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THE ROLE OF MOLECULAR MECHANISMS IN PREECLAMPSIA SCREENING

PhD THESIS SUMMARY

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INTRODUCTION

The present thesis addresses the epigenetic changes involved in pre-eclampsia, an obstetric condition of major importance, characterised by complex aetiopathogenesis and significant consequences for maternal and fetal health. The choice of this topic is justified by the persistence of pre-eclampsia as one of the leading causes of maternal and perinatal morbidity and mortality, as well as by current difficulties regarding prediction, early diagnosis and the individualisation of therapeutic management. Despite advances in maternal–fetal medicine, pre-eclampsia remains a heterogeneous clinical entity, with variable manifestations ranging from moderate forms to severe presentations associated with multi-organ involvement, placental dysfunction, intrauterine growth restriction and prematurity.

The topicality of the research derives from the need to integrate classical clinical data with information derived from molecular biology, epigenetics and digital medicine. Pre-eclampsia cannot be explained exclusively by gestational hypertension or by macroscopic placental alterations, but must be understood as the result of interactions between genetic, immunological, vascular, metabolic and epigenetic factors. In this context, epigenetics provides a relevant conceptual framework for explaining how maternal and environmental factors can influence gene expression without altering the DNA sequence, thereby contributing to placental dysfunction and the onset of the disease.

The general hypothesis of the thesis proposes that hypertensive disorders associated with pregnancy can be characterised more rigorously through an integrative approach that correlates angiogenic biomarkers, retrospective clinical and epidemiological data, and digital monitoring tools. The general objective is the integrated assessment of pre-eclampsia, eclampsia and pregnancy-associated hypertension through the analysis of the relevance of PlGF in suspected pre-eclampsia, the characterisation of cases admitted during the period 2020–2025, and the exploration of the usefulness of the Heart4Mom application in monitoring blood pressure in pregnant women.

The research is observational, retrospective and analytical in nature and is structured into three complementary studies. The first study investigates the distribution of PlGF values and the usefulness of this biomarker in obstetric risk stratification. The second analyses the clinical and epidemiological profile of cases of pre-eclampsia and eclampsia admitted to a hospital centre. The third study evaluates pregnancy-associated hypertension and introduces an innovative digital component represented by the Heart4Mom application.

I. GENERAL PART

1. Preeclampsia: general data, importance and pathophysiological basis

Pre-eclampsia represents one of the most important complications of pregnancy, with major implications for maternal, fetal and perinatal health. The classical definition, centred on the onset of hypertension and proteinuria after mid-pregnancy in previously normotensive pregnant women, describes only part of the complexity of this condition. At present, pre-eclampsia is regarded as a systemic disorder of placental origin, resulting from the interaction between defective placentation, angiogenic imbalance, inflammation, oxidative stress, endothelial dysfunction and alteration of the maternal immune response.

The central pathophysiological mechanism is insufficient trophoblastic invasion and incomplete remodelling of the spiral arteries. In normal pregnancy, the extravillous trophoblast transforms the uterine arteries into wide, low-resistance vessels capable of ensuring adequate placental perfusion. In pre-eclampsia, this transformation is incomplete, the arteries remain narrow and reactive, and the placenta is exposed to intermittent hypoxia and repeated episodes of reoxygenation. These processes induce oxidative stress, activate inflammatory pathways and promote the release into the maternal circulation of anti-angiogenic and pro-inflammatory factors responsible for systemic endothelial damage.

The classification of pre-eclampsia into early-onset and late-onset forms reflects the existence of distinct pathogenic mechanisms. Early-onset forms are more frequently associated with major defects in placentation, intrauterine growth restriction and a severe maternal–fetal prognosis. Late-onset forms are influenced to a greater extent by pre-existing maternal factors, such as obesity, diabetes, chronic hypertension and cardiovascular vulnerability.

From an epidemiological perspective, pre-eclampsia has an uneven global distribution, depending on access to prenatal care, medical infrastructure, socio-economic status and the prevalence of risk factors. The burden of the disease is more pronounced in resource-limited regions and in rural areas, where delayed diagnosis and insufficient monitoring increase the risk of complications. The clinical and socio-economic importance of pre-eclampsia derives from its association with prematurity, fetal growth restriction, maternal multi-organ involvement, increased duration of hospitalisation and high medical costs, particularly through neonatal intensive care.

At the same time, epigenetic mechanisms, through DNA methylation, histone modifications and non-coding RNAs, may influence the expression of genes involved in trophoblastic invasion, angiogenesis and the immune

response. Thus, pre-eclampsia should be approached as a complex, interdisciplinary condition in which understanding placental, vascular, immunological and epigenetic mechanisms is essential for prediction, prevention and personalised management.

2. Epigenetics and its role in the pathogenesis of pre-eclampsia

Epigenetics represents a fundamental field for understanding the pathogenesis of pre-eclampsia, as it explains how gene expression can be regulated without modifying the DNA sequence. Through mechanisms such as DNA methylation, histone modifications and regulation by non-coding RNAs, epigenetics ensures the fine control of gene activity, cellular differentiation, adaptation to stress and the response to environmental factors. In the context of pregnancy, these processes are of major relevance, because the placenta is a dynamic organ that depends on the coordinated activation and repression of genes involved in implantation, trophoblastic invasion, angiogenesis and vascular remodelling.

DNA methylation represents one of the most important epigenetic mechanisms, playing a role in gene silencing, the maintenance of genomic stability and the establishment of cellular identity. In pre-eclampsia, alterations in methylation profiles may modify the expression of genes involved in trophoblastic invasion, adaptation to hypoxia, angiogenesis and immunological regulation. In particular, abnormal methylation of the VEGF, FLT1 and ENG genes may disturb the balance between pro-angiogenic and anti-angiogenic factors, contributing to placental insufficiency, hypoxia and maternal endothelial dysfunction.

Histone modifications constitute another essential level of epigenetic regulation, influencing chromatin structure and gene accessibility for transcription. In the normal placenta, these modifications support trophoblastic invasion, angiogenesis and adaptation to hypoxia. In pre-eclampsia, histone dysregulation may reduce the expression of pro-angiogenic genes and activate pathways associated with inflammation and oxidative stress, amplifying shallow placentation and placental dysfunction.

A central role is also played by non-coding RNAs, particularly microRNAs, which regulate gene expression post-transcriptionally. MiR-210, miR-155 and miR-21 are relevant in pre-eclampsia through their involvement in hypoxia, the inflammatory response, angiogenesis, apoptosis and trophoblastic invasion. The overexpression of these molecules reflects insufficient placental adaptation to an environment characterised by hypoxia, inflammation and oxidative stress.

Therefore, the placental and circulating epigenome may function as a diagnostic and prognostic biomarker. Epigenetic signatures detectable in maternal blood may anticipate placental dysfunction, disease severity and the

risk of complications. Thus, epigenetics offers an integrative perspective on the relationship between genes, the environment and placental pathology, opening important directions for the prediction, monitoring and personalised management of pre-eclampsia.

3. Risk factors, epigenetic influences and the diagnosis of pre-eclampsia

The risk factors, epigenetic influences and diagnostic methods of pre-eclampsia highlight the multifactorial nature of this obstetric condition. Susceptibility to the disease is determined by the interaction between genetic predisposition, hereditary factors, the maternal environment and epigenetic changes that regulate the expression of genes involved in placentation, angiogenesis, endothelial function and the immune response. Although pre-eclampsia does not follow a simple hereditary pattern, family history, maternal genetic characteristics and the contribution of the fetal or paternal genome may influence trophoblastic invasion, remodelling of the spiral arteries and placental adaptation to hypoxia.

Environmental factors exert a significant impact on the placental epigenome. Diet, smoking, oxidative stress, exposure to toxins and socio-economic conditions may modify DNA methylation, histone structure and the expression of non-coding RNAs, thereby influencing placental function and the risk of pre-eclampsia. Prenatal and intergenerational exposures complete this perspective, demonstrating that the intrauterine environment and previous biological experiences may leave persistent epigenetic marks, with effects on maternal and fetal health and even on subsequent generations.

The diagnosis of pre-eclampsia requires the integration of clinical, biological and imaging assessment. Blood pressure monitoring, identification of signs of systemic involvement and laboratory investigations remain essential for assessing disease severity. Obstetric ultrasound and uterine and fetal Doppler examination allow the assessment of fetal growth, placental perfusion and vascular resistance, contributing to the early identification of defective placentation.

Traditional serum markers, such as PAPP-A, sFlt-1 and PlGF, provide relevant information regarding trophoblastic activity, angiogenic imbalance and placental function. In parallel, emerging epigenetic markers, particularly DNA methylation and circulating microRNAs, have considerable diagnostic and prognostic potential, as they reflect early molecular changes that can be detected in a minimally invasive manner in maternal blood. Thus, the modern diagnosis of pre-eclampsia is moving towards a predictive, integrative and personalised approach.

II. PERSONAL CONTRIBUTIONS

4. Working hypothesis and general objectives

The present research is based on the general hypothesis that hypertensive disorders of pregnancy, particularly pre-eclampsia, eclampsia and pregnancy-associated hypertension, can be characterised more accurately and with greater clinical utility through an integrated approach that combines placental angiogenic biomarkers, retrospective analysis of clinical data and the use of digital monitoring tools.

It is assumed that PlGF values reflect the functional status of the placenta and contribute to the stratification of pre-eclampsia risk, while the retrospective analysis of admitted cases highlights temporal variations, differences in severity and relevant clinical and demographic characteristics for understanding the profile of these conditions in a real hospital setting. At the same time, it is considered that the integration of a dedicated application, such as Heart4Mom, may complement traditional clinical assessment by facilitating continuous blood pressure monitoring and supporting the early identification of pregnant women at increased obstetric risk.

The general objective of the research is the integrated assessment of hypertensive disorders of pregnancy through the analysis of the relevance of the placental biomarker PlGF in suspected pre-eclampsia, the retrospective characterisation of cases of pre-eclampsia, eclampsia and pregnancy-associated hypertension during the period 2020–2025, and the exploration of the usefulness of the Heart4Mom application as a complementary tool for blood pressure monitoring in pregnant women.

Thus, the research proposes:

1. To evaluate the clinical relevance of PlGF as an angiogenic biomarker in the context of suspected pre-eclampsia, by analysing the distribution of its values and its usefulness in obstetric risk stratification.
2. To retrospectively characterise the clinical and epidemiological profile of pre-eclampsia and eclampsia in an obstetrics and gynaecology department, by analysing temporal distribution, frequency, severity and the structure of diagnostic categories.
3. To retrospectively analyse pregnancy-associated hypertension from the perspective of annual distribution, the main clinical forms and their relationship with the demographic characteristics of the patients, particularly maternal age.
4. To explore the applied potential of a digital monitoring solution, namely the Heart4Mom application, in monitoring blood pressure in pregnant women and in supporting the screening and monitoring of cases with hypertensive risk.

5. To outline an integrated model for understanding and monitoring hypertensive obstetric pathology, based on the correlation of biomarkers, retrospective clinical data and modern digital tools.

Study 1, entitled **"Distribution of PlGF values in a set of maternal samples analysed on DELFIA Xpress and their clinical relevance for suspected pre-eclampsia"**, is based on the hypothesis that serum values of placental growth factor (PlGF), determined using the automated DELFIA Xpress method, accurately reflect the placental angiogenic status and may be used as a useful tool for stratifying the risk of pre-eclampsia in clinical practice. It is assumed that the distribution of PlGF values in a cohort of patients investigated for suspected pre-eclampsia is characterised by increased variability and a non-Gaussian structure, determined by the biological heterogeneity of the population and by the presence of subgroups with different risks of placental dysfunction.

Another derived hypothesis is that low PlGF values are associated with angiogenic imbalance and an increased risk of placental pathology, whereas high values are suggestive of relatively preserved placental function. At the same time, it is considered that the use of PlGF as an isolated marker has limitations in the range of intermediate values, where interpretation requires integration with other clinical and biological parameters.

The general objective of the study was to evaluate the distribution of PlGF values in a set of maternal samples analysed using DELFIA Xpress and to assess the clinical relevance of this biomarker in the context of suspected pre-eclampsia.

The specific objectives included the statistical characterisation of PlGF values, assessment of the type of data distribution and identification of any deviations from normality, analysis of distribution by intervals and clinical stratification of values according to relevant thresholds, identification of extreme values and analysis of their impact on descriptive parameters, as well as assessment of differences between groups using statistical methods appropriate for non-parametric data. In addition, the study aimed to identify possible pre-analytical and administrative influences on the results and to integrate these into the final interpretation.

For Study 2, entitled **"Retrospective analysis of cases of pre-eclampsia and eclampsia during the period 2020–2025"**, the present research starts from the premise that pre-eclampsia and eclampsia are obstetric conditions with a major impact on maternal and fetal prognosis, whose clinical expression and distribution may vary from one year to another even within the same clinical department. In the context of current medical practice, the retrospective analysis of cases admitted to an obstetrics and gynaecology unit may provide relevant information regarding the frequency, structure and severity of these conditions, as well as the way in which they are recorded and classified in clinical documentation.

The working hypothesis of the study is that, during the period 2020–2025, the distribution of cases of pre-eclampsia and eclampsia admitted to the Obstetrics and Gynaecology I Department of Dr I. Cantacuzino Clinical Hospital in Bucharest was not uniform over time, with annual variations both in the number of cases and in the severity of the recorded clinical forms. It is also assumed that unspecified and moderate forms of pre-eclampsia accounted for an important proportion of the analysed cohort, while eclampsia remained a rare complication, but one of major clinical relevance.

The general objective of the research was the retrospective analysis of cases of pre-eclampsia and eclampsia identified during the period 2020–2025, in order to characterise their temporal distribution, the relative weight of diagnostic categories and variations in clinical severity.

In accordance with this general objective, the research pursued several main directions. The first consisted of describing the structure of the analysed cohort by identifying the number of patients, the number of clinical records and the age distribution. The second aimed to evaluate the annual distribution of cases, in order to highlight any variations in the frequency of pre-eclampsia and eclampsia over the analysed interval. Another objective was to quantify the relative weight of the included ICD-10 diagnostic categories, namely moderate pre-eclampsia, severe pre-eclampsia, unspecified pre-eclampsia and the different forms of eclampsia. The study also aimed to assess variations in clinical severity from one year to another, as well as the relationship between year of discharge and the distribution of clinical forms. In addition, the relationship between patients' age and diagnostic categories was analysed, in order to observe the extent to which age profile may influence the distribution or severity of cases.

By achieving these objectives, the study aims to contribute to a better understanding of the clinical profile of pre-eclampsia and eclampsia in a real hospital setting and to highlight the need for rigorous obstetric monitoring, standardised diagnostic coding and appropriate postpartum surveillance.

Study 3, entitled **"Retrospective analysis of cases of pregnancy-associated hypertension using the Heart4Mom application"**, is based on the premise that pregnancy-associated hypertension represents one of the most frequent and important obstetric conditions, with major implications for maternal and fetal prognosis, and that its clinical expression may vary over time even within the same obstetrics and gynaecology department. In current medical practice, the retrospective analysis of cases admitted to a clinical unit may provide relevant data regarding the frequency, structure and dynamics of the main forms of pregnancy-associated hypertension, as well as the way in which these are recorded, classified and reported in medical documentation. At the same time, the integration of a dedicated digital solution, such as the Heart4Mom application, opens up the prospect of modern and continuous blood pressure monitoring in pregnant women, with potential utility in the

early detection of blood pressure changes and in supporting the surveillance of cases with increased obstetric risk.

The working hypothesis of the study is that, during the period 2020–2025, the distribution of cases of pregnancy-associated hypertension admitted to the Obstetrics and Gynaecology I Department of Dr I. Cantacuzino Clinical Hospital in Bucharest was not uniform over time, with annual variations both in the number of cases and in the structure of the main recorded diagnostic categories. It is also assumed that gestational hypertension represented the dominant form in the analysed cohort, although in recent years there has been a relative increase in the proportion of pre-existing hypertension complicating pregnancy. At the same time, it is considered that more advanced maternal age was more frequently associated with forms of pre-existing hypertension than with gestational hypertension. As a complement to the retrospective component, it is assumed that the Heart4Mom application may constitute a feasible and useful tool for blood pressure monitoring in pregnant women, facilitating the longitudinal follow-up of blood pressure values and the early identification of changes suggestive of obstetric hypertensive risk.

The general objective of the research was the retrospective analysis of cases of pregnancy-associated hypertension identified during the period 2020–2025, in order to characterise their temporal distribution, the relative weight of the main diagnostic categories and the clinical and demographic characteristics of the cohort, as well as to explore the applicability of the Heart4Mom application in blood pressure monitoring in pregnant women.

The research pursued several main directions. The first consisted of describing the structure of the analysed cohort by identifying the number of patients, the number of diagnostic records and the age distribution. The second aimed to evaluate the annual distribution of cases, in order to highlight any variations in the frequency of pregnancy-associated hypertension over the analysed interval. Another objective was to quantify the relative weight of the main ICD-10 diagnostic categories included in the analysis, namely pre-existing essential hypertension complicating pregnancy, pre-existing secondary hypertension, unspecified pre-existing hypertension and gestational hypertension without significant proteinuria. The study also aimed to assess changes in diagnostic structure from one year to another, as well as to compare the dynamics of gestational hypertension with those of pre-existing hypertension. In addition, the relationship between patients' age and the main diagnostic categories was analysed, in order to observe the extent to which age profile may influence the distribution of the clinical forms of pregnancy-associated hypertension. At the same time, the applied component of the study aimed to explore the feasibility and clinical usefulness of the Heart4Mom application as a complementary tool for home blood pressure monitoring, in order to support the screening and surveillance of pre-eclampsia and other hypertensive disorders of pregnancy.

By achieving these objectives, the study aims to contribute to a better understanding of the clinical profile of pregnancy-associated hypertension in a real hospital setting and to highlight both the importance of rigorous diagnostic coding and careful obstetric monitoring, as well as the potential of modern digital tools to support the surveillance of pregnant women at hypertensive risk.

5. General research methodology

Study 1, entitled **"Distribution of PlGF values in a set of maternal samples analysed on DELFIA Xpress and their clinical relevance for suspected pre-eclampsia"**, is an observational, retrospective study based on the analysis of data obtained from laboratory determinations performed in a cohort of patients investigated for suspected pre-eclampsia. The research had an analytical and descriptive character, aiming to evaluate the distribution of PlGF values and interpret them in a clinical context.

The data were collected from the printout of the DELFIA Xpress automated analyser, corresponding to a series of determinations performed on a single day, thereby ensuring uniformity of analytical conditions. Only patient samples with the analytical status "Accepted" were included, while calibrators and technical replicates were excluded from the statistical analysis. The final analysed cohort comprised 42 valid determinations.

PlGF determination was performed using the automated immunochemical method based on fluoroimmunometric technology, according to the manufacturer's instructions. All samples were analysed using the same reagent lot and the same calibration curve, which contributed to reducing analytical variability.

Data processing was performed using IBM SPSS Statistics, version 26, and descriptive analysis methods were applied to characterise continuous variables. The normality of the distribution was assessed using the Shapiro–Wilk test, while comparative analysis between groups was performed using the non-parametric Kruskal–Wallis test, appropriate for data that do not meet the assumption of normality.

PlGF values were analysed both as a continuous and as a categorical variable, by dividing them into clinically relevant intervals and categories. The interpretation of the results was carried out in correlation with data from the specialised literature and with the pathophysiological mechanisms of pre-eclampsia.

In addition, pre-analytical and administrative aspects, such as the presence of haemolysis, lipaemia or identification inconsistencies, were evaluated in order to assess their impact on the validity of the results. All data were analysed in accordance with ethical and confidentiality principles, by anonymising patient-related information.

Study 2, entitled **"Retrospective analysis of cases of pre-eclampsia and eclampsia during the period 2020–2025"**, was designed as a retrospective, observational and descriptive study, conducted on the basis of clinical data available in the electronic archive of Dr I. Cantacuzino Clinical Hospital in Bucharest. The choice of this type of design was justified by the objective of the study, namely to evaluate the distribution and characteristics of cases of pre-eclampsia and eclampsia in a real clinical setting, without intervention in diagnostic or therapeutic management and without influencing the medical pathway of the included patients.

The analysed population consisted of all patients admitted to the Obstetrics and Gynaecology I Department between 1 January 2020 and 30 June 2025, in whom one of the ICD-10 codes corresponding to pre-eclampsia or eclampsia was recorded. Cases coded as O14.0, O14.1, O14.9, O15.0, O15.1, O15.2 and O15.9 were included, provided that essential clinical and administrative data were available. Incomplete or erroneous records, as well as those not belonging to the investigated department, were excluded.

The data were extracted retrospectively from the hospital information system, using electronic medical records and discharge registers. For each patient, information was collected regarding age, sex, medical record number, discharge date, ICD-10 code and type of recorded diagnosis. In order to ensure confidentiality, the data were anonymised and patients' names were converted into initials.

From a methodological point of view, two units of analysis were defined: the unique patient and the clinical record. This distinction was necessary because the same patient could appear more than once in the database, either through several diagnostic entries within the same admission or through different clinical episodes. The analysed variables included demographic, clinical, temporal and nosological parameters, and the cases were grouped by calendar year and diagnostic category.

The statistical analysis included both a descriptive and an inferential component. Categorical variables were expressed as absolute frequencies and percentages, while continuous variables were summarised using means and standard deviations or medians and interquartile ranges, depending on data distribution. The chi-square test of independence was used to assess the relationship between year of discharge and distribution of ICD-10 codes. One-way analysis of variance and the Kruskal–Wallis test were used to compare age between groups, depending on whether the assumptions for applying parametric tests were met. The threshold for statistical significance was set at $p < 0.05$.

The entire research process was conducted in accordance with the ethical principles applicable to studies using human clinical data. The data were used exclusively for scientific purposes, without the possibility of directly identifying the patients, and the study was carried out with the approval of the competent ethics committee.

Study 3, entitled **"Retrospective analysis of cases of pregnancy-associated hypertension using the Heart4Mom application"**, was designed as a study with a mixed structure, integrating a retrospective observational component and an applied pilot component focused on the use of a digital solution for blood pressure monitoring in pregnancy. Through this approach, the study aimed both to describe the real case profile of hypertensive disorders associated with pregnancy in an obstetric clinical centre and to explore the possibility of integrating a mobile application into the surveillance of pregnant women at hypertensive risk.

The analysed period covered the interval from 1 January 2020 to 16 July 2025, corresponding to the availability of data in the institutional system. All patients for whom diagnostic codes compatible with pregnancy-associated hypertension were recorded, according to the ICD-10 classification used in hospital reporting, were included. The analysis considered both the number of patients and the number of diagnostic records, since the same patient could appear with repeated admissions, multiple diagnoses or changes in diagnostic classification during the same clinical episode or in successive episodes.

From a methodological perspective, the research was built around two distinct units of analysis. The first unit was the reported patient, used to describe the general structure of the cohort and the annual clinical burden. The second unit was the diagnostic record, used to analyse the distribution of ICD codes, the annual dynamics of diagnosis and the type of diagnosis recorded in the system. This separation was necessary in order to avoid confusing the number of individuals with the number of diagnostic events and to allow a more accurate interpretation of data derived from retrospective administrative sources.

Cases were defined on the basis of ICD-10 codes recorded in hospital reports, without retrospective reclassification of the diagnosis according to modern clinical criteria. This approach was chosen in order to preserve fidelity to the reality of medical documentation and to evaluate how hypertensive pathology of pregnancy was reflected in routine clinical practice. Diagnoses were analysed both individually, by distinct codes, and by grouping them into major clinical categories, particularly gestational hypertension and pre-existing hypertension associated with pregnancy. In interpreting the results, it was taken into account that administrative diagnosis does not always capture the full pathophysiological complexity of hypertensive disorders of pregnancy and that overlaps or transitions may exist between different clinical entities.

The applied component of the study consisted of a pilot stage using the Heart4Mom application, integrated as a complementary digital tool for blood pressure monitoring in pregnant women. This stage had a translational role and aimed to assess the feasibility, acceptability and practical usefulness of the application in an obstetric context. Heart4Mom was used for the periodic recording of blood pressure values measured at home using a validated blood pressure monitor, after prior instruction of the participants regarding the

standardised measurement technique. The data entered into the application allowed longitudinal follow-up of the blood pressure profile and identification of elevated values or trends suggestive of worsening, with the possibility of referring the patient for clinical reassessment when the situation required it.

Therefore, the general methodology of the study combined retrospective analysis of hospital cases with the preliminary evaluation of a digital telemonitoring solution, in order to obtain an integrated perspective on pregnancy-associated hypertension. On the one hand, the study allowed the local burden of hypertensive obstetric pathology and its variations over time to be described. On the other hand, it provided a framework for exploring how digital tools may support the surveillance of at-risk pregnant women, complementing conventional monitoring and facilitating the early identification of blood pressure changes.

The data were extracted manually from institutional electronic reports and entered into a secure electronic database created specifically for this study. Subsequently, they were checked, standardised and recoded for analysis. Demographic, administrative and clinical coding variables were analysed, including maternal age, year of reporting, type of diagnosis and ICD code. In the case of missing data or records suspected of duplication, predefined validation and deduplication rules were applied, and incomplete information was not statistically imputed.

The statistical analysis included both descriptive and inferential methods. Categorical variables were expressed as absolute frequencies and percentages, while continuous variables were expressed using indicators of central tendency and dispersion. Appropriate comparative tests were used to assess differences between years and between the main diagnostic categories, while logistic regression models were applied to explore the relationship between clinical-demographic variables and diagnostic category.

The study has a pilot and translational character, being designed to verify the possibility of using a digital solution for blood pressure monitoring in pregnant women, an issue of particular relevance in the context of screening and follow-up of pre-eclampsia. Through this structure, the research methodology acquires both explanatory and practical value.

6. Study 1. Distribution of PlGF values in a set of maternal samples analysed on DELFIA Xpress and their clinical relevance for suspected pre-eclampsia

The study aimed to evaluate the distribution of PlGF values in a set of maternal samples analysed using the DELFIA Xpress system, with the purpose of assessing the clinical relevance of this biomarker in the context of suspected pre-eclampsia. The distribution of patients by age group showed a predominance of the 25–30-year interval, representing 33.3% of the cohort, followed by the 31–35-year group, with 29.6%. Thus, 62.9% of the patients

fell within the 25–35-year interval, corresponding to the optimal reproductive period.

The initial cohort included 54 patients; however, the final analysis was performed on the basis of analytically validated samples. After excluding calibrators, technical replicates and non-compliant results, 42 patient samples with the status "Accepted" were analysed, belonging to the same kit lot and the same calibration curve, which supports the uniformity of analytical conditions. The 12 determinations corresponding to calibrators were excluded from the statistical analysis, as they had an exclusively technical role.

PlGF values showed a heterogeneous distribution, with positive skewness and increased variability. The mean was 225.114 pg/mL, the median was 167.2 pg/mL, the standard deviation was 246.8783 pg/mL, the minimum value was 9.3 pg/mL, and the maximum value was 1323.1 pg/mL. The fact that the median was lower than the mean indicates the predominance of low and intermediate values, while extremely high values influenced the overall mean.

Clinical stratification identified 6 cases with PlGF <50 pg/mL, corresponding to an increased risk of placental dysfunction, 13 cases between 50–149 pg/mL, representing an intermediate-risk zone, and 23 cases ≥150 pg/mL, associated with a reduced short-term risk of pre-eclampsia. The Kruskal–Wallis test confirmed significant differences between groups, $H = 32.15$, $p < 0.001$.

Pre-analytical observations, such as haemolysis and lipaemia, together with certain administrative limitations, represent possible sources of variability. Overall, PlGF is confirmed as a useful tool for risk stratification, although its interpretation requires clinical integration and correlation with other biomarkers.

The present study highlighted the usefulness of determining placental growth factor (PlGF) as a tool for assessing placental function and stratifying risk in the context of suspected pre-eclampsia. The analysis of the investigated cohort showed that PlGF values had a wide and heterogeneous distribution, with important biological variability, reflected by the broad range of values, the presence of extreme values and significant deviation from normal distribution.

The statistical profile of the biomarker showed a predominance of low and intermediate values, whereas a smaller proportion of cases presented very high values. This distribution supports the existence of a mixed cohort, in which patients with potentially increased, intermediate and low risk of placental dysfunction coexist. The clinical stratification of PlGF values confirmed the presence of a subgroup with values <50 pg/mL, suggestive of angiogenic imbalance and increased risk of pre-eclampsia, as well as a majority group with values ≥150 pg/mL, associated with a reduced probability of severe short-term placental impairment.

The results obtained confirm the value of PlGF as a useful biomarker in obstetric practice, particularly for the triage and risk stratification of pre-eclampsia. However, its interpretation should not be performed in isolation, but integrated into a broader clinical context that includes gestational age, the maternal clinical picture, paraclinical investigations and, ideally, other angiogenic biomarkers. The study therefore supports the clinical relevance of PlGF, while also emphasising the need for a multidimensional approach to optimise medical decision-making.

7. Study 2. Retrospective analysis of cases of pre-eclampsia and eclampsia during the period 2020–2025

Study 2 aimed to perform a retrospective analysis of cases of pre-eclampsia and eclampsia admitted to the Obstetrics and Gynaecology I Department of Dr I. Cantacuzino Clinical Hospital in Bucharest, during the period 1 January 2020 – 30 June 2025. The research had an observational, descriptive and retrospective design, based on clinical and administrative data extracted from the hospital's electronic archive. Patients diagnosed according to ICD-10 codes O14.0, O14.1, O14.9, O15.0, O15.1, O15.2 and O15.9 were included, with both the unique patient and the clinical record being analysed in order to avoid overlap between the individual and the diagnostic episode.

The cohort comprised 95 patients and 107 clinical records. The annual distribution showed the highest number of cases in 2021, with 27 patients and 28 records, followed by a progressive decrease in subsequent years, with the note that 2025 included data only up to 30 June. The age of the patients was heterogeneous, including both adolescents and pregnant women of advanced maternal age; however, the statistical analysis did not reveal significant age differences between years or between diagnostic classes.

Over the entire study period, the dominant category was unspecified pre-eclampsia, O14.9, representing 41.1% of all records, followed by moderate pre-eclampsia, O14.0, with 36.4%, and severe pre-eclampsia, O14.1, with 20.6%. Eclampsia was rare, being identified in only two cases. The chi-square test demonstrated a significant association between year of discharge and the distribution of ICD-10 codes, indicating real variations in the clinical profile over time.

The results underline the heterogeneous nature of pre-eclampsia, the need to standardise diagnostic coding, the consolidation of prenatal monitoring and the importance of postpartum surveillance, especially in the context of severe forms and eclampsia during the puerperium [175–183].

8. Studiy 3. Retrospective analysis of cases of pregnancy-associated hypertension using the Heart4Mom application

Study 3 aimed to perform a retrospective analysis of cases of pregnancy-associated hypertension within the Obstetrics and Gynaecology I Department of Dr I. Cantacuzino Clinical Hospital in Bucharest, during the period 1 January 2020 – 16 July 2025, as well as to explore the usefulness of the Heart4Mom application as a complementary digital tool for blood pressure monitoring in pregnant women. Pregnancy-associated hypertension is approached as a complex clinical spectrum that includes gestational hypertension, pre-existing chronic hypertension, pre-eclampsia and superimposed forms, with major relevance for maternal and perinatal morbidity [184–191].

The research had an observational, retrospective, descriptive and analytical design, being based on hospital clinical and administrative reports. The retrospective cohort included 475 patients and 777 diagnostic records, the difference between these two values reflecting the existence of repeated admissions, multiple diagnoses or clinical reclassifications. The dominant diagnosis was O13 — gestational hypertension without significant proteinuria — representing 69.6% of all records and being recorded in 76.4% of patients. Pre-existing hypertension, represented by O10 codes, accounted for approximately one third of cases, with an important contribution from code O10.0 [203–209].

The temporal analysis revealed a clear predominance of gestational hypertension during the period 2020–2023, followed by an increase in the proportion of pre-existing hypertension in recent years, particularly in 2024 and 2025. Annual differences were statistically significant for O13 and O10.0, suggesting a real or administrative change in the structure of the case series. Maternal age was associated with the probability of a diagnosis of pre-existing hypertension, with patients in this category being older than those with gestational hypertension.

The Heart4Mom pilot component demonstrated the feasibility of home blood pressure telemonitoring through the recording of values, longitudinal follow-up and signalling of values exceeding pathological thresholds. Thus, the study supports the integration of digital tools into the surveillance of pregnant women at hypertensive risk, not as a replacement for clinical assessment, but as a complement to modern obstetric management [220–223].

9. Conclusions and personal contributions

This doctoral thesis aimed to provide an integrated assessment of hypertensive pathology associated with pregnancy, with an emphasis on pre-

eclampsia, eclampsia, angiogenic biomarkers and digital monitoring of pregnant women at risk. The research was structured into three complementary studies, conducted using data derived from real-world practice at Dr I. Cantacuzino Clinical Hospital in Bucharest, focusing on the analysis of PlGF values, the retrospective evaluation of cases of pre-eclampsia and eclampsia during the period 2020–2025, and the characterisation of pregnancy-associated hypertension, including through exploration of the Heart4Mom application.

The results demonstrated the usefulness of PlGF as a risk stratification tool in suspected pre-eclampsia, by delineating clinical categories corresponding to high, intermediate and low risk. Very low PlGF values were associated with possible placental dysfunction and angiogenic imbalance, and the differences between the analysed groups were statistically confirmed using the Kruskal–Wallis test, $H = 32.15$; $p < 0.001$. The retrospective analysis of cases of pre-eclampsia and eclampsia highlighted annual variability in frequency and clinical severity. Unspecified and moderate forms of pre-eclampsia predominated, while eclampsia was rare, but of major clinical relevance. The association between year of discharge and distribution of ICD-10 codes was statistically significant, $\chi^2(20) = 31.64$; $p = 0.046$, indicating variations in the case profile or in coding practices. In the study of pregnancy-associated hypertension, gestational hypertension represented the dominant category; however, a recent increase in pre-existing hypertension was observed, more frequently associated with advanced maternal age.

The Heart4Mom component demonstrated the feasibility of blood pressure telemonitoring as a complementary method to obstetric surveillance. The personal contributions consist of the establishment of original cohorts, the statistical analysis of biomarkers and clinical cases, the methodological distinction between patient and clinical record, the extended retrospective evaluation, and the proposal of an integrated biological, clinical and digital model for the management of hypertensive obstetric pathology.

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