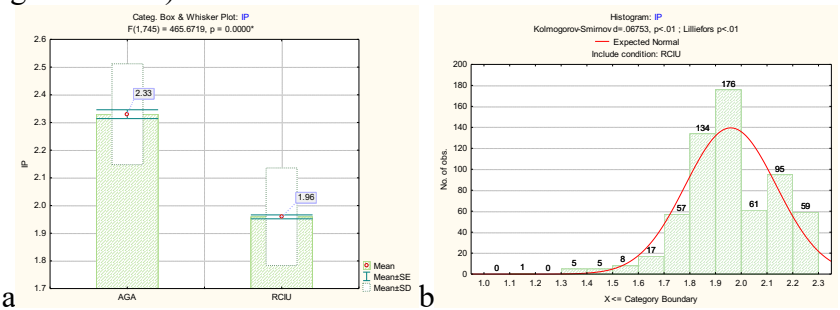
noted a significant association of IUGR cases in newborns younger than 32 weeks of gestation.

C. Ponderal index

The values of the weight index in the case of newborns with IUGR showed minimum values of 1.13 and maximum values of 2.29.

The mean value of the weight index was significantly lower (F = 465.67, p <.001) for IUGR infants (1.96 ± 0.18) compared to the mean value for newborns of AGA (2.33 ± 0.18) (Figure VI.11a).

Most cases of IUGR showed a weight index between 1.9 and 2 (Figure VI.11b).



a b

Figure VI.11. Mean value of birth weight index of newborns in study groups (a) and distribution of cases by IP of newborns with IUGR (b)

VI.5.3. Analysis of socio-economic factors involved in IUGR

A. Environment of origin

Table VI.18. Frequency of cases depending on the environment of origin in the studied groups

	Urban	Rural	Total
Lot martor (AGA)	72 55.81%	57 44.19%	**129**
Lot studiu (RCIU)	300 48.54%	318 51.46%	**618**
Total	**372**	**375**	**747**
Test statistic: Chi-square χ^2=2.2564, p=.1327, r=0.1449			

In the analyzed groups, the association of IUGR with the environment of origin indicated a very weak correlation highlighted by the frequency of 51.46% of cases with IUGR in rural areas, slightly higher value compared to the frequency of AGA cases in rural areas

(44.19%) (Table VI. 18). The risk of IUGR in the case of newborns in rural areas was insignificant - 1.34 times - higher compared to the risk of newborns in urban areas (OR = 1.34; CI: 0.91-1.96).

B. Following of the pregnancy

Pregnancy dispensation was a major issue, with case analysis showing that 83.8% of IUGR cases came from pregnancy follow, while 52.4% of SGA newborns came from non-dispensary pregnancy.

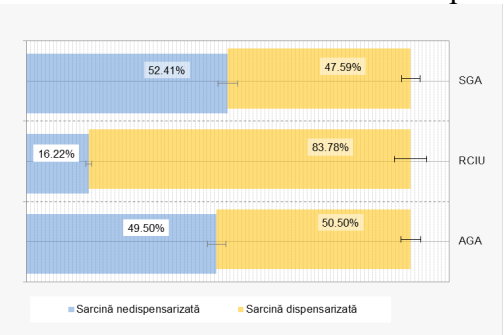


Figure VI.15. Distribution of cases according to the dispensation of the pregnancy

The results indicate a significant correlation (r = -0.3278, p <.001) between pregnancy follow and identification of cases with IUGR (Figure VI.15).

Based on the data, it was possible to evaluate the chance of detecting cases of IUGR in the dispensary task, which is 2.07 times higher.

C. Gestational age at diagnosis

The mean gestational age at the time of diagnosis of IUGR was 32.3 weeks, with a minimum of 18 weeks and a maximum of 39 weeks. Half of the newborns were diagnosed at gestational age less than 33 weeks

VI.5.4. Analysis of risk factors involved in IUGR

Univariate analysis of risk factors for IUGR demonstrated a statistically significant association with the following elements (Figure VI.17):

- maternal: extreme age of the mother (p = .0381), maternal infections (p = .0335), maternal hypertension (p = .0266);

- placental: considered in total, statistically significant association (p=.0104);
- fetal: considered as a whole, statistically significant association (p=.0193); in particular, twin pregnancy (p=.0285), TORCH infections and genetic impairment (p=.0156).

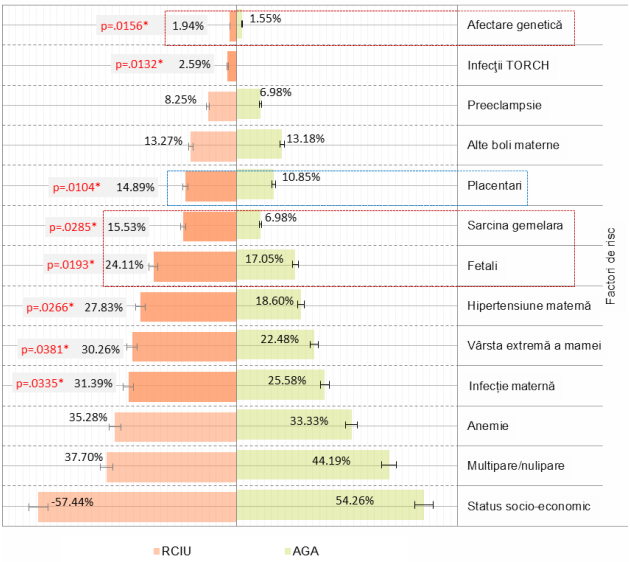


Figure VI.17. Distribution of cases according to risk factors in the studied groups

The analysis of the ROC curves allowed an objective assessment of the individual predictive power of each risk factor. Following this analysis, it can be seen that maternal infections (AUC = 0.632, 95% CI: 0.580-0.683), extreme age of the mother (AUC = 0.627, 95% CI: 0.574-0.680), placental risk factors - in overall (AUC = 0.785, 95% CI: 0.738-0.832), fetal risk factors - overall (AUC = 0.652, 95% CI: 0.600-0.703), genetic impairment (AUC = 0.874, 95% CI: 0.833-0.916) and TORCH infections (AUC = 0.592, 95% CI: 0.537-0.648) are significant risk factors for the occurrence of IUGR (Figure VI.18).

The comparative assessment of risk factors between the premature newborn and the full-term newborn revealed differences between the profiles of newborns with IUGR.

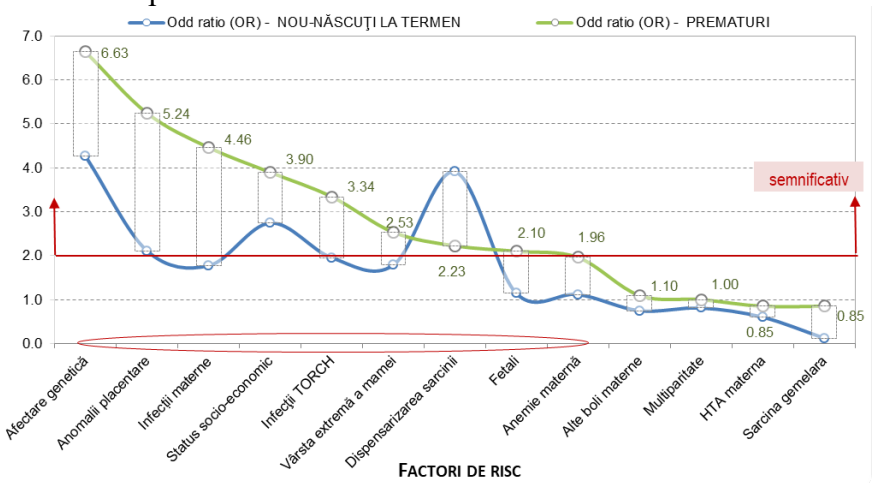


Figure VI.19. Comparative evaluation of RCIU predictive factors by Odds Ratio (OR) according to gestational age

Thus, if in the case of premature birth, the significant risk factors proved to be the socio-economic status of the mother, the extreme age of the mother, pregnancy pregnancy, maternal infections, maternal anemia, placental abnormalities, total fetal risk factors, but also impairment genetics and TORCH infections, in the case of the full-term newborn, for some of these risk factors the statistical significance has not been confirmed and, consequently, the risk for IUGR (extreme age of the mother, maternal anemia, fetal risk factors - total and TORCH infections) (Figure VI.19).

VI.5.5. Early complications in newborns with IUGR

We analyzed the early complications in the RCIU / SGA group, compared to the risk in the case of AGA newborns. A number of significant associations were noted between the frequency of complications and IUGR. Thus, asphyxia, respiratory distress syndrome due to surfactant deficiency, hemorrhagic disease, respiratory distress of other causes, ulcer-necrotic enterocolitis,

unspecified infections, hypocalcemia, difficult digestive tolerance and thrombocytopenia were significantly more common in newborns (Figure VI.20).

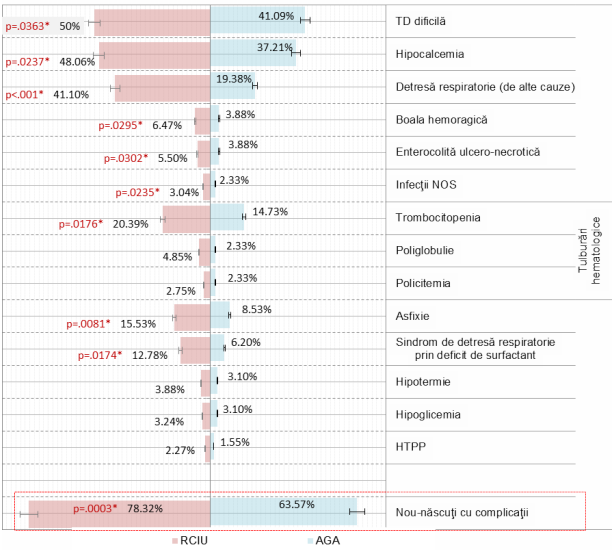


Figure VI.20. Distribution of cases according to the presence of early complications in the studied groups

Because newborns may have two or more associated complications, we applied a multivariate analysis to correctly assess the risk of early complications in the context of their association.

The results of the multivariate analysis indicated the following early complications of major importance for the newborn with IUGR: ulcer-necrotic enterocolitis, hemorrhagic disease, asphyxia, respiratory distress syndrome due to surfactant deficiency and other causes and thrombocytopenia. Their occurrence can often involve the association with polyglobulia, hypocalcemia or other complications.

VI.6. Discussions

The main purpose of this study, to define the clinical-epidemiological profile that characterizes IUGR in a level 3 maternity ward, with intensive care unit for newborns, was met. The

observational study documents the frequency of cases diagnosed with RCIU in the „Cuza Vodă" Maternity Hospital in Iași, being doubled by a comparative case-control study, between RCIU and AGA newborns.

The secondary goal, focused on identifying and evaluating the risk factors predisposing to the occurrence of IUGR, was also met, as through the case-control study we defined a number of maternal, placental and fetal risk factors, with an impact on the proper development of the fetus. In addition, through uni- and multivariate analysis, we established the risk factors with significant impact, but also the precocity of their occurrence.

Our data represent an important contribution to RCIU research, in the context of the current state of knowledge, providing an integrative picture of this pathology, from the perspective of experience developed in a center of expertise that provides medical services in obstetrics for patients in the Northeastern region of Romania.

In conclusion, our study provides a complex analysis of risk factors for the occurrence of IUGR and complications associated with this disease, based on the representative case of a hospital that serves, through the neonatal intensive care unit, the entire northeastern region of Romania.

The frequency of IUGR is constantly increasing in the five years studied. In the 5 years studied, out of a total of 31,683 newborns, 2.04% were diagnosed with RCIU or SGA. More importantly, most cases were diagnosed postnatal, which reveals a lack of proper follow-up of these cases, even in an important university center - an aspect attributable, in particular, to the non-presentation of patients.

The diagnosis of IUGR was established prenatally in 22.91% of cases and was established at an average gestational age of 32.3 weeks, with a minimum of 18 weeks and a maximum of 39 weeks. It is important to note that half of the newborns were diagnosed at gestational ages less than 33 weeks.

The most important risk factors for IUGR were genetic disorders, followed by the mother's socioeconomic status, maternal

infections, placental abnormalities, extreme maternal age, TORCH infections and pregnancy.

Early postnatal complications were 2.07 times more common in patients in the RCIU / SGA group, compared to newborns in the GMS group. These included, in order of importance: respiratory distress due to surfactant deficiency and other causes, hemorrhagic disease, asphyxia, thrombocytopenia and ulcer-necrotic enterocolitis.

Mortality in the study group was at a level of 1.62%, insignificantly higher than in the control group. Instead, we observed a longer hospitalization period for patients with IUGR / EMS compared to those on GMS.

CHAPTER VII
BIOCHEMICAL MARKERS OF OXIDATIVE STRESS AND PLACENTAL ANGIOGENESIS USEFUL IN THE ASSESSMENT OF INTRAUTERINE GROWTH RESTRICTION - PROSPECTIVE OBSERVATIONAL STUDIES

VII.1. Introduction

Pathologies such as pre-eclampsia, IUGR, gestational diabetes and premature birth can have important effects on the developing child as well as the mother. Although a multitude of risk factors (e.g., advanced maternal age, low nutrient intake, maternal obesity, other associated diseases, maternal parity, family history, and history of complications during a previous pregnancy) are known to play a role in these pathologies [215], there are many women who develop one of the conditions mentioned spontaneously, without any prior suspicion [108].

Pregnancies that present the risk factors listed above will be closely monitored, being considered to have a high risk of other diseases associated with pregnancy. Instead, pregnancies without risk factors will not benefit from the same monitoring, and the detection of these diseases will be done later, or not at all. In addition, the detection and monitoring of the aforementioned diseases is based on

measurements of physiological (blood pressure, glucose) and structural (AU Doppler indices) parameters [108]. Consequently, many conditions are detected late in pregnancy, although they are the pathophysiological results of events that occurred earlier during pregnancy. One such example is IUGR, as a consequence of oxidative stress that occurred much earlier.

In this context, we can discuss markers with predictive value for certain pathologies and markers with diagnostic value. The first mentioned markers can be detected early in pregnancy and provide information about the likelihood of developing certain conditions later, while diagnostic markers are evaluated after the onset of the disease.

Most biomarkers currently known are biomarkers with diagnostic value, being associated with the appearance of detectable symptoms and thus having a low predictive capacity, with limited clinical applicability [108].

VII.2. Motivation

Starting from the existing data in the literature on the value of biomarkers in relation to normal and pathological pregnancy, in addition to the study presented in detail in Chapter VI, doctoral research addressed the analysis of identifiable biomarkers in pregnant women or fetoplacental, seeking to identify tests with predictive value for the occurrence of IUGR or for its associated complications. To achieve this goal, we conducted two prospective observational studies and a mini-pilot study aimed at supporting the implementation of molecular biology techniques. These studies were defined as follows:

a. Study of oxidative stress and impact in RCIU (TAS Study);
b. Study of placental angiogenesis imbalances in IUGR (sFlt1 / PlGF study);
c. Pilot study for the analysis of placental proteomic expression.

VII.3. Aim and objectives

1) TAS study

The aim of this study was to investigate the markers of oxidative stress by evaluating the total antioxidant capacity, aiming to identify some markers for detecting IUGR with applicability in preventing complications and optimizing the treatment of this pathology.

A first objective of this study was to correctly identify and quantify specific markers of oxidative stress in both pregnant and newborns, in order to diagnose IUGR as early as possible and prevent the onset of lesions. As the literature shows a decrease in antioxidant factors and an increase in the production of reactive species in IUGR, the correct and early identification of oxidative stress markers provides significant information on the installation and progression of lesions.

The next goal was to assess changes in total antioxidant capacity depending on the presence of associated risk factors, and their impact on fetal development, thus making it possible to establish a standardized preventive behavior.

The third objective was to evaluate the frequency of oxidative stress in fetuses diagnosed with IUGR and to evaluate its association with perinatal pathology.

In parallel, the fourth objective was to compare the total antioxidant capacity of the newborn with the total antioxidant capacity of the mother, in order to establish a correlation between them.

2) sFlt1/PlGF study

The aim of this study was to evaluate the changes developed at the placental level in pregnant women with IUGR, based on variations in the values of antiangiogenic factor sFlt1 and angiogenic factor PlGF, following the significance of the dynamics of these markers in relation to the pathogenic mechanism of IUGR.

The first objective aimed at analyzing the values of the two factors, as well as the ratio between them, in newborns with IUGR and normal newborns. In parallel, for both groups, the association of PE

was analyzed, as an important pathological entity in generating an abnormal relationship between sFlt1 and PlGF.

The second objective considered was to evaluate the association of imbalances of these markers with oxidative stress, assessed by TAS values, in mothers and newborns with IUGR, respectively normal.

The third objective aimed at the correlation between abnormal angiogenesis, evaluated by the two factors mentioned above, and neonatal pathology.

3) Pilot study for the analysis of placental proteomic expression

In this study we aimed to evaluate the validity of the method of processing some placental fragments taken to obtain homogenates and to find the most appropriate method of storing them for later use. The perspective of this study is to identify, through proteomics, valid biomarkers for IUGR prediction.

The objective was to identify proteins obtained from a placental homogenate by electrophoresis and evaluate the best way to store placental homogenates in order to obtain valid samples.

VII.4. Material and method

1) TAS study

The TAS study was a case-control study, conducted over a period of 3 years (January 2016 - December 2018), in which we followed the evaluation of TAS and the significance of TAS by reference to the pathology of the mother and newborn with IUGR, respectively normal.

This study included 62 mother-newborn couples, for whom we performed TAS analysis in both mother and newborn.

The couples were grouped into two groups (Figure VII.2):

- group I (study group) - pregnant women and newborns with IUGR: 32 cases
- group II (control group) - normal pregnant women and newborns GMS: 30 cases.

2) sFlt1/PlGF study

The sFlt1 / PlGF study was a case-control study, conducted between January 2018 and June 2019 which aimed at analyzing markers of angiogenesis and their significance, by reference to TAS, and the pathology of mother and newborn with IUGR, respectively normal.

This study included 68 mother-newborn couples for whom we performed the sFlt1, PlGF and TAS analysis, both in the mother and in the newborn.

The couples were grouped into two groups:

- group I (study group) - pregnant women and newborns with IUGR: 38 cases from dispensary pregnancies;
- group II (control group) - normal pregnant women and newborns GMS: 30 cases of cases from dispensary pregnancies.

3) Studiul expresiei proteomice placentare (studiu pilot)

This pilot study was started in 2018, including 7 patients (2 patients with normal pregnancy, 5 patients with pregnancy associated with IUGR) whose placentas were harvested in order to identify specific classes of proteins by molecular biology techniques.

This study included 5 patients with IUGR and 2 normal patients.

VII.5. Results

VII.5.1. Study of oxidative stress and impact in IUGR

VII.5.1.1. Clinico-pathological characteristics in the studied groups

In this study, although the number of cases included was significantly lower than in the first study presented, both the clinico-pathological features presented previously, and the aspects related to risk factors and postnatal complications were similar.

Our results revealed that of the newborns confirmed postnatal with RCIU, 68.75% were detected antenatal in the second trimester,

and 28.13% in the third trimester. Also, our data indicated that 3.13% of newborns came from unattended pregnancies. The results obtained showed that a significant number of newborns with IUGR are diagnosed in the second trimester (p = .00001).

The most common risk factors encountered in the case of newborns with IUGR were maternal ones, with a frequency of 56.25%, significantly higher than in the GMS group, in which the frequency was 36.67% ($\chi 2 = 8.7391$, p =. 00312). Table VII.12 summarizes these risk factors and the association with the risk of RCIU.

Placental risk factors were present in 43.75% of patients with IUGR, with statistically significant differences compared to AGA newborns ($\chi 2 = 4.8394$, p = .03595). Similar results were recorded for fetal risk factors, whose frequency, although lower than the other categories of risk factors, was higher than in newborns AGA ($\chi 2 = 5.7138$, p = .01904).

VII.5.1.2. Evaluation of total antioxidant capacity (TAS)

A. Assessment of total antioxidant capacity in the mother (maternal TAS)

For newborns with IUGR, we noticed a high frequency of cases that showed values of maternal TAS lower than 1.3 mmol / L. The median value indicated that 50% of newborns with IUGR had corresponding maternal TAS values less than 1.3 mmol / L; moreover, for 25% of the newborns in this group they associated maternal TAS lower than 1.13 mmol / L. In the case of AGA newborns, 75% of cases associated maternal TAS values greater than 1.36 mmol / L.

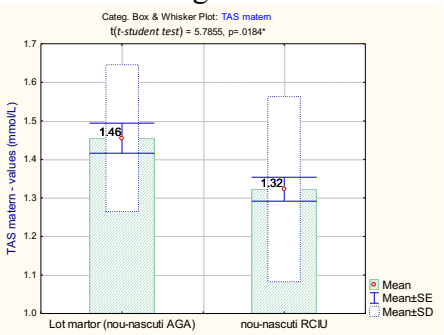


Figure VII.11. The average value of maternal TAS in the analyzed groups

Maternal TAS values were significantly lower (p = .0184) in newborns with IUGR (1.32 ± 0.23), compared to values in mothers with AGA newborns (control group: 1.46 ± 0.19) (Figure VII. 11). For maternal TAS in the case of newborns with IUGR, minimum values of 0.89 mmol / L were recorded.

B. Assessment of total antioxidant capacity in the newborn (newborn TAS)

In the case of newborns with IUGR, 50% of cases had TAS values less than 1.21 mmol / L. In the group of AGA newborns, the minimum value of TAS was 1.3 mmol / L, 10% of cases with values greater than 1.71 mmol / L. Also, in the control group 75% of the newborns had TAS values in the range of 1.3-1.71 mmol / L, normal reference values for the full-term newborn.

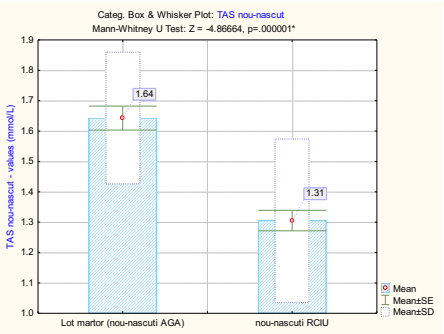


Figure VII.13. The average value of the newborn TAS from the analyzed groups

The results of the statistical analysis showed that the TAS values in newborns with IUGR (1.34 ± 0.25) were significantly lower (p=.000001), compared to the values recorded in the case of newborns AGA (1.61 ± 0.18).

VII.5.1.3. Correlation of maternal TAS with newborn TAS

In the case of both newborns in the study group (RCIU) and those in the control group, we obtained a significant direct correlation between the values of maternal TAS and newborn TAS. Low values of maternal TAS were associated with low TAS values of newborns.

VII.5.1.4. TAS predictability for IUGR

The maternal TAS value showed an increased predictivity for IUGR (AUC = 0.685, 95% CI: 0.563-0.806, p = .008). In the case of maternal SAR values lower than 1.32 mmol / L, we noted a significant increase in the risk of pregnancy with IUGR.

The descriptive analysis reveals that the TAS values of the newborn with IUGR, identified in the umbilical cord, are significantly lower, this aspect demonstrating the increased predictability for IUGR in case of a significant decrease (AUC = 0.813, 95% CI: 0.728-0.897, p <.001). If the umbilical cord TAS values are lower than the cutoff value of 1.25 mmol / L, the risk of the newborn exhibiting IUGR is significant.

VII.5.1.5. The influence of maternal risk factors on TAS values

The analysis of maternal TAS values showed that, both in the presence of maternal risk factors and in their absence, maternal TAS values in IUGR cases were significantly lower compared to AGA cases (p <0.03) (Figure VII.17). 50% of newborns with IUGR had a maternal TAS below the minimum reference threshold. The obtained results demonstrated that there is no predictability of maternal risk factors on maternal TAS (AUC <0.5, p> .05), both in the case of newborns with IUGR and in the case of AGA newborns.

The mean umbilical cord TAS value in newborns with IUGR was significantly lower (p <.001), below the minimum reference threshold (1.23 mmol / L), in the presence of maternal risk factors (1.32 ± 0.24 mmol / L). L), compared to AGA newborns (1.57 ± 0.14 mmol / L). The data obtained indicated that there is no predictability of maternal risk factors on TAS in the newborn (AUC <0.5, p> .05), both in the case of newborns with IUGR and in the case of AGA newborns.

VII.5.1.6. The influence of placental risk factors on TAS values

The results of the study showed that, although no significant differences in maternal TAS values were identified, in cases with IUGR associated or not with placental risk factors, there is a

significant predictability of placental risk factors on maternal TAS, both in the overall assessment of all newborns (AUC = .781; 95% CI: .630-.831, p = .032), as well as in the case of RCIU newborns (AUC = .854; 95% CI: .723-.985). , p = .021), respectively AGA (AUC = .8674; 95% CI: .651-.727, p = .037).

In addition, we identified a significant prediction of placental risk factors on the newborn TAS, both in the overall assessment of all newborns (AUC = .447; 95% CI: .166-.729, p = .027), as well as in the case of RCIU newborns (AUC = .914; 95% CI: .810-.927, p = .016), respectively AGA (AUC = .546; 95% CI: .265-.827, p = .036).

VII.5.1.7. The influence of fetal risk factors on TAS values

We identified a significant prediction of fetal risk factors on maternal TAS, both in the overall assessment of all newborns (AUC = 0.731; 95% CI: 0.680-0.882, p = .014), and in the case of RCIU newborns (AUC = .910; 95% CI: .796-.947, p = .001), respectively AGA newborns(AUC = .669; 95% CI: .612-.826, p = .018).

Regarding the newborn TAS, our results indicated a significant prediction of fetal risk factors on the newborn TAS in the overall assessment of all newborns (AUC = 0.796; 95% CI: 0.681-0.810, p =. 004), consequence of the predictive power confirmed exclusively in the case of RCIU newborns (AUC = .866; 95% CI: .689-.942, p = .003).

VII.5.1.8. Total antioxidant capacity and neonatal pathology

The values of maternal TAS and umbilical cord TAS were analyzed in relation to neonatal pathologies, including from the perspective of the predictive power of TAS to estimate the occurrence of neonatal morbid conditions.

A. Maternal TAS

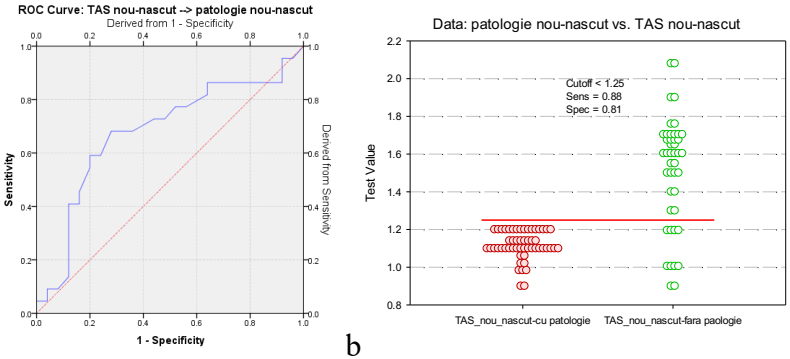
Maternal TAS values showed significantly lower values in cases associated with neonatal pathology, and in the case of association with IUGR, the values were very low, with an average of 1.28 ± 0.25 mmol / L, with lows of 0.89 and highs of 1.98.

The obtained results demonstrated the significant predictability of maternal TAS values on neonatal pathology (AUC = 0.709, 95% CI: 0.595-0.823, p = .001). We noticed that this predictive power increases significantly for newborns with IUGR (AUC = 0.869, 95% CI: 0.757-0.980, p <.001).

The cutoff value for maternal TAS for estimating the increased risk of the presence of the newborn pathology was 1.30 mmol / L. Our data indicated that values lower than this threshold significantly increase the probability of occurrence of newborn morbidity.

B. Newborn TAS

Unlike maternal TAS values (for which we recorded significantly lower values both in newborns with IUGR and in AGA newborns associated with perinatal pathology), in the case of TAS in newborns we obtained significantly lower values only in the context of the association of the pathology with IUGR (1.28 ± 0.25 mmol / L), with statistically significant differences compared to the values for AGA newborns with associated pathology.



a b

Figure VII.44. ROC curve (a) and even point histogram (b) for neonatal TAS cutoff values to assess the predictability of neonatal pathology

Umbilical cord TAS values were a good predictor in the overall assessment of the newborn's pathology (AUC = 0.677, 95% CI: 0.564-0.791, p = .003), a consequence of the predictive power of the newborn TAS in the group of newborns of IUGR (AUC = 0.707, 95% CI: 0.513-0.902, p = .022) (Figure VII.44a). To estimate the risk of perinatal or neonatal pathology, we calculated a cutoff value of umbilical cord

TAS - 1.25 mmol / L (Figure VII.44b). Our data indicated that values of the newborn TAS above this reference threshold significantly increase the risk of

The statistical analysis showed that the TAS values in the umbilical cord have a significantly better accuracy in predicting neonatal pathology (Odd ratio-Exp (β) = 2.07; 95% CI: 1.54-3.88), compared to maternal TAS values.

VII.5.2. Study of placental angiogenesis imbalances in IUGR

VII.5.2.1. Clinico-pathological characteristics in the studied groups

A. Arterial hypertension

Pre-existing hypertension in pregnancy was identified in 15.8% of pregnant women with IUGR, being absent in the control group.

In the case of pregnancy-induced hypertension, the frequency of cases was higher in pregnant women with IUGR (23.7%) compared to the frequency in those without IUGR (3.3%).

B. Preeclampsia

PE had a high frequency in pregnant women with IUGR, which was present in 26.3% of all cases. On the other hand, no case without RCIU showed preeclampsia.

C. Correlations between hypertension and preeclampsia

The results of the analysis showed a significant association between pre-existing hypertension in pregnancy and PE; 85.71% of pregnant women with pre-existing hypertension developed PE.

In contrast, our data did not confirm the association between gestational hypertension and PE. Although we recorded a frequency of 20% of cases with pregnancy-induced hypertension associated with PE, compared to only 13.79% of cases without pregnancy hypertension that presented PE, this difference was not statistically significant.

VII.5.2.2. The sFlt1 factor in IUGR

A. The sFlt1 factor in association with IUGR

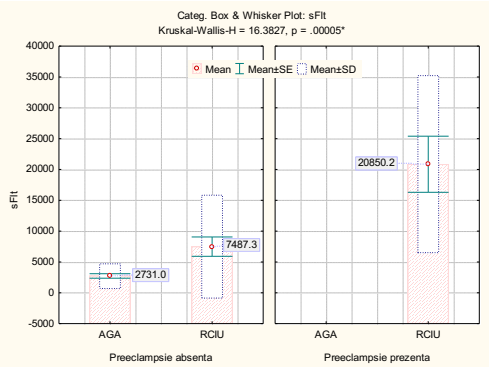


Figure VII.52. Mean values of sFlt1 depending on the presence of PE / RCIU

The results indicated significantly higher sFlt1 values in the case of IUGR. At the same time, we noticed that the association of PE in the case of IUGR determined significantly higher values of sFlt1 (20850.2 ± 14380.2) compared to those recorded in the case of IUGR for other causes (7487.3 ± 8304.4) (p = .00005) (Figure VII.52).

B. Correlation of sFlt1 factor values with TAS values

Maternal TAS values showed a significant inverse correlation with sFlt1 values. Thus, for lower values of TAS, significantly higher values of sFlt were encountered (r = -0.4982, p = .00002).

In the case of umbilical cord TAS values we also recorded a significant inverse correlation with sFlt values, explained by significantly lower umbilical cord TAS values in the case of high sFlt values (r = -0.7832, p = .00003).

The obtained results allowed to establish the cutoff reference value for which the probability of RCIU significantly increases. Thus, for values higher than the cutoff value of sFlt1 - respectively 3470 ng / mL, the probability of association of IUGR was very high. The sensitivity of this evaluation was 78.95% and the specificity of 86.67%.

VII.5.2.3. The PlGF factor in IUGR

A. The PlGF factor in association with IUGR

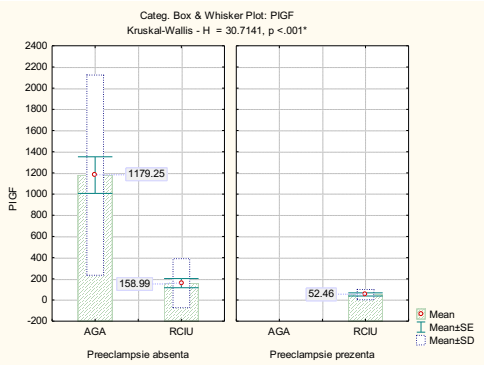


Figure VII.56. Mean PlGF values depending on the presence of PE / RCIU

The PlGF factor showed significantly lower values in the case of IUGR (159.0 ± 229.8) and, in particular, in the case of the association of IUGR with PE (52.5 ± 47.6) (p <.001) (Figure VII.56).

B. Correlation of PlGF factor values with TAS values

PlGF values showed a significant correlation (r = 0.526, p <.001) with maternal TAS values, explained by low maternal TAS values for low PlGF values.

In addition, in the case of TAS values from the umbilical cord we recorded a significant direct correlation (r = 0.618, p <.001) with PlGF values. Consequently, the TAS values in the newborn were low, given that the PlGF factor showed low values.

According to the AUC analysis, we obtained a high predictive power of PlGF for the occurrence of RCIU. As a result of establishing a cutoff value, PlGF values lower than the cutoff value of 143 ng / mL predict, with a very high probability, the presence of RCIU (sensitivity 84.21%, specificity of 93.3%).

VII.5.2.4. sFlt1 / PlGF ratio

We obtained a significant inverse correlation between the TAS values (maternal and newborn) and the value of the sFlt1 / PlGF ratio, for low TAS valuesm, high values of this ratio being recorded.

The values of the sFlt1 / PlGF ratio showed an increased predictive power, according to the ROC curve, having a sensitivity of 84.21% and a specificity of 93.3% for the cutoff of 36.07. Higher ratio values than this reference threshold indicated a very high probability of IUGR.

VII.5.2.5. sFlt1 and PlGF factors and the sFlt1 / PlGF ratio in correlation with neonatal pathology

The obtained results highlighted the fact that, based on the values of the sFlt1 / PIGF ratio, the risk of newborns presenting neonatal pathology can be estimated with the greatest precision.

To validate the result, AUCs were calculated for all three methods of predicting the occurrence of the pathology in the newborn.

Values of the sFlt / PIGF ratio higher than 85.23 predict the occurrence of neonatal morbidity with a sensitivity of 81.25% and specificity of 94.4%.

VII.5.2.6. Evaluation of the predictive power of sFlt1 and PlGF in patients without PE

The use of antiangiogenic factor - sFlt1 and serum placental growth factor - PlGF has a predictive value for PE and, implicitly, RCIU for this reason.

Consequently, we analyzed the predictive potential of IUGR and neonatal pathology, using the sFlt1 / PlGF ratio, if PE is not associated, as a risk factor.

The results indicated, in the case of RCIU, a cutoff value of the sFlt1 / PlGF ratio of 12.7. For the occurrence of neonatal pathology if RCIU was not associated with PE, we deduced a cutoff value of 85.23.

VII.5.3. Pilot study for the analysis of placental proteomic expression

VII.5.3.1. Separation of placental proteins by 1-D electrophoresis

The results obtained in the separation of placental proteins by 1-D electrophoresis showed both for the normal placentas (samples 2

and 5) and for those harvested from patients previously diagnosed with IUGR (samples 1, 3, 4, 6 and 7), several bands corresponding to proteins with a large molecular weight scale (between 15 kDa and 100 kDa) (Figure VII.69).

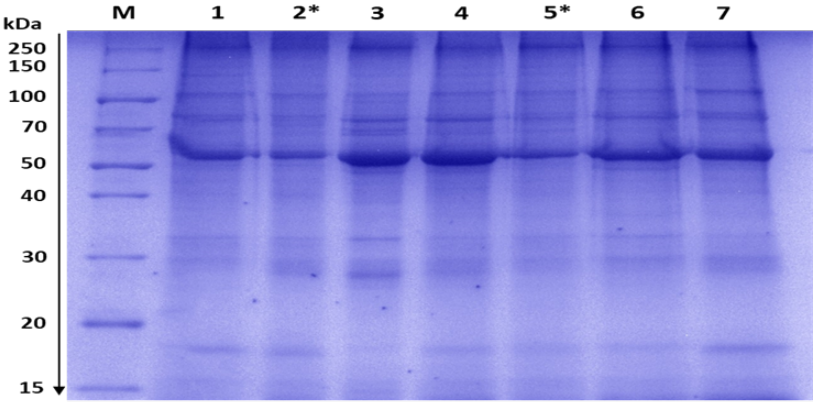


Figure VII.69. Separation of placental proteins by immediate electrophoretic method (appearance of placentas with and without pathology)

VII.5.3.2. Optimal storage conditions for proteomics studies

We studied the optimal storage conditions (time) of the placental homogenates. Thus, we analyzed the differences in the processing of placental samples from pregnant women diagnosed with IUGR, at an interval of one year from protein isolation and, respectively, in fresh homogenates - in both cases the storage conditions being identical (-28 ° C).

Although we did not observe any qualitative differences between the samples, the analysis of the samples indicated quantitative differences between the gel migration lines. We thus verified the negative effect of freezing / thawing samples with complex protein content, as well as isolated proteins, by the method of precipitating proteins with trichloroacetic acid.

VII.6. Discussions

VII.6.1. Study of oxidative stress and impact in RCIU (TAS Study)

Doctoral research focused on the analysis of oxidative stress in IUGR has achieved its objectives, as we have confirmed a number of correlations between IUGR and decreased TAS, which reflect the increase in oxidative status in both mother and newborn. We also highlighted the most important risk factors in the production of TAS variations and, last but not least, we demonstrated the TAS variation, as a marker of oxidative stress, in newborns with secondary perinatal complications of IUGR.

In conclusion, this study led to the identification of lower TAS values (maternal and neonatal) in pregnancies with IUGR, compared to pregnancies resulting in newborns on GMS. These values proved to be predictive, below the value of 1.32 mmol / L (maternal TAS), respectively 1.25 mmol / L (newborn TAS), for the occurrence of IUGR.

Also, an important aspect is the direct, significant correlation between the values of maternal TAS and those of the newborn, which means a reflection of the oxidative processes occurring at the fetal level in the maternal blood.

Placental and fetal risk factors had a significant influence on the variation of TAS, in both groups investigated (RCIU and GMS), with some peculiarities related to the association of RCIU. Maternal risk factors have not been shown to significantly influence TAS values.

Urea, creatinine and uric acid values are inversely correlated with TAS values in the newborn, while LDH values are in direct correlation with TAS in the newborn.

Last but not least, maternal and neonatal TAS values are predictive of perinatal complications - overall, the highest strength is with umbilical cord TAS, which is an important predictive marker for digestive or pulmonary hemorrhage and respiratory distress.

VII.6.2. Study of placental angiogenesis imbalances in IUGR (sFlt1 / PlGF study)

Doctoral research focused on changes in placental angiogenesis has achieved its goal, as we confirmed the association between factors expressing placental angiogenesis - sFlt1 and PlGF - and imbalances of oxidative factors in maternal and fetal serum and, consequently, the occurrence of IUGR.

Consequently, the contribution of our study is reflected in the identification of additional markers that have predictive value on the occurrence of IUGR, in addition to ultrasound indicators known and already used in clinical practice. Our results are relevant in the constant effort of specialists to refine and increase the sensitivity and specificity of diagnostic tests for IUGR, so that the established treatment is best adapted to the cases.

In conclusion, this study confirmed that pregnancy-induced hypertension and, in particular, pre-existing hypertension are associated with IUGR as well as PE.

The TAS values were significantly higher in the AGM group compared to the RCIU and, in addition, higher in patients with RCIU without PE than in those with PE.

The values of sFlt1, PlGF and the sFlt1 / PlGF ratio correlate with the presence of associated pathologies, thus observing an imbalance in the sense of increasing antiangiogenic factors and decreasing angiogenic ones.

Also, sFlt1, PlGF and the sFlt1 / PlGF ratio correlate with TAS values. The increase in the sFlt1 / PlGF ratio has a significant inverse correlation with TAS values; thus, we can appreciate that an oxidizing status is correlated with a deficient angiogenesis.

Angiogenic factors correlate with the presence of neonatal pathology. Thus, the sFlt1 / PlGF imbalance is predictive of neonatal morbidity.

In patients without PE, the cutoff value of the sFlt1 / PlGF ratio is 12.7 for the RCIU prediction and 85.2 for the neonatal morbidity prediction.

VII.6.3. Pilot study for the analysis of placental proteomic expression

Doctoral research focused on placental proteomic expression has achieved its goal because, by electrophoresis of placental proteins, we have shown that there are differences between the results obtained in cases with IUGR and normal ones.

In the future, we will consider the separation of these homogenates by the 2D-gel technique that allows the loading of gels with 200-300 μg of homogenate, and the separation will be done according to two dimensions: the isoelectric, after which of the molecular mass.

In our study, we demonstrated the validity of the method in dosing placental proteins and determined that it would be ideal for the method to be performed on the fresh sample, as storage (freezing and thawing) of the sample causes irreparable protein damage.

Perspectives set by the doctoral research

The studies in this paper approached the problem of biomarkers from two points of view: markers of oxidative stress and markers of placental angiogenesis. An in-depth study of these issues could be of interest in the future, as increasing the study group could help to refine the results and, consequently, to identify those markers that will be of interest both in terms of yield and economic aspect.

Proteomics is an area with important potential in the study of biomarkers. In perspective, we will consider the separation by 2D-gel technique of the homogenates initially examined by the 1-D technique, thus being possible to load the gels with 200-300 μg of homogenate, consecutively the separation being done according to two dimensions: the electrical and molecular weight.

Considering the results obtained following the doctoral study, the following directions are established that would be required in the future:

- implementation of an IUGR screening program at regional, or even national level;
- establishing at regional level teams of professionals to manage the cases diagnosed with IUGR;
- conducting in-depth studies of IUGR prophylaxis, applicable in risk tasks;
- creation of working protocols for the diagnosis and treatment of IUGR, which could represent, in the future, a possible starting point for the development of practice guidelines in this pathology; an example of such a protocol that we propose following the doctoral study can be found in the Annex section of this paper (Annex 1).

FINAL CONCLUSIONS

1. The frequency of IUGR is constantly increasing at the maternity hospital "Cuza Voda" in Iasi, so that, out of a total of 31683 newborns, in a period of 5 years, 2.04% were diagnosed with RCIU or SGA. More importantly, most cases were diagnosed postnatal, which reveals a lack of proper follow-up of these cases, even in an important university center - an aspect attributable, in particular, to the non-presentation of patients.
2. The diagnosis of IUGR was established at an average gestational age of 32.3 weeks, with a minimum of 18 weeks and a maximum of 39 weeks. It is important to note that half of the newborns were diagnosed at gestational ages less than 33 weeks.
3. The most important risk factors for IUGR include genetic disorders, followed by the mother's socio-economic status, maternal infections, placental abnormalities, extreme maternal age, TORCH infections and pregnancy.
4. Early postnatal complications were 2.07 times more common in patients in the RCIU / SGA group, compared to newborns in the AGA group. These included, in order of importance: respiratory distress due to surfactant deficiency and other causes, hemorrhagic disease, asphyxia, thrombocytopenia and ulcer-necrotic enterocolitis.
5. Mortality among newborns with IUGR was at a level of 1.62%, insignificantly higher than that of AGA newborns. Instead, we observed a longer hospitalization period for patients with IUGR / SGA compared to those with AGA.
6. We identified lower TAS values (maternal and neonatal) in pregnancies with IUGR, compared to pregnancies resulting in AGA newborns. These values proved to be predictive, below the value of 1.32 mmol / L (maternal TAS), respectively 1.25 mmol / L (newborn TAS), for the occurrence of IUGR. Also, an important

aspect is the direct, significant correlation between the values of TAS in the maternal serum and that of the newborn, which means a reflection of the oxidative processes occurring at the fetal level in the maternal blood.

7. Placental and fetal risk factors had a significant influence on the variation of TAS for both pregnant women and AGA/IUGR newborns. Maternal risk factors have not been shown to significantly influence TAS values.
8. Maternal and neonatal TAS values are predictive for the occurrence of perinatal complications, the highest predictive power having a TAS at the level of the umbilical cord; it is an important predictive marker for the occurrence of digestive or pulmonary hemorrhages and respiratory distress.
9. Pre-existing hypertension in pregnancy is a risk factor for PE. The timing of PE diagnosis correlates significantly with the values of the sFlt1 / PlGF ratio, so the elevated values of this ratio will be predictive for PE in association with IUGR.
10. The values of sFlt1, PlGF and the sFlt1 / PlGF ratio correlate with the presence of neonatal pathologies, thus observing an imbalance in the sense of increasing antiangiogenic factors and decreasing angiogenic ones.
11. The factors sFlt1, PlGF and the ratio sFlt1 / PlGF correlate with the TAS values. The increase in the sFlt1 / PlGF ratio has a significant inverse correlation with TAS values; thus, we can appreciate that an oxidative status is correlated with a deficient angiogenesis.
12. Angiogenic factors correlate with the presence of neonatal pathology. Thus, the sFlt1 / PlGF imbalance is predictive of neonatal morbidity. In patients without preeclampsia, the cutoff value of the sFlt1 / PlGF ratio is 12.7 for the RCIU prediction and 85.2 for the perinatal morbidity prediction.

13. Proteomics is an avant-garde way of identifying biomarkers, with diversified applications. In our study, we demonstrated the validity of the method in dosing some placental proteins and determined that it would be ideal for the method to be performed on the fresh sample, as storage (freezing and thawing) of the sample causes irreparable protein damage.
14. Pregnancy follow-up, early detection of preeclampsia and identification of risk factors for IUGR, associated with a panel of appropriately targeted investigations (TAS, sFlt1, PlGF) could help to improve the progress of pregnancies with IUGR.

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Scientific activity in the field of doctoral thesis carried out during the Doctoral Studies:

The first scientific report: 11.06.2018 - Obstetric diagnosis of intrauterine growth restriction. Early complications and risk factors involved in intrauterine growth restriction
Second scientific report: 28.02.2019 - The role of oxidative stress in perinatal and neonatal pathology

Articles published in the field of Thesis in ISI indexed journals:

- Șadiye-Ioana Scripcariu, Andreea Avasiloaiei, Demetra Socolov, Elena Mihălceanu, Daniela-Cristina Dimitriu, Mihaela Moscalu, Maria Stamatin. **Total antioxidant status as marker of oxidative stress in infants with intrauterine growth restriction**. *Revista Româna de Medicină de Laborator*. 2020;28(2):145-52. (IF=0.8)
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Participation in international congresses with posters in the field of thesis:

ECPM (European Congress of Perinatal Medicine) 5-8 September 2018, Sankt Petersburg

- **Şadiye-Ioana Scripcariu**, Andreea Avasiloaiei, Mihaela Moscalu, Maria Stamatin – „The intrauterine growth restricted neonate – epidemiological issues, immediate outcome"

The XXVth European Congress of Perinatal Medicine, 15-18 June 2016, Maastricht

- **Şadiye-Ioana Scripcariu**, Andreea Avasiloaiei, Maria Stamatin – „Risk Factors of IUGR in a Level III Maternity Center from Romania"

Participation in the national congress with oral presentation in the field of the thesis:

SOGR (National Congress of the Romanian Society of Obstetrics and Gynecology) 20-22 September 2018, Iași

- Maria Stamatin, Andreea Avasiloaiei, Mădălina Gradinaru-Popa, Șadiye-Ioana Scripcariu – „Rolul stresului oxidativ în patologia materno-fetală și neonatală”, ISBN 978-973-0-27806-4