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Assessment of changes in cardiac structure and function in pregnant women with preeclampsia and gestational hypertension

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The materials related to the defense are available at the Department of Science and Research of Medical University – Plovdiv, 15A Vasil Aprilov Blvd.

The Summary of the thesis is published online at the website of the university - https://mu-plovdiv.bg/

*The numbering of the tables and figures in the summary does not correspond to that in the thesis.

I would like to express my gratitude:

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To my family

And to all who helped and supported the realization of this work in personal and professional terms

CONTENTS

List of abbreviations

2D	2-dimensional
A	Peak velocity of the A wave of the late diastolic filling
a‟	Peak velocity of the a‟ wave of the late diastolic filling
AH	Arterial hypertension
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AT	Acceleration time
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM	Diabetes mellitus
E	Peak velocity of the E-wave of the early diastolic filling
E/A	The ratio of the waves E and A
e‟	Peak velocity of the e‟ wave of the early diastolic filling
e‟/a‟	The ratio of the waves e‟ and a‟
EDD	End-diastolic dimension of the left ventricle
E-DT	Deceleration time of E-wave
EDV	End-diastolic volume of the left ventricle
E/e‟	The ratio of the waves E and e‟
EF	Ejection fraction
ESD	End-systolic dimension of the left ventricle
ESV	End-systolic volume of the left ventricle
FAC	Fractional area change
g.w.	Gestational week
Gal-3	Galectin-3
GH	Gestational hypertension
GLS	Global longitudinal strain
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
IHD	Ischemic heart disease
IL-6	Interleukin-6
Ind.	Indexing to BSA
LA	Left atrium
LA1	The anterior-posterior diameter of LA
LPM	Last menstrual period
LV	Left ventricle

LVMI	Left ventricular mass index;
MAPSE	Mitral annular plane systolic excursion
MV	Mitral valve
NT-proBNP	N-terminal B-type natriuretic peptide
OB/GYN	Obstetrics and gynecology
OR	Odds ratio
PE	Preeclampsia
PlGF	Placental growth factor
PV	Pulmonary valve
PW	Pulsed-wave Doppler
PWLV	Posterior wall of the left ventricle
RA	Right atrium
RIMP	Right ventricular index of myocardial performance
RR	Risk ratio
RV	Right ventricle
RV EDA	Right ventricular end-diastolic area
RV ESA	Right ventricular end-systolic area
RVOT	Right ventricle outflow track
RWT	Relative wall thickness
S,,	Peak velocity of the S-wave
SBP	Systolic blood pressure
SF	Shortening fraction
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler Imaging
TV	Tricuspid valve
VTI	Velocity time integral
WHO	World Health organization

1. INTRODUCTION

According to the World Health Organization, hypertension is the most common complication of pregnancy in the modern world. About 4-10% of all pregnant women are affected. Two of the main forms of hypertension during pregnancy are gestational hypertension and preeclampsia – systemic diseases, which in their more severe forms can lead to serious complications, both on the part of the mother and on the part of the fetus.

In a significant number of studies of women with preeclampsia and gestational hypertension, abnormalities in the cardiac structure and function have been identified, which are expressed to varying degrees - as assessed echocardiographically and with the use of various biomarkers. The registered changes are usually asymptomatic, but they, similarly to the high blood pressure, can persist for some time after the end of pregnancy and recur in subsequent pregnancies.

There is evidence of a higher risk for the development of arterial hypertension, ischemic heart disease, cerebrovascular disease, diabetes, venous thromboembolism, and even a higher cardiovascular mortality in women with hypertensive disorders of pregnancy. This risk is increased in the first few years after the pregnancy and also at a later age. However, the role of hypertensive disorders of pregnancy as a long-term risk factor is often overlooked and post-pregnancy follow-up is not usually performed.

The current study aims to answer the question of whether and to what extent the presence of preeclampsia and gestational hypertension has affected certain echocardiographic parameters and biomarkers related to cardiac structure and function. This would improve the management of women, both during and after pregnancy, and predict an increased risk of cardiovascular diseases in some of them in the longer term. Based on the results, recommendations can be made for stricter control of individual risk factors for cardiovascular disease, as well as to draw conclusions about the population of women at risk. When analyzing the studied parameters, those of them with the largest deviations in the respective pathologies could be identified and subsequently introduced into the clinical obstetrics and gynecology and cardiology practices to optimize the care provided to pregnant women.

The choice of the study population is favorable for several reasons:

1. A real opportunity for prevention: Consists of a young population with the opportunity to change their lifestyle with an appropriate and scientifically supported motivation;

2. A specific, early risk factor: From the point of view of women's health, the occurrence of preeclampsia/gestational hypertension is an interesting phenomenon, as it is a specific, sex-related cardiovascular risk factor that manifests years before the classic risk factors could lead to detectable changes. The presence of preeclampsia/gestational hypertension could be considered a very early prodrome for the development of cardiovascular pathologies, and efforts in the study of those disorders would improve cardiac care for women.

3. Minimization of the influence of other risk factors: The population consists of premenopausal women who are known to have a lower cardiovascular risk compared to men of similar age. After the menopause, however, the protective effect of estrogen is depleted and the CVD morbidity and mortality are similar between men and women. In older populations, it is more difficult to rule out the presence of asymptomatic atherosclerotic disease that is confounding of the reported changes. With age, the exposure to other significant risk factors becomes longer - LDL-cholesterol levels, smoking, sedentary lifestyle, and obesity. In the selected population, it is feasible to register changes, determined mainly by the presence of preeclampsia/gestational hypertension, as those women are outside of the scope of the two main risk factors for CVD, namely male gender and age. In order to further ensure that the results are predominantly influenced by the designated hypertensive disorders, we have excluded from the study women with the pre-pregnancy established diagnoses of arterial hypertension or diabetes mellitus – also classic risk factors for cardiovascular diseases.

2. AIM AND OBJECTIVES OF THE STUDY

2.1 Aim

To determine the changes in the cardiac structure and function of women with preeclampsia and with gestational hypertension in order to improve the evaluation and the prognosis of the conditions.

2.2 Objectives

1. To analyze data on patients with preeclampsia, gestational hypertension and healthy pregnant women concerning anamnesis, including obstetric anamnesis, risk factors and family history for cardiovascular disease, physical status, laboratory tests.

2. To analyze echocardiographic parameters giving information on cardiac morphology, systolic and diastolic function in women with preeclampsia and gestational hypertension and to compare them with those in healthy pregnancies.

3. To determine the serum concentration of certain biomarkers (NT-proBNP, Galectin-3, hs-CRP, IL-6 and PlGF) for cardiac, endothelial and placental dysfunction in women with the studied pathologies and compare to those in healthy pregnant controls.

4. To determine whether there are associations between:

4.1 Some echocardiographic parameters (global longitudinal strain of the left ventricle) and certain characteristics of the women (as set out in point 1);

4.2. The biomarkers and certain characteristics of the women;

4.3. The echocardiographic parameters and the biomarkers.

3. MATERIALS AND METHODS

3.1. Study population and design of the study

We carried out a prospective, monocentric, clinico-epidemiological study in the period from 15.08.2018 until 15.01.2020. The study was conducted on the territory of two of the departments of Medical University Plovdiv – the First Department of Internal diseases, section of Cardiology and the Department of Obstetrics and Gynecology. The study was approved by the Committee on Scientific Ethics at the Medical University - Plovdiv with protocol N 3/26.09.2019. All women signed informed consent prior to inclusion in the study after being provided with an information bulletin and after a conversation with the chief investigator. The study involved 123 pregnant women recruited on the basis of hospitalizations at the Clinic of Obstetrics and Gynecology of the Multiprofile University Hospital "Sveti Georgi", Plovdiv, Bulgaria as well as OB/GYN outpatients. For the purposes of the study, the following three groups were formed (Fig. 1):

- Group 1 (women with gestational hypertension) – 36 (29,3%);
- Group 2 (women with preeclampsia) – 37 (30,1%);
- Control group – 50 (40,7%).

In order to be included in groups 1 or 2 the respective diagnostic criteria had to be met:

1. **For gestational hypertension** – newly-appeared arterial hypertension after the 20th gestational week with lack of significant proteinuria (<300mg for 24 hours).

2. **For preeclampsia -** newly-appeared arterial hypertension after the 20th gestational week with the presence of significant proteinuria (>300mg for 24 hours).

3. **Control group –** women without the presence of preeclampsia or gestational hypertension as well as fetal growth restriction due to other reasons as assessed by an OB/GYN specialist.

The mean age of the participants in the study was 29.93±5.71 years in the range of 18-43 years. With the highest percentage (34.1%) were the women in the 25-29 age group, followed by those aged 30-34 years with 26.8%, and with the lowest relative share were the women in the ranges 18-19 and 40-43 years both with 5.7%. 114 of the participants had a singleton pregnancy and 9 of them had a bigeminal pregnancy. Three of the women with a bigeminal pregnancy were in the control group, two in the gestational hypertension group and four in the preeclampsia group. According to the gestational week, the interval was 22.00 – 39.29 weeks with an average of 33.72±4.47 weeks.

Criteria for the selection of patients

Inclusion criteria:

- Pregnant women over the age of 18 years;
- Diagnostic criteria for the presence of either preeclampsia or gestational hypertension according to the established definitions;
- Signed informed consent.

Exclusion criteria – apply for all three groups:

- Pregnant under 18 years of age;
- Pre-pregnancy hypertension (persistently elevated blood pressure at rest above 140/90 mmHg) that required long-term treatment with antihypertensive agents;
- Arterial hypertension due to another systemic disease (secondary arterial hypertension)
- Other pre-existing cardiovascular diseases, including congenital heart malformations (regardless of performed corrections); echocardiographic or other data for the presence of significant valvular lesions – valvular regurgitations above mild/functional; ischemic heart disease; primary or secondary cardiomyopathies; myocarditis, pericarditis, endocarditis, significant rhythm and conduction disorders (ventricular and supraventricular tachycardias, sinoatrial blocks, atrioventricular blocks, bundle branch blocks);
- Presence of pre-pregnancy diabetes mellitus;
- Women diagnosed with other significant systemic diseases that could potentially influence the studied parameters, such as: uncontrolled hypothyroidism, hyperthyroidism, cortisol-related diseases, polycystic ovary syndrome, connective tissue diseases, malignancies and malignancy-associated radiation therapy or chemotherapy (exception – some forms of skin cancer), renal, hepatic or respiratory failure, alcoholism, drug addiction, storage diseases, etc.;
- Poor acoustic window for the echocardiographic examination;
- For ethical reasons, the study did not include women, who were in need of urgent OB/GYN or other interventions, as well as women considered unstable or non-transportable to perform echocardiography.

3.2. Materials

1. A questionnaire with anthropometric data, anamnestic data relevant to the pregnancy, the diagnosis of preeclampsia/gestational hypertension, the presence of accompanying diseases, risk factors and family history for CVD.
2. Approximately 8.1 ml of venous blood taken on the day of the echocardiographic examination.
3. Echocardiographic examination following a certain protocol.

3.3. Methods

3.3.1. Anamnesis

3.3.2. Physical examination

High blood pressure was defined as values of SBP≥140 mmHg and/or DBP≥90 mmHg measured at least twice by a healthcare professional with a period between the two measurements of at least 4 hours. The measurement was performed with sphygmomanometry in a sitting position after at least 15 minutes of rest.

The height and weight of the participants were based on current measurements at the Clinic of Obstetrics and Gynecology. The weight before pregnancy was according to anamnesis.

3.3.3. Electrocardiogram

3.3.4. Echocardiographic study

A two-dimensional (2D) echocardiographic study was performed on the territory of the Clinic of Cardiology, the images were recorded following a specific protocol on an Ultrasound System General Electric Vivid 9.5, and were analyzed using EchoPAC Clinical Workstation Software version 201 of General Electric (General Electric Medical System, Milwaukee, WI, USA). The recordings and measurements were done according to the instructions of the current recommendations for echocardiographic studies, endorsed by the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE), namely the following three documents:

1. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am SocEchocardiogr 2015; 28:1-3; European Heart Journal – Cardiovascular Imaging (2015)16, 233–271.

2. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016;29:277-314. Eur Heart J Cardiovasc Imaging. 2016 Dec;17(12):1321-1360. Epub 2016 Jul 15.

3. Echocardiographic reference ranges for normal cardiac Doppler data: results from the NORRE Study. European Heart Journal – Cardiovascular Imaging (2015)16, 1031–1041.

The parameters for which indexing is recommended, were indexed to BSA using DuBois & DuBois formula: $BSA = 0.007184 \times weight^{0.425} \times height^{0.725}$

Left ventricular mass is calculated using Devereux‟s formula:

$LV\ mass = 0{,}8\{1{,}04[([EDD + septum\ in\ diastole + PWLV\ in\ diastole]^3 - EDD^3)]\} + 0{,}6$

Cut-off values for the echocardiographic parameters are presented in the related tables.

3.3.5. Laboratory tests and biomarkers

Routine laboratory tests:

Laboratory tests available from current hospitalizations of the patients in groups 1 and 2 were used, namely: hemoglobin, erythrocytes, total serum protein, serum albumin, uric acid, amount of protein in urine, AST and ALT.

Tests for specific biomarkers:

1. **Galectin-3** – Double Antibody Sandwich ELISA (MyBioSource, Inc. San Diego, California, USA).

2. **Interleukin-6** – Solid Phase Sandwich ELISA (Diaclone, Besançon, France).

3. **High-sensitivity CRP** –Sandwich ELISA (DIAsource ImmunoAssays S.A., Louvain-la-Neuve – Belgium).

4. **PlGF** – sandwich ELISA (BioVendor Research and Diagnostic Products, Brno, Czech Republic).

5. **NT-proBNP -** the tests were carried out on an automated Abbott Alinity system (Abbott Park, Illinois, U.S.A.). The reaction for determination is a two-stage, hemiluminescent microparticle immunoassay (CMIA).

3.3.6. Methods for statistical data analysis

The data was entered into and processed with IBM SPSS Statistics 25.0 (IBM SPSS Statistics for Windows, SPSS Inc., Chicago, IL, USA) and MedCalc Version 14.8.1 (MedCalc Software, Mariakerke, Belgium). $P<0.05$ was accepted as a level of significance for rejecting the null hypothesis.

The following methods were applied:

1. **Descriptive analysis** – the frequency of the analyzed parameters in the study groups is given in tables.

2. ***Variation analysis*** – to assess the characteristics of the central tendency and statistical dispersion.

3. ***Graphic analysis*** – for visualization of the results.

4. ***Chi-squared test and Fisher's exact test -*** *to check dependency between categorical variables.*

5. ***Comparison of proportions.***

6. ***Non-parametric test of Kolmogorov-Smirnov and Shapiro-Wilk –*** *to check the distribution of data for normality.*

7. ***One-way ANOVA –*** to compare the mean values of more than two independent samples.

8. ***Independent samples t-test of Student –*** parametric test for checking hypotheses for difference between two independent samples.

9. ***Non-parametric test of Kruskal-Wallis –*** to compare more than two independent samples.

10. ***Non-parametric test of Mann-Whitney –*** to check hypotheses for difference between two independent samples.

11. ***Correlation analysis*** – to determine if there is a linear relationship between two quantitative variables.

12. ***Binary logistic regression analysis –*** for quantitative assessment of the influence of the studied factors.

13. ***ROC curve analysis*** *– to determine cut-off values for the quantitative variables.*

14. ***Criteria for validation of the screening tests.***

4. RESULTS

4.1. Characteristics of the studied population

Figure 1 represents the distribution of the women according to age group, gestational week interval and study group.

The three groups were maternal and gestational age-matched, ensuring the accuracy of subsequent comparisons (table 1).

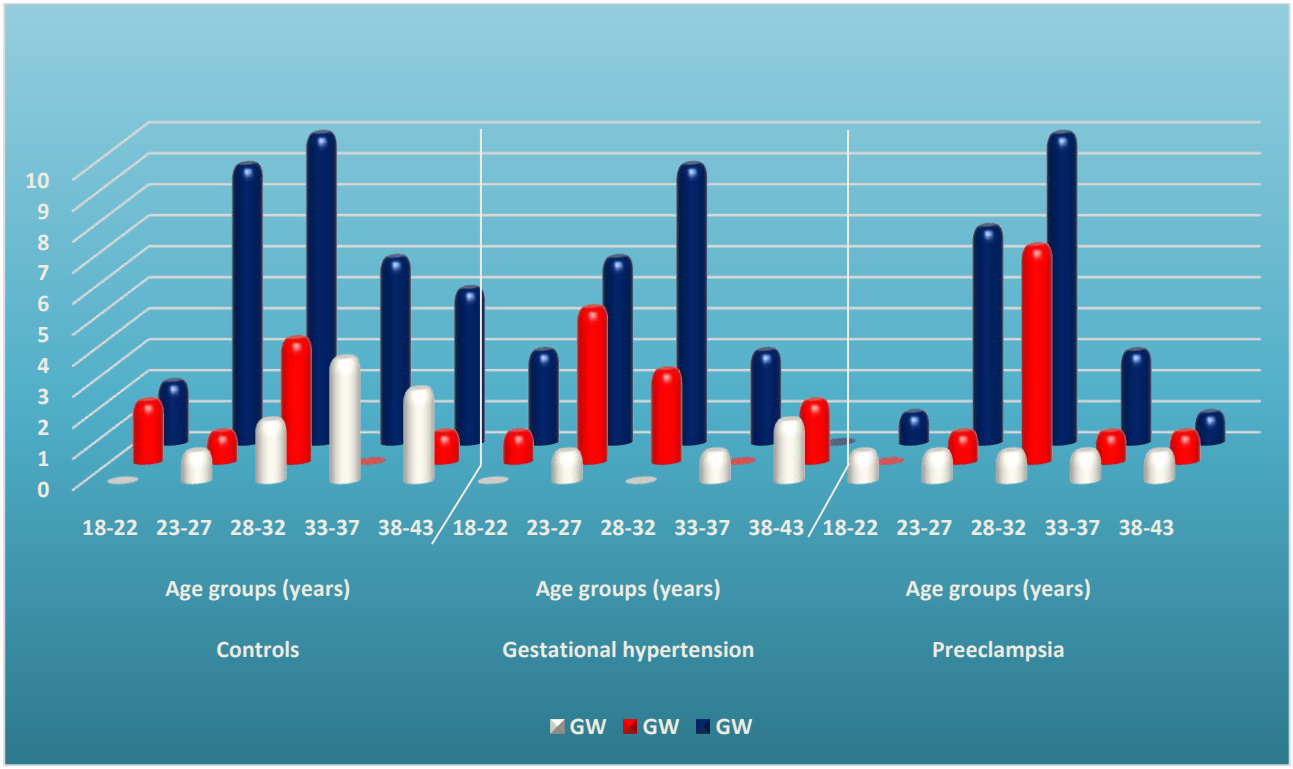

Figure 1: Distribution of the women according to age group, gestational week interval and study group

Table 1: Comparative analysis of the studied groups by maternal age and gestational week

Groups	Parameters					
	Maternal age			Gestational week		
	n	$\overline{X}$	SD	n	$\overline{X}$	SD
Controls	50	30,82[a]	6,02	50	34,08[a]	5,23
Gestational hypertension	36	28,83[a]	5,78	36	33,71[a]	4,08
Preeclampsia	37	29,81[a]	5,14	37	33,24[a]	3,74

* - Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

Table 2: Distribution of the early and severe forms in the pathological groups

Form \ Group	Frequency	GH	PE	P	All
Early form	n	26	31	0,269	57
	%	72,2	83,8		78,1
Severe form	n	13	13	1,000	26
	%	36,1	35,1		35,6

The results in table 2 show that the pathological groups are not statistically different as far as the presence of women with an early onset (defined as such after 20th gestational week, but before the 34th gestational week) and severe forms of the disorders are concerned.

The most common criterion used for defining the severe forms of PE and GH in our study population was the arterial blood pressure values (forms are considered severe if the measured maximum systolic pressure ≥160 mmHg and/or diastolic ≥110 mmHg). One of the women in the GH group, additionally to having SBP ≥160 mmHg, had values of ALT more than two times higher than the laboratory reference limit. One of the women with PE was assigned to the severe PE group due to elevation of both AST and ALT more than two times above the upper reference limit. For ethical reasons, we did not include women who at the time of recruitment had clinical manifestations of severe preeclampsia (defined as presence of any of the following: persistent epigastric pain or right upper quadrant pain, cerebral symptoms, visual disturbances or pulmonary congestion), as well as HELLP syndrome, as these conditions were considered high risk for performing activities other than those related to the treatment process.

Analysis of the number of pregnancies and distribution in the groups

The results showed that the participants with a first pregnancy had the highest relative share (39%), followed by those with a second (33.3%), a third (21.1%) and more than three pregnancies (6.5%). The comparative analysis of the control and pathological groups according to the number of pregnancies showed that there was a significant difference only for first pregnancy, with the relative proportion of patients with a first pregnancy in the mixed pathological group significantly higher than that in the controls (table 3).

History of a previous hypertensive disorder of pregnancy

9.8% of the women in the study reported having a previous hypertensive disorder of pregnancy. In the mixed pathological group those women were significantly more – 15,1% (16.7% (6 women) in the gestational hypertension group and 13.5% (5 women) in the preeclampsia group) compared to only 4% (2 women) with a normotensive pregnancy. This finding confirms the risk of recurrence of hypertensive disorders of pregnancy in a next pregnancy.

Table 3: Distribution of women according to the history of a previous hypertensive disorder of pregnancy in the groups (p=0,027)

History of a HDP	Frequency	Group		All
		Controls	Gestational hypertension + Preeclampsia	
No	n	49	62	111
	%	98,0	84,9	90,2
Yes	n	1	11	12
	%	2,0	15,1	9,8
All	n	50	73	123
	%	100,0	100,0	100,0

Smoking

The majority (50.8%) of the women were smokers, followed by the non-smokers with 36.4% and the ex-smokers with 12.7%. Altogether 63.5% of the women were ever-smokers. There was no statistical difference in the percentage of women who smoked in the three groups (44,4% in the gestational hypertension group; 45,9% in the preeclampsia groups and 54,0% in the controls; p>0,05). Of the current smokers, almost half (46.7%) admitted to also smoked during the target pregnancy. When analyzed separately into groups, the highest percentage (51,9%) of women who smoked during the pregnancy were in the controls, followed by the preeclampsia group - 47.1 % and by those with gestational hypertension with 37.5%, but the difference was not statistically significant (p>0,05).

Family history for arterial hypertension, ischemic heart disease, cerebrovascular disease, diabetes mellitus 2nd type and hypertensive disorders of pregnancy

Family history was taken for the presence of arterial hypertension, ischemic heart disease, cerebrovascular disease, diabetes mellitus 2nd type, as well as hypertensive disorders of pregnancy in female relatives in 1st to 3th degree of kinship (parents, siblings, siblings of parents and grandparents).

Only 16 (13%) of the study participants indicated no family predisposition for any of the listed diseases. With the highest percentage (76.4%) for the whole sample was the family history for arterial hypertension, followed by that for cerebrovascular disease with 38.2%, diabetes mellitus 2nd type with 37.4%, ischemic heart disease with 31.7%, and for hypertensive disorders of pregnancy in closely related women with 13.8%. The comparative analysis of the pathological groups and the controls for family predisposition gave the following results (table 4): A significant difference between the three groups was found only for the family history of arterial hypertension and hypertensive disorders of pregnancy in closely related women. The women from the mixed pathological group were with a significantly higher relative incidence.

Table 4: Comparative analysis of the pathological groups and the controls according to family history

Family history	Controls			Gestational hypertension and preeclampsia			P
	n	%	Sp	n	%	Sp	
None	9	18,0	5,4	7	9,6	3,4	0,277
Arterial hypertension	33	66,0	6,7	61	83,6	4,3	**0,041**
Ischemic heart disease	15	30,0	6,5	24	32,9	5,5	0,887
Cerebrovascular disease	19	38,0	6,9	28	38,4	5,7	0,886
Diabetes mellitus 2nd type	21	42,0	7,0	25	34,2	5,6	0,491
Hypertensive disorder of pregnancy in closely related women	2	4,0	2,8	15	20,5	4,7	**0,019**

* - Response percentages exceed 100%, as many of the patients indicated family history for more than one disease

Discussion: Gestational hypertension and preeclampsia occur statistically more often in the **first pregnancy** than in the subsequent pregnancies. This confirms the first pregnancy as one of the well-known risk factors for preeclampsia and other hypertensive disorders of pregnancy (Kenny et al. 2017).

The prevalence of **smoking** among these young women of childbearing age is alarming. 23.7% of all the women stated that they smoked during the current pregnancy which is undoubtedly a marker for a poor health culture among Bulgarian gravidas. The smoking rate varies from country to country and yet for Bulgaria it is clearly above the average for Europe and the world, something that is also evident from our data. In a literature analysis and meta-analysis performed by Lange et al. (2018), covering data from 295 studies from 43 countries and statistical remodeling for 133 countries from the period between 1st of January 1985 and 1st of February 2016, it became clear that that the top three countries with the higher percentage of active smokers during pregnancy were Ireland (38.4%), Uruguay (29.7%) and Bulgaria (29,4%). For comparison, the incidence of smoking during pregnancy is reported to be 1.7% globally; 8.1%, in the European region, 5.9% in the Americas, 1,2% in Southeast Asia and 0.8% in the African region. Because of those worrisome results, we believe measures should be taken in order to promote health culture among the Bulgarian population in terms of the harmful impact that smoking has on pregnancy.

An interesting result from a cardiological perspective is the influence of **family history of arterial hypertension** on the occurrence of hypertensive disorders of pregnancy. There is not much research in this regard. We found similar observations in a Brazilian

population study of Bezerra et al. (2010) who included 412 pregnant women and analyzed family history for arterial hypertension, preeclampsia and eclampsia only in gravidas‟ mothers and sisters. A higher risk was present for the occurrence of a severe form of preeclampsia during pregnancy for each of the reported disorders in the relatives. Endeshaw et al. (2016) examined the risk factors for the occurrence of preeclampsia in 453 pregnant women in Ethiopia and also found an increased risk in the presence of family predisposition for arterial hypertension, even reporting it as the riskiest compared to the other studied factors such as advanced maternal age, diabetes mellitus, urinary tract infections, multiparity and anemia. It is possible that common genetic mechanisms determine the predisposition for arterial hypertension in women and the occurrence of hypertensive disorders of pregnancy. In this aspect, the data from the current study allows us to support the hypothesis of HDPs as a failed "stress test" of the female organism and to assume that the occurrence of PE or GH is not an isolated event, but is probably part of a broader adverse cardiovascular profile.

In summary, we can assume that there would be some benefits for the obstetrics and gynecological practice when including family history for arterial hypertension as a regular question in the anamnesis of women in early pregnancy.

Analysis of women by groups according to mean values of physical examination parameters at the inclusion in the study.

Results given in table 5 show that the mean values of 5 out of the 9 analyzed parameters (systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI) – before pregnancy and current; and BSA (current)) were significantly lower in the controls compared to the two pathological groups.

Table 5: Comparative analysis of the groups according to SBP, DBP, Heart rate, BMI, weight gain and BSA

Parameter \ Groups	Controls			Gestational hypertension			Preeclampsia		
	n	$\bar{X}$	SD	n	$\bar{X}$	SD	n	$\bar{X}$	SD
SBP [mmHg]	46	107,07[a]	10,36	33	121,91[b]	12,28	34	126,32[b]	12,20
DBP [mmHg]	46	68,70[a]	7,92	33	78,12[b]	10,91	34	83,18[b]	9,80
Heart rate [bpm]	50	86,00[a]	11,06	35	85,66[a]	12,28	35	81,74[a]	14,11
BMI – before pregnancy [kg/m^2]	49	22,58[a]	5,11	35	28,58[b]	6,14	35	27,26[b]	5,68
BMI – current [kg/m^2]	50	27,81[a]	5,49	36	33,66[b]	5,75	36	31,77[b]	5,32
Weight gain [kg]	49	14,05[a]	6,18	35	13,69[a]	6,54	36	12,94[a]	7,51
BSA (current) [m^2]	50	1,83[a]	0,20	36	1,97[b]	0,20	37	1,96[b]	0,18

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

Discussion: The presence of a **significantly higher BMI in women with HDPs** has been proven in numerous other studies. Extensive literature analysis and meta-analysis of Motedayen et al. (2019) encompassing data of 5,946 women who participated in 16 studies between 2000 and 2016 showed significantly lower BMI values in healthy pregnant women than in mild or severe preeclampsia. The interpretation of BMI in preeclampsia and gestational hypertension is complex because on the one hand, this indicator reflects the degrees of obesity, but also the retention of fluids which is more pronounced in preeclampsia than in normotensive pregnancies. Our current study also found a **significantly higher BMI before pregnancy** in women in both of the pathological groups compared to the controls. This, in turn, has also been confirmed by other authors, such as Shao et al. (2017) who analyzed 9,863 pregnant women, 347 of which with preeclampsia.

We did not establish a significant difference in the **weight gain** from the beginning of pregnancy till the inclusion in the study between the groups in the sample. The relationship between weight gain during pregnancy and the development of a hypertensive disorder is ambiguous, as some studies found it to be a risk (Wei et al. (2015), Hillesund et al. (2018), Pare et al. (2014), Magann et al. (2013)), but according to others the relation was weak or completely lacking (O'Dwyer et al. (2013), Gaillard et al. (2013)).

4.2. Echocardiographic parameters

In tables 6-8 the results from the comparative analysis between the groups according to the studied echocardiographic parameters are presented.

In tables 10-12 the results from the comparative analysis between the groups according to the percentage of abnormal values of the studied echocardiographic parameters are presented. The cut-off values for abnormality are given according to the currently endorsed echocardiography guidelines, described in methods.

For a more convenient reading, the significant differences between the groups are marked with a different color.

Table 6: Comparative analysis of left-chamber structural parameters and their derivatives in the groups

Groups / Parameters	Controls			Gestational hypertension			Preeclampsia		
	n	$\bar{X}$	SD	n	$\bar{X}$	SD	n	$\bar{X}$	SD
LA1 [mm]	50	34,84[a]	3,90	36	36,75[ac]	3,06	37	37,95[bc]	3,80
Ind. LA1 [mm]	50	19,18[a]	2,23	36	18,81[a]	1,81	37	19,49[a]	2,46
Ind. LA volume [ml/m^2]	50	21,89[a]	4,91	36	24,28[b]	5,00	37	25,76[b]	7,61
Septum in diastole [mm]	50	8,88[a]	1,57	36	9,97[b]	1,28	37	10,41[b]	1,21
PWLV in diastole [mm]	50	8,90[a]	1,40	36	9,36[a]	1,25	37	10,08[b]	1,28
EDD of LV [mm]	50	43,02[a]	4,30	36	43,89[a]	4,21	37	44,76[a]	5,85
ESD of LV [mm]	50	28,36[a]	3,33	36	29,42[a]	3,32	35	29,23[a]	4,45
Ind. EDD [mm]	50	23,72[a]	2,72	36	22,49[a]	2,66	37	23,00[a]	3,53
Ind. ESD [mm]	50	15,61[a]	1,86	36	15,07[a]	1,97	35	14,94[a]	2,56
EDV biplane [ml]	50	83,48[a]	16,04	36	92,38[ac]	16,79	37	95,70[bc]	20,18
ESV biplane [ml]	50	29,62[a]	7,42	36	34,04[ac]	8,51	37	35,03[bc]	9,30
Ind. EDV bi [ml/m^2]	50	45,72[a]	7,28	36	47,04[a]	7,57	37	48,75[a]	8,65
Ind. ESV bi [ml/m^2]	50	16,23[a]	3,71	36	17,31[a]	3,95	37	17,91[a]	4,54
LVMI [g/m^2]	50	66,20[a]	12,69	36	70,09[ac]	11,48	37	73,31[bc]	15,74
RWT	50	0,42[a]	0,07	36	0,45[ac]	0,07	37	0,47[bc]	0,14

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

Table 7: Comparative analysis of parameters of left ventricular systolic and diastolic function in the groups

Parameters \ Groups	Controls			Gestational hypertension			Preeclampsia		
	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD
SF [%]	50	33,96[a]	5,11	36	33,00[a]	5,07	37	34,65[a]	6,30
EF [%]	50	64,64[a]	5,20	36	63,32[a]	5,21	37	63,40[a]	5,71
Stroke volume [ml]	50	70,64[a]	15,84	36	71,96[a]	15,61	34	74,65[a]	18,45
Ind. stroke volume [ml/m^2]	50	37,54[a]	7,32	36	36,65[a]	6,99	36	37,82[a]	8,17
Stroke volume VTI [ml]	50	68,55[a]	15,42	36	71,67[a]	13,24	36	74,24[a]	18,42
Cardiac output [L]	50	5,85[a]	1,31	35	6,12[a]	1,46	35	6,17[a]	1,88
Cardiac index [L]	50	3,20[a]	0,63	35	3,14[a]	0,77	35	3,11[a]	7,76
MAPSE med. [mm]	50	12,92[a]	1,01	35	12,74[a]	1,07	36	12,56[a]	0,97
MAPSE lat. [mm]	50	14,16[a]	0,96	35	13,86[a]	1,29	36	13,86[a]	0,87
S med. [cm/sec]	50	9,74[a]	1,55	36	9,03[b]	1,42	36	8,56[b]	1,56
S lat. [cm/sec]	50	11,08[a]	1,76	36	10,00[b]	1,47	36	9,92[b]	1,70
LV GLS [%]	50	-22,37[a]	2,15	36	-19,75[b]	2,20	37	-19,32[b]	2,40
E (MV) [cm/sec]	50	78,10[a]	15,62	36	77,94[a]	15,10	36	82,72[a]	13,51
E/A (MV)	50	1,26[a]	0,32	36	1,24[a]	0,26	36	1,32[a]	0,33
E-DT [msec]	50	184,16[a]	24,08	36	172,97[b]	21,38	36	172,83[b]	28,53
e‟med. [cm/sec]	50	12,30[a]	2,54	36	10,39[b]	2,54	36	9,97[b]	2,20
e‟lat. [cm/sec]	50	15,62[a]	3,58	36	13,72[b]	2,55	36	13,61[b]	2,70
E/e‟ med.	50	6,50[a]	1,35	36	7,75[b]	1,73	36	8,58[c]	1,91
E/e‟ lat.	50	5,21[a]	1,47	35	5,81[b]	1,23	36	6,24[b]	1,32
E/e‟ mean	50	5,73[a]	1,24	36	6,62[b]	1,25	36	7,19[b]	1,44

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

Table 8: Comparative analysis of right-chamber structural parameters, right ventricular systolic and diastolic disfunction in the groups

Groups	Controls			Gestational hypertension			Preeclampsia		
Parameters	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD
Ind. Volume of RA [ml/m^2]	50	15,35^{ac}	4,01	35	13,88^{a}	3,61	37	17,79^{bc}	6,60
RV basal diameter [mm]	50	33,34^{a}	3,74	36	33,81^{a}	2,79	37	34,11^{a}	4,30
RVOT proximal [mm]	50	28,94^{a}	3,66	36	28,78^{a}	3,09	37	30,54^{b}	3,74
RVOT distal [mm]	50	24,40^{a}	3,11	36	24,89^{a}	2,82	37	26,92^{b}	3,22
Ind. RV EDA [cm^2/m^2]	50	84,3^{a}	1,25	36	80,2^{a}	1,19	37	83,8^{a}	1,42
Ind. RV ESA [cm^2/m^2]	50	41,2^{a}	0,64	36	40,7^{a}	0,73	37	41,1^{a}	0,69
RV free wall [mm]	50	3,74^{a}	0,69	36	3,44^{a}	0,73	37	3,65^{a}	0,75
RV longitudinal diameter [mm]	50	66,40^{a}	6,88	36	67,92^{a}	6,64	37	67,11^{a}	7,22
RV mid diameter [mm]	50	29,96^{a}	3,50	36	31,33^{a}	4,30	37	30,78^{a}	4,81
FAC [%]	50	50,91^{a}	5,13	36	49,20^{a}	5,35	37	50,74^{a}	4,29
TV S wave [cm/sec]	50	14,26^{a}	1,85	35	13,54^{a}	2,09	36	13,75^{a}	2,63
TAPSE [mm]	50	24,54^{a}	3,16	36	24,67^{a}	3,56	37	24,51^{a}	4,00
RIMP	47	0,53^{a}	0,20	32	0,48^{a}	0,09	34	0,49^{a}	0,12
GLS of RV [%]	50	-30,19^{a}	4,81	36	-25,79^{b}	5,14	37	-26,09^{b}	3,52
TV E/A	50	1,16^{a}	0,27	33	1,13^{a}	0,27	33	1,10^{a}	0,19
TV E-DT [msec]	50	176,92^{a}	26,64	33	184,85^{a}	28,53	33	184,73^{a}	33,37
TV e‘ [cm/sec]	50	16,00^{a}	2,89	35	14,89^{ac}	2,75	36	14,39^{bc}	2,45
TV E/e‘	50	3,63^{a}	1,05	33	3,98^{ac}	1,16	33	4,07^{bc}	1,05
TV e'/a'	48	1,13^{a}	0,44	32	1,14^{a}	0,43	34	1,06^{a}	0,33
Pulmonary valve acceleration time [msec]	50	121,32^{a}	13,43	36	117,33^{a}	16,78	37	124,43^{a}	20,46

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

Left ventricular geometry patterns according to left ventricular mass index (LVMI) and relative wall thickness (RWT)

The pregnant women fell into four categories of left ventricular geometry pattern according to the calculated left ventricular mass index and the relative wall thickness.

Table 9 illustrates the following:

- In the controls, women with normal LV geometry were with the highest relative share (58%), followed by those with concentric remodeling (40%), and concentric hypertrophy (2%). No women had eccentric hypertrophy;
- In the pathologic groups, women with concentric remodeling had the highest relative share (54,8), followed by those with normal LV geometry (37%), and the fewest were the women with concentric (5,5%) and eccentric hypertrophy (2,7%).

Table 9: Left ventricular geometry patterns according to RWT and LVMI values in the controls and the mixed pathological group

Relative wall thickness		Controls		Gestational hypertension + preeclampsia	
	> 0,42	Concentric remodeling N=20 (40%)	Concentric hypertrophy N=1 (2%)	Concentric remodeling N=40 (54,8%)	Concentric hypertrophy N=4 (5,5%)
	≤ 0,42	Normal LV geometry N=29 (58%)	Eccentric hypertrophy N=0 (0%)	Normal LV geometry N=27 (37%)	Eccentric hypertrophy N=2 (2,7%)
		≤ 95 g/m^2	> 95 g/m^2	≤ 95 g/m^2	> 95 g/m^2
		Left ventricular mass index			

Table 10: Comparative analysis of the incidence of abnormal values of left-chamber structural parameters and their derivatives in the groups

Parameters	Normal	Controls		Gestational hypertension		Preeclampsia		All	
		n	%	n	%	n	%	n	%
LA1 [mm]	≤ 38 mm	8	16,0[a]	12	33,3[ac]	15	40,5[bc]	35	28,5
Ind. LA1 [mm]	≤ 23 mm	2	4,0[a]	1	2,8[a]	6	16,2[a]	9	7,3
Ind. LA volume [ml/m^2]	16–34 ml/m^2	4	8,0[ac]	1	2,8[a]	8	21,6[bc]	13	10,6
Septum in diastole [mm]	≤ 9 mm	17	34,0[a]	24	66,7[b]	28	75,7[b]	69	56,1
PWLV in diastole [mm]	≤ 9 mm	19	38,0[a]	16	44,4[ac]	25	67,6[bc]	60	48,8
EDD of LV [mm]	≤ 52 mm	1	2,0[a]	1	2,8[a]	1	2,7[a]	3	2,4
ESD of LV [mm]	≤ 35 mm	0	0,0[a]	2	5,6[a]	4	11,1[a]	6	4,9
Ind. EDD [mm]	≤ 31 mm	1	2,0[a]	0	0,0[a]	0	0,0[a]	1	0,8
Ind. ESD [mm]	≤ 35 mm	1	2,0[a]	0	0,0[a]	0	0,0[a]	1	0,8
EDV biplane [ml]	≤ 106 ml	4	8,0[a]	9	25,0[ac]	10	27,0[bc]	23	18,7
ESV biplane [ml]	≤ 42 ml	1	2,0[a]	5	13,9[ac]	8	21,6[bc]	14	11,4
Ind. EDV bi [ml/m^2]	≤ 61 ml/m^2	1	2,0[a]	1	2,8[a]	3	8,1[a]	5	4,1
Ind. ESV bi [ml/m^2]	≤ 24 ml/m^2	0	0,0[a]	2	5,6[a]	3	8,1[a]	5	4,1
LVMI [g/m^2]	≤ 95 g/m^2	1	2,0[a]	1	2,8[a]	5	13,5[a]	7	5,7
RWT	≤ 0,42	21	42,0[a]	23	63,9[a]	21	56,8[a]	65	52,8

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

Table 11: Comparative analysis of the incidence of abnormal values of left ventricular systolic and diastolic function in the groups

Parameters	Normal	Controls		Gestational hypertension		Preeclampsia		All	
		n	%	n	%	n	%	n	%
SF	> 28%	3	6,0[a]	3	8,3[a]	3	8,1[a]	9	7,3
EF	≤ 54%	1	2,0[a]	0	0,0[a]	1	2,7[a]	2	1,6
MAPSE med.	≥ 11 mm	1	2,0[a]	1	2,9[a]	2	5,6[a]	4	3,3
MAPSE lat.	≥ 13 mm	1	2,0[a]	6	17,1[bc]	1	2,8[ac]	8	6,6
LV GLS	≤ 21%	11	22,0[a]	25	69,4[b]	29	78,4[b]	65	52,8
E/A (MV)	< 0,8	1	2,0[a]	1	2,8[a]	0	0[a]	2	1,6
E-DT	≤ 217 msec (age 18-40) ≤ 227 msec (age over 40)	3	6,0[a]	0	0,0[a]	3	8,3[a]	6	4,9
e‟med.	≥ 8 [cm/m^2]	0	0,0[a]	5	13,9[bc]	3	8,3[ac]	8	6,6
e‟lat.	≥ 10 [cm/m^2]	2	4,0[a]	2	5,7[a]	2	5,6[a]	6	5,0
E/e‟ med.	≤ 15	0	0,0[a]	0	0,0[a]	1	2,8[a]	1	0,8
E/e‟ lat.	≤ 13	0	0,0	0	0,0	0	0,0	0	0,0
E/e‟ mean	≤ 14	0	0,0	0	0,0	0	0,0	0	0,0

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

The currently endorsed European echocardiography guidelines do not state cut-off values for the following parameters: stroke volume (calculated using VTI), indexed stroke volume, cardiac output, cardiac index, mitral annulus lateral and medial S-wave velocity and therefore we did not analyze the incidence of abnormal values.

Table 12: Comparative analysis of the incidence of abnormal values of right-chamber parameters and right ventricular systolic and diastolic function between the groups

Parameters	Normal	Controls		Gestational hypertension		Preeclampsia		All	
		n	%	n	%	n	%	n	%
Ind. Volume of RA	21+/-6 ml/m^2	25	50,0^a	23	65,7^a	17	45,9^a	65	53,3
RV basal diameter	≤ 41 mm	0	0,0^a	0	0,0^a	1	2,7^a	1	0,8
RV mid diameter	≤ 35 mm	1	2,0^a	7	19,4^b	8	21,6^b	16	13,0
RV longitudinal diameter	≤ 83 mm	0	0	0	0	0	0	0	0
RVOT proximal	≤ 35 mm	3	6,0^a	0	0,0^a	3	8,1^a	6	4,9
RVOT distal	≤ 27 mm	25	50,0^a	19	52,8^a	27	73,0^a	71	57,7
Ind. RV EDA	≤ 11,5 cm^2	1	2,0^a	0	0,0^a	1	2,7^a	2	1,6
Ind. RV ESA	≤ 6,4 cm^2	0	0,0	0	0,0	0	0,0	0	0,0
RV free wall	≤ 5 mm	0	0,0	0	0,0	0	0,0	0	0,0
FAC	≥ 35%	0	0,0	0	0,0	0	0,0	0	0,0
TAPSE	≥ 17 mm	0	0,0	0	0,0	0	0,0	0	0,0
TV S wave	≥ 9,5 [cm/m^2]	0	0,0^a	2	5,7^a	1	2,8^a	3	2,5
RIMP	≤ 0,54	19	40,4^a	7	21,9^a	8	23,5^a	34	30,1
RV GLS**	> -23,3%	3	6,0^a	12	33,3^b	9	24,3^b	24	19,5
TV E/A	≥ 0,8	2	4,0^a	3	9,1^a	0	0,0^a	5	4,3
TV e‟	≥ 7,8 [cm/m^2]	0	0,0	0	0,0	0	0,0	0	0,0
TV E/e‟	≤ 6	1	2,0^a	2	6,1^a	2	6,1^a	5	4,3
TV e'/a'	≥ 0,52	3	6,3^a	1	3,1^a	1	2,9^a	5	4,4

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

** - Sex-specific cut-off value according to Muraru et al. (2016)

Discussion: When analyzing the data, significant differences were found for a great number of the echocardiographically measured left-chamber and right-chamber structural and functional parameters between the groups.

Comparison of the mean values of the left-chamber structural parameters

As far as the mean of the absolute values of the left-chamber structural parameters were concerned, statistically significant differences were observed for the following: the anterior-posterior diameter of the left atrium (LA1), the indexed volume of the left atrium, septum thickness in diastole (septum in diastole), thickness of the posterior wall of the left ventricle in diastole (PWLV in diastole), mean end diastolic volume of the left ventricle from two- and four-chamber planes (EDV biplane), mean end systolic volume of the left ventricle from two- and four- chamber planes (ESV biplane), left ventricular mass index (LVMI, using Devereux formula) and relative wall thickness (RWT).

We found that when indexing to BSA is applied to the parameters LA1, EDV biplane and ESV biplane, the differences between the mean values for the groups become non-significant. The difference is significant, however, for the indexed LA volume - it is significantly higher for both preeclampsia and gestational hypertension when compared to controls, but despite a trend for higher values in preeclampsia, there is no significant difference between the two pathological groups.

Based on those results, we can assume that indexing plays an important role in assessing the cardiac structural parameters in the studied pathologies. This lack of significant difference for some of the measurements after indexing has to be taken into account when attempting to determine structural differences in preeclampsia and gestational hypertension. This phenomenon may be due to the already significantly higher current BMI of the hypertensive participants in the sample. We performed correlation analysis between the current BMI and BSA and it showed a strong positive correlation, both in the whole sample and in the separate groups (table 13).

Table 13: Correlation coefficients between the current BMI and BSA (in the whole sample and in the groups)

Groups	Parameters	BSA
Whole sample		0,830***
Controls		0,833***
GH	Current BMI	0,786***
PE		0,840***
GH+PE		0,785***

* - p<0,05, ** - p<0,01, *** - p<0,001

This gives us reason to believe that the higher absolute values for the left-chamber size measurements are likely to be influenced by the higher BMI, which in turn is a characteristic feature of hypertensive disorders of pregnancy; and they are not necessarily a result of other pathophysiologic mechanisms associated with the hypertensive conditions at issue. Therefore, we recommend the use of indexed parameters when examining women with PE and GH. Comparing only the absolute values for the listed measurements may lead to overlooking the influence of BMI in samples with a strong correlation between BMI and BSA.

In an extensive meta-analysis of De Haas et al. (2017), analyzing echocardiographic changes in normotensive and hypertensive pregnancies, no significant differences were found in regard to the increase in EDD in norm and pathology, and in regard to ESD, all, but two studies included in the analysis, found no significantly different increase either. The same meta-analysis also found a significantly greater increase in the absolute value for the anterior-posterior size of LA in hypertensive versus normotensive pregnant women.

In our study, only the increased septal thickness differentiated both of the pathologies from the controls, while the increase in the posterior wall thickness was apparently not as pronounced in gestational hypertension compared to preeclampsia and could not distinguish women with gestational hypertension from the controls. A parallel can be drawn with ventricular remodeling in arterial hypertension outside of pregnancy, where in the majority of cases the septum is more markedly hypertrophied than the posterior wall, occasionally even to the point of forming a localized sigmoid thickening. The observed more pronounced hypertrophy in preeclampsia is supportive of the hypothesis of compensatory reaction to the increased afterload, which is characteristic of this pathology.

Gaudron et al. (2015) established the role of local hypertrophy of the basal septum as an early manifestation of hypertension, with its presence being a predictor of arterial hypertension in the sample with a sensitivity of 93% and specificity of 86%. The mechanisms leading to a more pronounced basal septum hypertrophy are not fully understood, but a possible explanation is the larger radius of longitudinal fibers localized in this ventricular segment, and according to Laplace's law, the larger radius also determines a higher ventricular wall tension. Mechanisms, related to additive strain to the septum from the tension in the right ventricle (Kelshiker et al., 2013), are also discussed.

Similar to our study are the results of Melchiorre et al. (2010), who found a significant difference between the control group and the preeclampsia group in terms of left ventricular mass index and relative wall thickness. Such were also the results of Pan et al. from a 2019 study that included 33 women with severe preeclampsia and 20 healthy pregnant women – both the septum and the PWLV were significantly more hypertrophied in the pathological group, and there was also a higher left ventricular mass index in those women. In a 2017 meta-analysis De Haas et al. reported a trend towards hypertrophic remodeling in hypertensive pregnancies, which is present in our study.

Comparison of mean values of left ventricular functional parameters

Out of the parameters indicating systolic function, differences were observed when comparing the TDI-derived medial and lateral mitral annulus S-waves - these were significantly lower in both of the pathological groups compared to the controls, with no significant difference when comparing preeclampsia to the gestational hypertension group. Another parameter with a significant difference in the mean values was the left ventricular global longitudinal strain (LV GLS) with better (lower) values in the controls compared to the two pathologies, which again, did not differ from each other.

Regarding diastolic function, women with preeclampsia and gestational hypertension had lower values of the medial and lateral e' waves of mitral annulus; higher values of the ratios E/e' lateral and E/e‟ mean, with these differences being significant when comparing both PE and GH to the controls, but the differences between the two pathological groups were only algebraic despite a tendency for worsened parameters in PE compared to GH. The E/e' medial ratio was significantly different between all three groups, with the lowest values recorded in the control group and the highest in the PE group. Other studies also found worse indicators of diastolic function in women with hypertensive pregnancies as assessed with e' medial, e' lateral and the ratio E/e' (Pan et al. (2019), Vaught et al. (2018), Naidoo et al. (2013)).

It is interesting to note that in our study the values of the deceleration time of the E-wave of the mitral inflow were significantly higher in the control group, compared to the two pathological groups that did not differ significantly from each other. In the majority of studies slower deceleration of E-wave was reported in the pathological groups. However, in a 2012 study, Tyldum et al. reported no difference in the E-wave deceleration time between controls and untreated preeclampsia and explained this result with possible pseudonormalization, since at the same time significantly lower e' and higher E/e ratios were also present in their pathological group. We could also accept a similar hypothesis of pseudonormalisation as the reason for the significantly lower deceleration time in our pathological groups, since all other significant differences were indicative of worsened diastolic function in the pathological groups.

It is clear from the results that the older, classic echocardiographic parameters for assessing the left ventricular systolic function, relying on two-dimensional measurements, such as shortening fraction, ejection fraction and MAPSE, cannot distinguish normotensive from hypertensive pregnant women. This lack of difference in the left ventricular systolic function, assessed with those methods, is confirmed by many authors (Vaught et al. (2018), Naidoo et al. (2013), Tyldum et al. (2012), Mostafavi et al. (2019)). When using more recent and complex methods, such as the global longitudinal strain (based on speckle tracking) and the S-waves of medial and lateral mitral annulus (TDI), there were obvious differences between the groups showing a more impaired LV systolic function in both of the studied hypertensive disorders of pregnancy, compared to the normotensive gravidas. These changes are likely a sign of asymptomatic left ventricular systolic dysfunction resulting from poor myocardial adaptation to the pathological pregnancy. However, our study does not identify a possibility of discrimination between PE and GH based on these two parameters. Numerous

studies have shown worse LV GLS values in preeclamptic pregnancies, as well as lower TDI S-wave velocities (Shahul et al. (2012), Vaught et al. (2018), Tyldum et al. (2012), Shahul et al. (2016), Chow et al. (2020)).

Comparison of the mean values of the right-chamber structural parameters

In regard to the right-chamber structural parameters, there is a significant difference in the mean values between the groups for the indexed right atrial volume, as well as for two of the dimensions of the right ventricle – the proximal and distal diameter of the right ventricular outflow tract. For the indexed right atrial volume there was a significant difference only between the PE and the GH groups with higher values in PE, but the GH group did not differ significantly from the controls. No significant difference was present when comparing this parameter between PE and the controls, despite the presence of an algebraically higher mean value in PE.

We observe some similarities between our results and those of Çağlar et al. (2015), who analyzed 67 women with untreated PE and 46 controls and found significantly higher values of the basal diameter of the RV, the proximal and the distal diameter of the RV outflow tract and the RV free wall in PE. The presence of differences in more of the parameters in their study compared to ours, may be due their inclusion of only untreated women with PE. In the same study, the authors also reported a significantly larger indexed area of the right atrium in PE, but did not analyze the indexed RV volume, like it is done in our study.

Comparison of the mean values of the right ventricular functional parameters

With regard to the right ventricular systolic function, there were significant differences only for the mean values of the RV global longitudinal strain (RV GLS), and it was significantly more impaired (higher) in both groups with hypertensive pregnancies compared to the controls, but again they did not differ significantly between PE and GH. In the assessment of diastolic function, significantly lower rates of the e' wave of tricuspid annulus and higher values of the E/e' ratio were observed when comparing PE with the controls. The mean values of these two parameters in the GH group were algebraically between the other two groups, without being significantly different with either of them. Similar observations were made by Vaught et al. in a 2018 study with 63 women with severe preeclampsia features and 36 healthy controls. They did not find any significant differences regarding the S-wave of tricuspid annulus and RV FAC, but there were such when the RV GLS was analyzed and it was significantly lower (better) in controls. The TAPSE values in their study were lower in their pathological group, which is not confirmed by our study.

Analysis of the incidence of abnormal values in the groups

Left-chamber structural parameters

Significant differences existed in the percentage of women with abnormal values for the following parameters: the anterior posterior diameter of LA, the EDV and the ESV of the LV when comparing PE to the controls, while the GH group did not differ significantly from either of the other two groups, but was algebraically in the middle. When comparing the indexed values of the same parameters, the difference between the incidence of abnormal values was no longer significant. Significant differences were present regarding the prevalence of abnormal values for the indexed LA volume – the difference was present when comparing

preeclampsia to both the controls and the GH group, while the GH group did not differ from the controls.

Regarding septum thickness in diastole, there was a significantly higher number of patients with abnormal values in the GH and PE groups, which did not differ statistically from each other, despite the algebraic difference. For the thickness of the PWLV in diastole, there was a significant difference between the controls and the PE group, while the GH group did not differ from either and the percentage was algebraically in the middle. There was no statistical difference in the percentage of abnormal values for EDS and ESS of LV and their indexed values, LVMI or RWT between the groups.

Left ventricular functional parameters

In left ventricular functional parameters, significant differences were observed in the percentage of abnormal values for the LV global longitudinal strain, the e' medial wave and lateral MAPSE. The rest of the parameters - shortening fraction, ejection fraction, the mitral inflow ratio E/A, the deceleration time of the E-wave, e' lateral, the ratios E/e' medial, E/e' lateral and E/e' mean, did not differ significantly between the groups for the prevalence of abnormal values. Additionally, all of those parameters have very low percentages of abnormal values, in the majority of them even equal to zero.

The global longitudinal strain of the LV was the parameter with the greatest difference in terms of abnormal values in the pathological groups, compared to the controls. The percentage distribution according to abnormal values was as follows: 22% in the control group, 69.4% in the gestational hypertension group and 78.4% in the preeclampsia group. An additional advantage of its use is that the significant difference in the prevalence of abnormal values between normotensive and hypertensive pregnancies occurs at the standardized cut-off for abnormality in non-pregnant populations. While not replacing the classically measured left ventricular ejection fraction in the current guidelines, the left ventricular GLS has been proven in multiple studies to be a more sensitive and an earlier marker for detecting asymptomatic systolic dysfunction. It is likely that the large percentage of abnormal values in the pathological groups accurately reflects the presence of such dysfunction, and therefore we could assume that the hypertensive disorders of pregnancy are indeed a functional manifestation of the "failed stress test" in these women. Moreover, unlike most of the classically measured echocardiographic parameters, it is not affected by preload or afterload, which makes it especially valuable in pregnancy.

As far as the other parameters with significant differences are concerned – lateral MAPSE and the medial e'-wave, the prevalence of abnormal values in women with the pathologies is much lower than that for LV GLS - only 2% for lateral MAPSE in the controls, 2.8% in PE and 17.1% in GH; and 13.9% for medial e‟ wave in GH and 8.3% in PE group, none in the controls. We deduce that the abnormal values of these parameters are not as typical of the two studied pathologies as the LV GLS.

Right-chamber structural parameters

Out of the right-chamber parameters, significant differences in the percentage of women with abnormal values were observed for the mid diameter of the RV, which was higher in both pathological groups, without a significant difference between them. The

guidelines, however, do not indicate a cut-off for its indexed value, which is why we have reservations in the interpretation of this phenomenon, since we cannot establish to what extent this value significantly reflects the higher BMI in the pathological groups.

No significant difference was observed between the groups for the percentage of abnormal values for the rest of the structural right-chamber parameters. With a high percentage of abnormal values (close to and above 50%) for all groups were the indexed volume of the right atrium and the distal right ventricular outflow tract.

Right ventricular functional parameters

Out of all studied right-ventricular functional parameters, a statistically significant difference in the percentage of abnormal values was present only for the right ventricular GLS. It is also important to note that the RV GLS is not yet a standardized parameter, unlike the LV GLS, and that the current guidelines for echocardiographic evaluation do not recommend a universal cut-off value. At >-23.3% (as is the female sex-specific cut-off value for the General Electric vendor, with an assessment of only the RV free wall (Muraru et al., 2016)) in the control group 6% had abnormal values, in the GH group - 33.3% and in the PE group - 24.3%, with a statistically significant difference present when comparing each of the two pathological groups with the controls, but not between themselves. The percentage of abnormal RV GLS values in pathological groups is much lower than that for the LV GLS and therefore we cannot claim that the abnormal values of this parameter constitute a typical characteristic of the pathologies.

The rest of the functional parameters did not show significant differences between the groups, and again the percentage of abnormal values is low for all three groups, for some of them even equal to zero. We could not identify another study comparing the percentage of echocardiographic abnormal values in hypertensive pregnancies to that in normotensive ones.

Conclusion:

1. In samples where a strong correlation between BMI and BSA is present, we recommend the use of indexed values of the echocardiographic parameters in order to avoid the influence of BMI, which is typically higher in hypertensive disorders of pregnancy. From the literature review that we conducted, the authors usually provide comparisons of only the absolute values of the parameters, despite reporting higher BMI in the pathological groups in most cases.

2. The gestational hypertension group had more echocardiographic parameters being statistically equal to those in the preeclampsia group, than to the controls, both as mean and abnormal values; a smaller proportion of the parameters occupied a mid-algebraic position between the preeclampsia and the control groups without reaching a statistical difference with either of them, and was rarely statistically the same as the control group, but different from the preeclampsia group. It is worth noting that there was no significant difference between the two hypertensive groups regarding the left ventricular GLS - a parameter, considered to be highly informative for myocardial function assessment; as well as for the majority of diastolic functional parameters.

3. An interesting result concerning the abnormal values, is that structural or functional cardiac changes were not present in all of the women with a hypertensive pathology. At the

same time, not all women with preeclampsia or gestational hypertension will develop cardiovascular diseases, despite having a higher risk. The results of this study enable us to make a more precise risk assessment within the pathological groups, and thus to pay special attention to the women with the most pronounced changes. A scientific assumption can be made that the women with the most pronounced structural or functional deviations during pregnancy will also be at the highest risk for developing cardiovascular disease in the future.

4. Regarding the left-chamber parameters:

- A significant difference was present only in the indexed volume of left atrium between the hypertensive and the normotensive pregnant women;
- There was a more pronounced left ventricular hypertrophy and remodeling in hypertensive pregnancies; and for the mixed pathological group concentric remodeling was the most common left ventricular geometry pattern;
- There were significant differences in multiple parameters of systolic and diastolic left ventricular function between women with hypertensive and normotensive pregnancy, evidencing a more impaired left ventricular function in the studied pathologies;
- Additionally, in comparison to the classically measured shortening fraction, ejection fraction (Simpson's biplane method) and the M-mode measured MAPSE, the LV global longitudinal strain and the S-wave velocities were superior in the assessment of this population for a more precise detection of abnormal systolic function and contractility, and should therefore be preferred;
- Based on our results, the echocardiographic parameter with the greatest difference in the percentage of abnormal values between the pathological groups and the controls, in terms of function is the LV GLS, while in terms of structure are the thickness of the septum in diastole, and to a lesser extent and only for the preeclampsia group - the thickness of the PWLV in diastole and indexed volume of the left atrium.

5. Regarding the right-chamber parameters:

- A significant difference was observed for the mean values of the indexed volume of the right atrium between the preeclampsia and the gestational hypertension group, and there were also significantly higher means for the proximal and distal diameters of the right ventricular outflow tract for both of the pathological groups compared to the normotensive pregnant women;
- Hypertensive pregnancies do not lead to the development of a significant hypertrophy of the right ventricular free wall, unlike to the left ventricular walls;
- There were significant differences in the parameters of systolic and diastolic right ventricular function between the women with hypertensive versus those with normotensive pregnancies, again with evidence of a more impaired right ventricular function in the pathologies;
- Additionally, we have observed benefits of the use of the global longitudinal strain to evaluate the right ventricular systolic function versus the more classical methodologies such as FAC, TAPSE and the S-wave velocity of the tricuspid annulus;
- Regarding the functional parameters, statistical difference was present for the prevalence of abnormal values between normotensive and hypertensive pregnancies only for

right ventricular global longitudinal strain (at a cut-off value of >-23,3%), but the incidence of abnormal values is far lower than that of abnormal values of the left ventricular global longitudinal strain.

4.3. Biomarkers

The following results are presented in table 14:

- For Galectin-3 and IL-6, the mean arithmetic values of the controls were significantly lower than those of the two pathological groups (analyzed separately and as a mixed pathological group), but the two pathological groups did not differ statistically from each other;
- The mean value of hs-CRP in the controls was significantly lower than that in the gestational hypertension group, but not than the value in preeclampsia, which itself did not differ significantly from the other two groups.
- The mean value of PlGF in the controls was significantly higher than that of the two pathological groups (analyzed separately and as a mixed pathological group), but the pathological groups did not differ statistically from each other.
- The mean value of NT-proBNP of the gestational hypertension group was significantly lower than that of controls and preeclampsia patients and the latter two did not differ significantly from each other. There was a trend for the presence of higher levels in women with preeclampsia compared to controls, but it did not reach significance.

Table 14: Comparison analysis of the biomarkers in the groups

Biomarkers	Controls			Gestational hypertension			Preeclampsia			GH + preeclampsia			All		
	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD
Galectin-3 (ng/ml)	50	6,53[a]	1,87	36	7,31[b]	1,92	37	7,59[b]	1,63	73	7,45[b]	1,77	123	7,08	1,86
hs-CRP (ng/ml)	50	5095,61[a]	3086,67	36	6441,12[bc]	3124,17	37	5581,02[ac]	3036,28	73	6005,18[a]	3088,96	123	5635,44	3107,89
IL-6 (pg/ml)	50	2,77[a]	2,43	36	5,08[b]	5,16	37	8,06[b]	12,48	73	6,59[b]	9,65	123	5,04	7,80
PlGF (pg/ml)	50	215,89[a]	175,14	36	81,34[b]	43,50	37	88,60[b]	97,87	73	85,02[b]	75,64	123	138,22	140,94
NT-proBNP (pg/ml)	49	50,11[a]	40,31	34	41,30[b]	51,50	36	86,19[a]	74,74	70	64,39[a]	67,92	119	58,51	58,38

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

Cut-off values of the studied biomarkers and values of the validation criteria for differentiation between the controls and the women with preeclampsia and gestational hypertension

In order to determine the optimal cut-off values of the examined biomarkers, ROC curve analysis was performed. Statistically significant cut-off values could be established for all five of the biomarkers for differentiating the GH group from the controls and for 3 of them for differentiating between the preeclampsia group and the controls (no significant cut-off value could be established for hs-CRP and NT-proBNP). When choosing a cut-off value, the optimization criteria were high sensitivity and precision. The biomarkers with the best cut-off values were PlGF and hs-CRP for gestational hypertension and PlGF and IL-6 for preeclampsia (tables 15 and 16).

Table 15: Cut-off values of the studied biomarkers for differentiation between the patients with gestational hypertension and the controls, AUC and values of the validation criteria for screening tests

Biomarker	Cut-off value	AUC	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	% of correct answers
Galectin-3 (ng/ml)	≥7,15	0,646	67	64	57	73	65
hs-CRP (ng/ml)	≥5446	0,628	72	56	54	74	63
IL-6 (pg/ml)	≥4	0,648	56	72	59	69	65
PlGF (pg/ml)	≤122	0,849	75	70	64	80	72
NT-proBNP (pg/ml)	≤16,35	0,643	50	84	68	71	70

Table 16: Cut-off values of the studied biomarkers for differentiation between the patients with preeclampsia and the controls, AUC and values of the validation criteria for screening tests

Biomarker	Cut-off value	AUC	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	% of correct answers
Galectin-3 (ng/ml)	≥7,25	0,681	70	68	62	76	69
IL-6 (pg/ml)	≥2,82	0,696	73	64	60	76	68
PlGF (pg/ml)	≤82	0,846	70	92	87	81	83

Binary logistical regression was used in order to quantify the association between the cut-off values of the biomarkers and the presence of gestational hypertension or preeclampsia. The highest odds ratio (OR) for the presence of gestational hypertension (tabl. 17) was for the

chosen cut-off value of PlGF (=7), followed by that for NT-proBNP, Galectin-3, hs-CRP and the lowest was for IL-6; while for the presence of preeclampsia (table 18) the highest values of the OR was for PlGF (~27), followed by that for Galectin-3 and the lowest was for IL-6. The results also show that the biomarkers PlGF, Galectin-3 and IL-6 are considerably more precise for the differentiation of preeclampsia from the controls, than for gestational hypertension from the controls.

Table 17: Odds ratios and 95% confidence intervals (CI) of the studied biomarkers as indicators for gestational hypertension

Biomarker	Comparison	OR	95% CI		P
			Lower limit	Upper limit	
PlGF (pg/ml)	≤ 122 / > 122	7,000	2,662	18,409	**<0,001**
NT_proBNP (pg/ml)	≤ 16,35 / > 16,35	5,125	1,861	14,111	**0,002**
Galectin-3 (ng/ml)	≥ 7,15 / < 7,15	3,556	1,443	8,763	**0,006**
hs-CRP (ng/ml)	≥5446 / < 5446	3,309	1,321	8,291	**0,011**
IL-6 (pg/ml)	≥ 4 / < 4	3,214	1,304	7,920	**0,011**

Table 18: Odds ratios and 95% confidence intervals (CI) of the studied biomarkers as indicators for preeclampsia

Biomarker	Comparison	OR	95% CI		P
			Lower limit	Upper limit	
PlGF (pg/ml)	≤ 82 / > 82	27,182	7,856	94,053	**<0,001**
Galectin-3 (ng/ml)	≥ 7,25 / < 7,25	5,023	1,998	12,628	**0,001**
IL-6 (pg/ml)	≥ 2,82 / < 2,82	4,800	1,899	12,133	**0,001**

Discussion

PlGF is an established marker of abnormal placentation and its inclusion in the study aimed to eliminate possible subjective errors in patient selection. The low PlGF values in pathological groups of our study confirm the importance of its use in many European countries as a screening and diagnostic marker for preeclampsia, also due to the fact that its lower levels indicate earlier and more severe forms of preeclampsia (Kleinrouweler et al. (2016), Hodel et al. (2019), Herraiz et al. (2018)). In our study, PlGF ≤130 pg/ml accurately separated the pathological groups from controls with an area under the curve of 0.84, a sensitivity of 84% and a specificity of 66%. Our results are similar to those observed by other authors over the years - in an extensive meta-analysis (Agrawal et al., 2019) of PlGF‟s ability to predict preeclampsia that included 40 studies, the AUC for hierarchical summary ROC (HSROC) model was 0.83.

Galectin-3 is a protein with an established role in the inflammatory process, immunity and carcinogenesis. Its main function is the binding and activation of fibroblasts, which form collagen and fibrous tissue. In human studies, its up-regulation has been proven in patients with left ventricular hypertrophy. There are very few studies of Galectin-3 in pregnant women. A study by Taha et al. (2019) analyzed 60 women with preeclampsia and 30 healthy controls in Iraq and found significantly higher levels in the pathological group. The authors also established a correlation between the higher levels and a worse lipid profile. We could not identify studies, other than ours, that provide an AUC, sensitivity and specificity for a Galectin-3 cut-off value for the presence of preeclampsia or gestational hypertension.

It is worth noting that there is a proven connection between the increased levels of Galectin-3 and the development of various cardiac pathologies. Such a link exists between its levels and the presence of cardiac fibrosis, ventricular remodeling, arterial hypertension, pulmonary hypertension, as well as the manifestations of heart failure, metabolic disorders and dyslipidemia (Gehlken et al. (2018), Amin et al. (2017), Meijers et al. (2014), Li et al. (2016), Ho et al. (2012), Martínez et al. (2015)).

High-sensitivity CRP

The significantly higher hs-CRP values only in the gestational hypertension group were likely the result of a more pronounced inflammatory response in these women compared to the controls, but not to women with preeclampsia, from whom they did not differ statistically. In our study hs-CRP showed no statistical difference between the preeclampsia group and the controls, although algebraically its levels were higher in preeclampsia. In a 2019 study, Rout et al., analyzed data on 160 women with gestational hypertension and 190 controls, demonstrating significantly higher levels of CRP in gestational hypertension during the 2nd and 3rd trimester, respectively 10.01 mg/L and 10.28 mg/L, compared to the controls with 1.85 mg/L and 3.06 mg/L. Of interest is also Brown's 2013 study, in which, when analyzing data on 2,463 women who had given birth, years after the pregnancy the levels of hs-CRP in those with past history of hypertensive pregnancies were significantly higher than in those with normotensive pregnancies, even after adjustment for BMI.

Interleukin-6

There are studies demonstrating elevated serum interleukin-6 levels in preeclampsia (Teran et al. (2001), Sharma et al. (2007), Jonsson et al. (2006), Singh et al. (2009)), as well as studies in which no link has been established between high values and the presence of preeclampsia (Borekci et al. (2007)). Dimitrakova et al. (2016) also found higher levels of interleukin-6, as well as interleukin-2 and TNF-alpha in 40 pregnant women with hypertension after the 20^{th} gestational week, compared to 30 normotensive pregnant women. In their study, a positive, moderate in strength correlation of interleukin-6 levels with the diastolic and a positive weak correlation with the systolic blood pressure has also been demonstrated. In a relatively large study by Xiao et al. from 2012, data were analyzed on 104 women with preeclampsia and 75 healthy pregnant women, and it was found that the interleukin-6 levels were increased in the early, late and severe forms of preeclampsia, but not in the mild ones, and did not differ in the presence of intrauterine growth restriction.

We believe that the increased levels of this biomarker in the pathological groups of our study confirm the inflammatory component and the presence of endothelial dysfunction that are characteristic of the hypertensive disorders of pregnancy. Low-level inflammation is a known risk factor for the occurrence and progression of atherosclerosis (Koenig et al. (2018)).

NT-proBNP

In our study, there was a tendency for higher NT-proBNP levels in preeclampsia compared to normotensive pregnancies, but the difference was not significant. An explanation may be sought in the lower representation of severe forms of preeclampsia in the sample (13 severe vs. 24 mild), as well as their classification as severe mainly based on blood pressure criterion. Similar to our study are the results of Resnik et al. (2005), who did not observe significantly higher levels of NT-proBNP in the mild forms of preeclampsia, but only in the severe forms that prevailed in their study.

In our gestational hypertension group, significantly lower NT-proBNP values were present compared to the preeclampsia group and the controls. Given that, natriuretic peptides are excreted in an attempt to reduce systemic vascular resistance and central venous pressure, and their final effect is a decrease in the blood pressure, we can assume that their production is less pronounced in women with gestational hypertension than in those with preeclampsia due to the more pronounced vasoconstriction in preeclampsia. The lower levels in the gestational hypertension group compared to the controls, may be the result of an inadequate response to the increased requirements of pregnancy and may ultimately be co-involved in the increase of blood pressure in these women.

Over the course of the growing use of natriuretic peptides for the diagnostics of heart failure, one limitation of theirs was established - their values appear to be lower in patients with obesity. It is assumed that specifically for BNP, lower levels are due to a greater adipocytic expression of receptors associated with the clearing of natriuretic peptides - natriuretic peptide clearance receptors-C (NPR-C), but lower levels of NT-proBNP have also been identified in obese people, the clearance of which is not associated with these receptors. In view of these data, another possible explanation for the absence of significantly higher levels of NT-proBNP in the preeclampsia group and for lower ones in the gestational hypertension group could be the presence of a significantly higher BMI in both pathological groups compared to the controls.

Conclusion:

There were no statistical differences in the levels of some of the studied biomarkers – PLGF, IL-6 and Galectin-3 between gestational hypertension and preeclampsia. This is also consistent with the echocardiography results, where the values of the parameters in gestational hypertension in their majority were statistically equal to those in preeclampsia. The two hypertensive pathologies, however, differed from each other as far as the levels of the other two biomarkers were concerned – hs-CRP and NT-proBNP. This gives us a reason to assume that there are probably differences in the underlying pathophysiologic mechanisms, indicating a more pronounced inflammation in gestational hypertension and a lower secretion of the vasodilatory natriuretic peptides. While preeclampsia is considered to be more unfavorable for the course of the pregnancy itself, we believe that more studies are needed to assess the cardiovascular risk in women after gestational hypertension as well, since their profile of pathological reaction during pregnancy does not suggest a more “benign” effect on the cardiovascular system. Following the theory of hypertensive disorders of pregnancy being a "stress test," it would be worth investigating whether the women with greater deviations in the biomarker levels in our study would eventually be more likely to develop cardiovascular disease.

4.4. Associations

4.4.1. Analysis of the characteristics of the women associated with the development of abnormal global longitudinal strain of the left ventricle

Given the large number of echocardiographic parameters in the dissertation, it was decided that at this point only the associations between the left ventricular GLS and the basic characteristics of the women are to be analyzed, as the LV GLS is the echocardiographic marker with the highest number of abnormal values in pathological groups, and also considering its promising role in the detection of asymptomatic left ventricular dysfunction.

In order to test for the presence of correlations between the LV GLS and the following characteristics: gestational week of first detection of high blood pressure, time from the detection of high blood pressure to the inclusion in the study, the maximum measured systolic and diastolic BP in the pathological groups (analyzed separately and as a mixed pathological group), a correlation analysis was performed and it showed a lack of correlation in all groups. Comparative analysis showed a lack of significant correlation between the LV GLS values and the number of pregnancies.

Considering the high percentage of smoking women, including during pregnancy, we deemed it necessary to test if smoking has an effect on the left ventricular GLS values, as well as whether the pack-years in smokers also influence its values. A significant difference was present only between the non-smokers and the former smokers, while the current smokers did not differ statistically from either of the other two groups (table 19).

Table 19: Association between smoking status and LV GLS values

Smoking status	LV GLS		
	n	$\overline{X}$	SD
Non-smokers	43	-20,44[a]	2,55
Former smokers	15	-22,41[bc]	2,78
Current Smokers	60	-20,55[ac]	2,54

* -Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)

A correlation was not established between the pack-years for former and current smokers and the LV GLS, neither in the controls nor in the mixed pathological group. For the current smokers, there was no significant association between the LV GLS values and smoking during the current pregnancy.

Weight

The correlation analysis revealed a correlation between the LV GLS and weight before the pregnancy and at the time of the inclusion in the study only in the control group (table 20). The correlation was positive and moderate in strength.

Table 20: Correlation coefficients between the LV GLS and the following characteristics of the women: pre-pregnancy BMI, BMI at the inclusion in the study, pre-pregnancy weight, weight at the inclusion and weight gain up until the inclusion

Characteristics	LV GLS		
	Gestational hypertension	Preeclampsia	Controls
Pre-pregnancy BMI	0,128	0,242	0,221
BMI at inclusion	0,194	0,213	0,149
Pre-pregnancy weight	0,261	0,228	0,385**
Weight at inclusion	0,262	0,255	0,316*
Weight gain at inclusion	0,031	0,267	-0,070

* - p<0,05, ** - p<0,01

Laboratory markers

Correlation analysis did not establish a significant correlation between the LV GLS values and the following laboratory markers: protein in urine, total serum protein, serum albumin, hemoglobin and uric acid in the pathological groups and between the LV GLS and hemoglobin in the controls.

Binary logistic regression analysis of the factors associated with abnormal left ventricular GLS

A binary logistical regression analysis was conducted in order to establish the factors influencing the appearance of abnormal LV GLS values and to assess their quantitative impact. The following were tested as potential factors: maternal age, pre-pregnancy weight and BMI, weight and BMI at the inclusion in the study, weight gain until the inclusion, patient group (gestational hypertension, preeclampsia, controls), smoking, pack-years, smoking during pregnancy, maximum measured SBP and DBP, days from the detection of high blood pressure (for gestational hypertension and preeclampsia), gestational week of the detection of high blood pressure (for gestational hypertension and preeclampsia).

The requirement for the use of quantitative variables in binary logistical regression analysis is that they have a normal distribution. In our study only the weight and the BMI were with Gaussian distribution. ROC curve analysis was then applied to determine, where possible, cut-off values differentiating between the normal and abnormal LV GLS values. Significant cut-off values could be established for the following: pre-pregnancy weight and BMI, weight and BMI at the inclusion in the study. For the established cut-offs, the validation criteria values were very good – sensitivity 79-80% and precision 68-71% (table 21).

Table 21: Cut-off values for pre-pregnancy weight and BMI, and weight and BMI at the inclusion in the study, AUC and the values of the validation criteria for screening tests for differentiation between normal and abnormal LV GLS values

Parameter	Cut-off	AUC	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	% of correct answers
Pre-pregnancy weight	≥ 64 kg	0,769	79	54	66	70	68
Weight at inclusion	≥ 76 kg	0,748	80	55	67	71	68
Pre-pregnancy BMI	≥ 28 kg/m^2	0,777	79	56	66	71	68
BMI at inclusion	≥ 28 kg/m^2	0,775	80	62	70	73	71

The results from the binary logistic regression analysis were the following:

Individually (tabl. 22):

- The highest odds ratio was for the presence of preeclampsia – compared to the control group, the risk for abnormal LV GLS was about 13 times higher in preeclampsia, and the second highest odds ratio was for the presence of gestational hypertension, associated with about 8 times higher risk compared to the controls;
- The third highest odds ratio was for BMI at inclusion – those with BMI ≥ 28 kg/m2 compared to those with a lower one, had about a 6.4 times higher risk for abnormal LV GLS. The same parameter, represented quantitatively, showed a risk increase of about 6% with an increase of 1 kg/m2. In this form, however, BMI is more difficult to interpret, so its cut-off value was used in the multiple regression equation instead;

In order to take into account their combined influence and to eliminate the confounders, we composed a regression equation with the parameters that had significant odds ratios. We removed highly correlating parameters such as pre-pregnancy weight (correlated with pre-pregnancy BMI, weight at inclusion (correlated with BMI at inclusion), pre-pregnancy BMI (correlated with BMI at inclusion). After applying the procedure "Backward conditional" two of the parameters remained in the final version of the equation – the study group and BMI at inclusion.

Table 22: Odds ratios and 95% CI of the analyzed parameters as factors associated with abnormal LV GLS

Parameter	Comparison	Individually				Combined			
		OR	95% CI		p	OR	95% CI		p
			Lower limit	Upper limit			Lower limit	Upper limit	
Study group	Gestational hypertension/ controls	8,058	3,039	21,362	**<0,001**	4,776	1,671	13,648	**0,004**
	Preeclampsia/ controls	12,852	4,590	35,991	**<0,001**	8,582	2,921	25,216	**<0,001**
BMI at inclusion	≥ 28 kg/m^2 / < 28 kg/m^2	6,420	2,863	14,395	**<0,001**	3,556	1,416	8,930	**0,007**
	Increase with 1 kg/m^2	1,212	1,115	1,317	**<0,001**				
Pre-pregnancy weight	≥ 64 kg / < 64 kg	4,586	2,055	10,231	**<0,001**				
Weight at inclusion	≥ 76 kg / < 76 kg	4,923	2,216	10,937	**<0,001**				
	Increase with 1 kg	1,060	1,032	1,089	**<0,001**				
Pre-pregnancy BMI	≥ 23 kg/m^2 / < 23 kg/m^2	4,825	2,158	10,788	**<0,001**				
Smoking	Former smokers / Non-smokers	0,101	0,020	0,503	**0,005**				
	Smokers / Non-smokers	0,855	0,386	1,896	0,700				
Smoking during the pregnancy	Yes / No	2,241	0,868	5,786	0,095				

Discussion:

A potentially useful for the clinical practice observation is the fact that the LV GLS values are not significantly affected by the values of hemoglobin, uric acid, amount of protein in urine, total serum protein and albumin, uric acid, number of pregnancies, maximum measured SBP and DBP, the gestational week in which increased BP was first reported, as well as the time from the detection of increased BP until the inclusion in the study. In our study, the risk for the occurrence of abnormal LV GLS is determined by the presence of either of the two pathologies, as well as current BMI ≥ 28 kg/m2.

We could not identify studies that investigate the factors involved in the occurrence of abnormal LV GLS or provide cut-off values in pregnant populations.

4.4.2. Associations between some characteristics of the women and the biomarkers

In order to investigate the presence of an association between the studied biomarkers and certain characteristics of the women (maternal age, gestation week, BMI – pre-pregnancy and at the inclusion in the study, weight gain up until the inclusion in the study, the maximum measured SBP and DBP, pack-years, days from the detection of high blood pressure, weight and length of the newborn) we conducted correlation analysis, the results of which are presented in tables 23-26. For the gestational hypertension, the preeclampsia and the mixed pathological group, the tables are abridged in this document, showing the rows only for the characteristics with significant correlations.

Table 23: Correlation coefficients between the biomarkers and some characteristics of the controls

Characteristics	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Maternal age	0,101	0,035	-0,105	-0,123	-0,427**
Gestational week	-0,175	0,176	0,488***	-0,523***	-0,198
Pre-pregnancy BMI	-0,093	0,360*	0,305*	-0,189	-0,025
BMI at inclusion	-0,189	0,442**	0,466**	-0,245	-0,096
Weight gain	-0,107	0,125	0,382**	-0,258	-0,061
Pack-years (smokers)	0,211	0,198	-0,008	0,015	-0,371
Days from initial high BP detection	-	-	-	-	-
Weight of the newborn	0,040	0,180	0,228	-0,295*	-0,023
Length of the newborn	0,028	0,119	0,155	-0,220	0,029

* - p<0,05, ** - p<0,01, *** - p<0,001,

Table 24: Correlation coefficients between the biomarkers and some characteristics of the women with gestational hypertension (abridged table)

Characteristics	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Weight gain	-0,245	0,118	0,069	0,004	-0,408*
Days from initial high BP detection	-0,085	0,255	0,344*	0,286	-0,074
Weight of the newborn	-0,314	0,108	0,379*	0,143	-0,162
Length of the newborn	-0,339	-0,002	0,454**	0,195	-0,230

* - p<0,05, ** - p<0,01, *** - p<0,001,

Table 25: Correlation coefficients between the biomarkers and some characteristics of the women with preeclampsia (abridged table)

Characteristics	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Weight of the newborn	-0,164	0,107	0,054	0,553**	-0,274
Length of the newborn	-0,095	0,073	-0,104	0,598***	-0,138

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 26: Correlation coefficients between the biomarkers and some characteristics of the women with gestational hypertension or preeclampsia (abridged table)

Characteristics	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Weight of the newborn	-0,229	0,123	0,188	0,388**	-0,260*
Length of the newborn	-0,209	0,071	0,154	0,433***	-0,248*

* - p<0,05, ** - p<0,01, *** - p<0,001

No significant difference was present in the mean values of PlGF, Galectin-3, hs-CRP or NT-proBNP according to smoking status in the groups (table not given in this document). The mean values of Interleukin-6 were significantly higher in smokers compared to non-smokers in all women and when analyzed separately, in the controls and in gestational hypertension, but not in preeclampsia (table 27). The comparative analysis did not establish a significant difference in any of the biomarker levels between those who continued to smoke during the pregnancy and those who did not in any of the groups (table not given in this document).

Table 27: Analysis of the association between smoking status and the levels of interleukin-6 (pg/ml) by groups

Group	Smoking status								
	Non-smoker			Former smoker			Smoker		
	n	$\bar{X}$	**SD**	**n**	$\bar{X}$	**SD**	**n**	$\bar{X}$	**SD**
Whole sample	43	5,07[a]	10,68	15	2,60[a]	2,68	60	5,70[b]	6,36
Controls	13	1,64[a]	1,68	10	2,69[ac]	2,17	27	3,34[bc]	2,69
Gestational hypertension	15	2,88[a]	2,48	2**	4,57	6,41	16	7,41[b]	6,43
Preeclampsia	15	10,22[a]	17,00	3**	1,00	1,03	17	7,84[a]	9,03

* Same letters in the rows signify a lack of a statistical difference, while different letters signify the presence of a significant difference ($p<0,05$)
** The category „Former smoker“is not analyzed due to lack of statistical representability

Discussion: The greatest number of correlations between the biomarkers and the basic characteristics of women were established in the control group, and the biomarker with the most correlations was Interleukin-6. In the pathological groups, fewer associations were present, which could probably mean that the influence of factors, other than the main pathology is negligible. In the controls, **the maternal age** had a moderate negative correlation with the NT-proBNP values. It can be assumed that in younger women, the release of NT-proBNP due to cardiac stress is more pronounced than in older women, but the limited age range of our sample (18-43 years) should also be taken into account.

A moderate negative correlation existed between **the gestational week** and the PlGF levels in the controls, which probably reflects the normal decline in the levels of this growth factor after its peak is reached around the 30th gestational week (Chau et al., 2017), given that the earliest gestational week registered for the group is 22nd and the majority of women included were after the 30th gestational week (40 women after the 30th gestational week vs. 10 women before). Interleukin-6 levels also increased with the progression of pregnancy in the control group. No correlations were established between the gestational age and the biomarkers levels in the pathological groups.

Pre-pregnancy BMI and BMI at the inclusion in the study both showed positive correlations with the levels of hs-CRP and IL-6 in the controls, but not in the pathological pregnancies. A similar result for a positive association between hs-CRP levels and BMI was reported by two other authors in non-pregnant populations (Lavanya et al. (2017), Kawamoto et al. (2013)), and a study by Friis et al. (2013) found higher serum levels of hs-CRP, IL-6 and other inflammatory markers in pregnant women with higher BMI. NT-proBNP has a negative correlation with weight gain only in the gestational hypertension group, where the lowest levels of this biomarker were registered. For the natriuretic peptides, it is now known that lower levels are observed in higher BMI (Madamanchi et al. (2014), Reinmann et al. (2020)).

The maximum measured values of SBP and DBP did not correlate with any of the studied biomarkers in the groups, and therefore, we can assume that they are not reliable as a stand-alone indicator for the severity of cardiovascular involvement in the hypertensive disorders of pregnancy.

Interleukin-6 levels were significantly higher in smokers for the entire sample, the controls and in gestational hypertension, but not in preeclampsia. This result is consistent with a 2017 study by Jamil et al. who reported higher IL-6 values in smokers, and there was also a moderate positive correlation between its levels and those of serum amyloid A-LDL, which in turn is a marker of oxidative stress.

Weight and length of the newborn: PlGF had the most pronounced positive correlation with the weight and length of the newborn in the preeclampsia group, which is expected given the role of this hormone in placentation. The negative correlation between the weight of the newborn and this biomarker in the control group is probably associated with its lower levels in more advanced pregnancy when the weight of the fetus is expected to be higher, and the PlGF levels are already declining. NT-proBNP correlated slightly and negatively with the length and weight of the newborn in the mixed pathological group. These observations show that although hypertensive disorders of pregnancy are viewed as a two-step process – hypoperfusion of the fetus, which provokes a response in the maternal organism; and with this study, we seek to clarify first and foremost the changes and implications for the maternal organism, some of the cardiovascular markers could simultaneously reflect the suffering of the fetus. Sadlecki et al. (2016) also found a weak negative correlation ($r=-0.2177$, $p=0.0088$) between maternal serum NT-proBNP levels and fetal weight at birth when analyzing women with preeclampsia, gestational hypertension, gestational diabetes, as well as healthy pregnant women.

In the gestational hypertension group, a moderate in strength positive correlation between interleukin-6 levels and length and weight of the newborn was established. We believe that this result could be due to more complex interactions, since specifically in the gestational hypertension group there was also a moderate positive correlation between IL-6 levels and the time from the first detection of high BP, i.e. as the pathological pregnancy progresses, higher levels of IL-6 are expected; but at the same time, the longer the presence of the pathology could be tolerated without necessitating delivery, the lighter its course; hence closer to term deliveries, resulting in the birth of larger fetuses. On the other hand, there were also significant positive correlations in the control group between IL-6 values and gestational week, BMI and weight gain, which implies the presence of a more complex formation of its levels. Unfortunately for the gestational hypertension group, the distribution was not normal and multiple linear regression analysis could not be performed.

A positive correlation between IL-6 levels and newborn weight was also reported in a study by Szczerba et al. (2017) in a sample consisting of 40 pregnant women with BMI < 30 kg/m2 and 24 pregnant women with BMI ≥ 30kg/m2, and in that study the other factor with a significant impact on fetal weight at birth was maternal BMI.

Conclusions:

1. The greatest number of correlations between the characteristics of the women and the biomarkers were observed in the control group

2. In the pathological groups, the biomarkers, with the exception of IL-6 and NT-proBNP in gestational hypertension, showed no correlations with the characteristics of the mother, which allows us to hypothesize that the influence of factors other than the underlying pathology is negligible, and their levels are determined mainly by the presence of the hypertensive disorder.

3. It is possible that some of the cardiovascular biomarkers also carry information about the impact of the hypertensive disorders on the fetus and potentially be used to predict the outcome of pregnancy, but this is not set as a goal of the current study.

4.4.3. Associations between the echocardiographic parameters and the biomarkers

Tables 28-37 present the results for the correlations between the studied biomarkers and the echocardiographic parameters by groups. For the controls, the gestational hypertension, the preeclampsia and the mixed pathological group, the tables are abridged, showing the rows only for the parameters for which there were significant correlations.

Table 28: Correlation coefficients between the biomarkers and certain left-chamber parameters (whole sample)

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
LA1	0,069	0,070	0,239**	-0,301**	0,018
Ind. LA1	0,088	-0,187*	0,032	-0,139	-0,056
Ind. LA volume	0,041	-0,123	0,094	-0,219*	0,045
Septum in diastole	0,050	0,122	0,184*	-0,310***	-0,127
PWLV in diastole	-0,051	-0,021	0,118	-0,217*	-0,071
EDD of LV	0,052	0,033	0,293**	-0,212*	0,145
ESD of LV	0,051	0,080	0,317***	-0,192*	0,100
Ind. EDD	0,011	-0,160	0,085	-0,044	0,087
Ind. ESD	-0,005	-0,132	0,128	-0,062	0,036
EDV biplane	-0,052	0,174	0,186*	-0,194*	0,000
ESV biplane	0,037	0,166	0,146	-0,262**	-0,016
Ind. EDV bi	-0,062	-0,015	0,061	-0,131	0,036
Ind. ESV bi	0,043	0,060	0,047	-0,193*	-0,007
LVMI	0,021	-0,016	0,234**	-0,224*	-0,015
RWT	-0,067	-0,046	-0,090	-0,052	-0,134
EF (S)	-0,153	-0,125	-0,023	0,193*	0,052
Stroke volume VTI	0,020	0,097	0,215*	-0,049	-0,009
Cardiac output	-0,108	0,109	0,250**	0,046	-0,135
Cardiac index	-0,162	-0,012	0,162	0,124	-0,154
E (MV)	0,100	-0,044	0,126	0,045	0,031
E/A (MV)	0,150	-0,090	0,069	0,029	0,234*
E-DT	-0,031	-0,067	-0,128	0,108	0,027
e‟med.	0,069	-0,080	-0,097	0,318***	0,068
e‟lat.	-0,038	-0,079	-0,133	0,354***	0,151
e‟ mean	-0,006	-0,056	-0,112	0,360***	0,112
E/e‟ mean	0,075	-0,020	0,206*	-0,315***	-0,053
S med.	-0,120	-0,112	-0,174	0,272**	-0,028
S lat.	-0,054	-0,146	-0,255**	0,447***	0,152
LV GLS	0,121	0,121	0,211*	-0,387***	-0,188*

* - p<0,05, ** - p<0,01, *** - p<0,001,

Table 29: Correlation coefficients between the biomarkers and certain left-chamber parameters (controls) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
LA1	-0,072	0,016	0,318*	-0,340*	-0,128
Ind. LA1	-0,028	-0,364**	-0,122	0,081	-0,042
Septum in diastole	-0,030	0,203	0,285*	-0,239	-0,237
PWLV in diastole	-0,070	0,120	0,210	-0,169	-0,262
EDD of LV	-0,039	-0,003	0,356*	-0,139	0,179
ESD of LV	0,026	0,222	0,401**	-0,277	0,048
Ind. EDD	-0,005	-0,274	-0,065	0,298*	0,291*
EDV biplane	-0,079	0,292*	0,179	-0,243	-0,034
ESV biplane	0,008	0,289*	0,225	-0,211	0,028
LVMI	-0,078	0,202	0,441**	-0,311*	-0,083
RWT	-0,007	0,066	-0,029	-0,095	-0,326*
Stroke volume VTI	0,160	0,137	0,322*	-0,122	0,012
Cardiac output	0,095	0,140	0,343*	-0,149	-0,066
E/A (MV)	0,007	-0,181	0,007	0,226	0,350*
E-DT	0,075	-0,084	0,032	-0,295*	-0,175
e‘‘med.	0,275	-0,257	-0,271	0,344*	0,248
e‘‘lat.	0,113	-0,124	-0,232	0,306*	0,248
e‘‘ mean	0,182	-0,188	-0,258	0,324*	0,243

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 30: Correlation coefficients between the biomarkers and certain left-chamber parameters (gestational hypertension) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Ind. LA1	0,028	-0,068	0,171	-0,341*	-0,353*
E (MV)	0,035	-0,121	0,376*	0,054	-0,292
e‘‘med.	-0,002	-0,041	0,357*	0,016	-0,136

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 31: Correlation coefficients between the biomarkers and certain left-chamber parameters (preeclampsia) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Septum in diastole	-0,398*	-0,045	-0,158	0,102	-0,230
EDD of LV	0,092	-0,028	0,358*	-0,387*	0,224
ESD of LV	-0,010	-0,049	0,333*	-0,276	0,314
Ind. EDD	0,262	-0,099	0,316	-0,527**	0,178
Ind. ESD	0,093	-0,201	0,353*	-0,511**	0,285
EDV biplane	-0,245	0,036	0,327*	-0,093	-0,010
ESV biplane	-0,154	-0,044	0,227	-0,412*	-0,095
Ind. EDV bi	-0,100	-0,124	0,407*	-0,295	0,007
Ind. ESV bi	-0,012	-0,036	0,220	-0,468**	-0,103
EF (S)	-0,041	0,127	0,040	0,432**	0,084
Cardiac output	-0,478**	-0,025	0,216	0,285	-0,233
Cardiac index	-0,506**	-0,062	0,237	0,227	-0,258
E/A (MV)	0,567***	-0,061	-0,129	-0,170	0,270
e‟ med.	0,384*	0,192	0,079	-0,004	0,218
e‟ lat.	0,223	-0,036	0,047	0,206	0,416*
e‟ mean	0,299	0,065	0,074	0,126	0,344*
S med.	0,136	-0,273	-0,178	0,410*	0,146
S lat.	0,045	-0,144	-0,300	0,579***	0,165
LV GLS	-0,227	-0,179	-0,140	0,035	-0,398*

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 32: Correlation coefficients between the biomarkers and certain left-chamber parameters (gestational hypertension and preeclampsia) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Ind. LA1	0,170	-0,068	0,132	-0,325**	-0,080
EDD of LV	0,035	0,025	0,238*	-0,191	0,143
ESD of LV	-0,005	-0,030	0,268*	-0,112	0,135
Ind. EDD	0,084	-0,065	0,257*	-0,402***	-0,027
Ind. ESD	0,013	-0,130	0,278*	-0,342**	-0,023
Ind. ESV bi	0,348	0,655	0,011	-0,255*	-0,055
RWT	-0,148	-0,167	-0,237*	0,163	-0,043
EF (S)	-0,021	-0,029	0,099	0,234*	0,078
Stroke volume VTI	-0,150	0,011	0,097	0,124	-0,028
Cardiac output	-0,293*	0,047	0,185	0,249*	-0,180
Cardiac index	-0,335**	0,003	0,219	0,137	-0,263*
E/A (MV)	0,247*	-0,030	0,089	-0,032	0,173
S lat.	0,071	-0,072	-0,143	0,408***	0,119

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 33: Correlation coefficients between the biomarkers and certain right-chamber parameters (whole sample)

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Ind. Volume of RA	0,029	-0,078	0,133	0,079	0,167
RV EDA	0,102	0,076	0,071	-0,023	0,189*
RV ESA	0,185*	0,051	0,120	-0,051	0,207*
Ind. RV EDA	0,103	-0,050	-0,058	0,065	0,153
Ind. RV ESA	0,224*	-0,110	-0,004	0,025	0,233*
FAC	-0,206*	0,002	-0,139	0,105	-0,126
TAPSE	0,117	0,087	0,021	0,017	-0,126
TV E	-0,016	0,052	-0,020	0,080	0,182
RV E-DT	0,009	-0,009	-0,046	-0,211*	0,088
TV E/A	-0,049	-0,124	-0,034	0,133	0,330***
TV e‟	0,048	-0,198*	-0,033	0,246**	0,216*
TV E/e‟	-0,010	0,135	-0,021	-0,070	-0,003
TK e'/a'	0,126	-0,070	-0,018	0,122	0,195*
TV S wave	-0,044	0,047	-0,052	0,246**	0,092
RV GLS	-0,050	0,116	0,147	-0,233**	-0,156
Pulmonary valve acceleration time	-0,023	-0,184*	-0,016	0,068	0,221*

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 34: Correlation coefficients between the biomarkers and certain right-chamber parameters (controls) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
RV ESA	0,145	0,319*	0,286*	0,004	0,267
Ind. RV EDA	0,090	-0,004	-0,249	0,332*	0,313*
Ind. RV ESA	0,176	0,106	0,022	0,271	0,413**
FAC	-0,261	-0,210	-0,389**	0,140	-0,085
TV E/A	-0,059	-0,156	0,001	0,231	0,388**
TV e‟	0,041	-0,231	-0,052	0,196	0,329*
RV GLS	-0,384**	0,182	0,139	-0,046	-0,105

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 35: Correlation coefficients between the biomarkers and certain right-chamber parameters (gestational hypertension) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
FAC	-0,380*	0,212	-0,146	-0,017	-0,270
TV e'/a'	0,001	-0,275	0,023	0,377*	0,204

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 36: Correlation coefficients between the biomarkers and certain right-chamber parameters (preeclampsia) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Ind. Volume of RA	0,218	-0,093	0,178	-0,057	0,357*
FAC	0,040	0,156	0,325*	0,055	-0,218
TV E/e‟	-0,146	0,097	-0,367*	0,202	0,128
TV S wave	0,253	0,005	-0,122	0,367*	0,048

* - p<0,05, ** - p<0,01, *** - p<0,001

Table 37: Correlation coefficients between the biomarkers and certain right-chamber parameters (gestational hypertension and preeclampsia) – abridged table

Parameters	Galectin-3	hs-CRP	IL-6	PlGF	NT-proBNP
Ind. RV ESA	0,283*	-0,213	-0,029	-0,055	0,166
TV E/A	-0,043	-0,083	0,007	-0,009	0,308*
TV S wave	0,161	-0,046	-0,026	0,318**	0,082
Pulmonary valve acceleration time	-0,045	-0,197	0,040	0,054	0,301*

* - p<0,05, ** - p<0,01, *** - p<0,001

Discussion: In our study, the biomarker with the most echocardiographic correlations in the groups was PlGF, with higher values generally associated with smaller heart chamber sizes, thinner left ventricular walls, lower left ventricular mass index and better left ventricular systolic function (Simpson), higher e' and S wave velocities, lower E/e' ratio and lower (better) LV GLS. In regard to the right-chamber parameters, it was associated with lower deceleration time of the tricuspid E-wave, higher S wave velocities and lower (better) RV GLS. It is interesting to note that only in the control group there was a positive correlation between PlGF values and greater EDD of the left ventricle and indexed EDA of the right ventricle. A possible explanation is that normotensive pregnancy, in which **PlGF** levels are higher, is associated with hypervolemia, which determines the larger sizes of cardiac chambers.

In a small study by Pearce et al. (2015) involving 10 women with mild and 13 with severe preeclampsia, a link was found between the increased ratio of cathepsin D/PlGF with a worsened LV GLS before delivery, and postpartum with a decreased fraction of shortening, which the authors interpreted as a subclinical cardiac dysfunction. In another study, Benschop et al. (2019) examined PlGF levels 6 years after pregnancy in 5,475 women and found that its low mid-pregnancy values were associated with a larger diameter of the aortic root and the left atrium, higher left ventricular mass and systolic blood pressure.

NT-proBNP had more correlations with the right-chamber parameters than with the left ones, and the results were confirmative of the secretion of the natriuretic peptides due to stretching of the myocardium. In the preeclampsia group, there was a correlation between higher NT-proBNP levels and a better diastolic function (higher mitral e' lateral and e' mean) and a better systolic function (lower LV GLS). Such associations were not present in the group of gestational hypertension, where its levels, as already reported, were significantly lower than in preeclampsia. This result can be explained by a similar improvement in the cardiac parameters in the course of sacubitril/valsartan treatment – sacubitril blocks BNP degradation, which leads to higher circulating levels (Jhund et al. (2016)). In the preeclampsia group, in otherwise healthy women, it is quite likely that the natriuretic peptides are exhibiting their beneficial clinical effect – as is the goal of the activation of this protective in nature mechanism over the course of heart failure progression.

Higher **Interleukin-6 levels** correlated with larger sizes of the left chambers, a more hypertrophied septum, higher LV mass index, greater stroke volume and cardiac output; in the whole sample it had a positive correlation with the E/e' mean ratio of the mitral valve and a negative correlation with the S' lateral velocity. In terms of right-chamber parameters, the associations were fewer – a positive correlation with right ventricular EDA and a negative one with right ventricular FAC existed in the control group. It is worth noting that IL-6 had a positive correlation with the right ventricular fractional area change (FAC) and a negative one with the E/e‟ ratio of the tricuspid valve in the preeclampsia group alone. Although those particular results might seem paradoxical, they could be explained by the presence of an increased contractility, which is believed to be the initial right ventricular response to a higher afterload (Dewachter (2018), Vonk Noordegraaf (2017), likely happening as a result of the generalized vasoconstriction in preeclampsia, which is not as pronounced in gestational hypertension and not present in the controls. Thus, higher levels of IL-6 could indicate the initial compensatory stages of right ventricular involvement in the systemic syndrome of preeclampsia, despite corresponding to a seemingly better function.

Compared to the other biomarkers, **hs-CRP** showed no correlations with the echocardiographic parameters in the pathological groups, while in the control group it had a positive correlation with the right ventricular EDA, as well as the left ventricular EDV and ESV; there was no correlation with the anterior-posterior diameter of the LA, but a negative one appeared after indexing. Sarojini et al. (2013) established a positive correlation between hs-CRP levels and the left ventricular EDD and ESD and a negative one with the ejection fraction in 46 women with established peripartial cardiomyopathy.

Galectin-3 in the preeclampsia group on the one hand had moderate negative correlations with the cardiac output and the cardiac index, but on the other also had a negative correlation with septum thickness in the diastole and a positive one with the e' wave of medial mitral annulus. With regard to the right-chamber parameters, its levels corresponded positively with the indexed and non-indexed right ventricular EDA in the whole sample and negatively with the right ventricular FAC in the gestational hypertension group; only in the control group there was a negative correlation with RV GLS. A study by Zaborska et al. (276) found a negative correlation between galectin-3 levels and certain parameters of RV systolic function (TAPSE and tricuspid S' wave) in patients with reduced

left ventricular ejection fraction, but the authors did not observe any differences in the left ventricular parameters according to the levels of this biomarker.

5. CONCLUSIONS

1. Pregnant women with gestational hypertension and preeclampsia had a more unfavorable baseline cardiovascular profile - higher BMI, more frequent family history for arterial hypertension, and nearly half of them were also smokers.

2. Compared to normotensive pregnancies, hypertensive disorders of pregnancy were characterized by the presence of significantly more pronounced changes in the echocardiographically evaluated cardiac structure and function.

3. The two-dimensional global longitudinal strain (GLS), based on speckle tracking, is more sensitive to detecting changes in the systolic function of both ventricles than the classically measured parameters – the left ventricular ejection fraction, shortening fraction and MAPSE; and the right ventricular FAC and TAPSE.

4. For the majority of the echocardiographic parameters and biomarkers - PlGF, Galectin-3, Interleukin-6 and hs-CRP, the women with gestational hypertension showed no statistical difference from those with preeclampsia, which leads to the interpretation that the lack of registered proteinuria likely does not equal a more favorable cardiovascular reaction to the hypertensive pregnancy.

5. NT-proBNP levels were higher in preeclampsia compared to gestational hypertension, while hs-CRP levels were higher when comparing gestational hypertension with the controls, but not preeclampsia with the controls, which may suggest differences in the underlying pathophysiologic mechanisms.

6. The presence of preeclampsia, gestational hypertension and BMI $\geq$ 28 kg/m^2 were independently associated with the occurrence of abnormal left ventricular GLS.

7. With the exception of IL-6, there were not many correlations between the biomarkers‟ levels and the basic characteristics of the women, which would allow the differences in their values to be attributed mainly to the presence or not of the studied pathologies.

8. The correlation analysis gave the following results:

- PlGF had the greatest number of correlations with the echocardiographic parameters, with higher values corresponding to less pronounced changes;
- Isolated for the preeclampsia group, higher NT-proBNP values corresponded to better indicators of left ventricular systolic (GLS) and diastolic function;
- For the rest of the biomarkers, higher values mostly corresponded to more pronounced structural and functional changes.

6. CONTRIBUTIONS

Contributions of a primarily scientific and theoretical nature:

1. Original contribution for Bulgaria is the identification of echocardiographic parameters applied in clinical practice, which detect differences between groups with gestational hypertension, preeclampsia and normotensive pregnant women.
2. For the first time in Bulgaria, the discriminatory abilities of the listed five biomarkers for distinguishing between pregnant patients with gestational hypertension and preeclampsia, and healthy normotensive pregnant women were studied.
3. A comprehensive analysis was carried out and associations were established between the echocardiographic parameters and the five biomarkers in pregnant women with gestational hypertension, preeclampsia and normotensive pregnancies.

Contributions of a primarily applied nature

1. An input document for the generation of a database and a database "Pregnant women with gestational hypertension/preeclampsia" were created.
2. The study encompassed a young population with an early, gender-specific risk factor for cardiovascular events, allowing for a further follow-up and control of other risk factors for the purpose of primary prevention.
3. The global longitudinal strain, based on speckle tracking, has been proven as a more sensitive modality for the detection of changes in the systolic function of both ventricles in this population, compared to the classically measured parameters.
4. Based on the observed differences in the echocardiographic parameters and biomarker levels, models for the evaluation and prediction of cardiovascular risk in such populations can be subsequently created.

7. PUBLICATIONS, PARTICIPATIONS IN SCIENTIFIC FORUMS AND PROJECTS RELATED TO THE DISSERTATION

Publications

1. **Gencheva DG,** Nikolov FP, Uchikova EH, Mihaylov RD, Pencheva BG, Ivanova KI. High-sensitivity CRP levels in women with gestational hypertension, preeclampsia and in normotensive pregnant women and its correlations. Folia Med (Plovdiv) 2021;63(4):511-8. doi: 10.3897/folmed.63.e56489.

2. **Dolina Gencheva**, Fedya Nikolov, Ekaterina Uchikova, Krasimira Hristova, Rosen Mihaylov, Blagovesta Pencheva. Hypertension in pregnancy as an early sex-specific risk factor for cardiovascular diseases: evidence and awareness. Folia Medica. ISSN 0204-8043, SJR (2019) =0.252, Q 3. ID 64741. Accepted 18.03.2021.

3. **Dolina Gencheva**, Fedya Nikolov, Ekaterina Uchikova, Krasimira Hristova, Rosen Mihaylov, Blagovesta Pencheva. Cardiac biomarkers in pregnancies, complicated by hypertensive disorders. Open Access Macedonian Journal of Medical Sciences. Open Access Maced J Med Sci. 2021 Apr 16; 9F:137-144. E-ISSN:1857-9655, SJR (2019) =0.260, Q 3. ID OJS5913.

Participations in scientific forums

1. Participation with a presentation "Hypertension during pregnancy and cardiac function" at the scientific forum “ARTERIALE 2020 – the circle from target organ damage to a clinically manifested event”, 31 January-02 February 2020, Sofia.

2. Participation with an e-poster in the Jubilee Scientific Conference "Medicine of the Future 2020" at the Medical University of Plovdiv, 29-31 Sept. 2020, virtual congress - "High-sensitivity CRP is elevated in women with gestational hypertension, while in normotensive pregnancy it correlates with BMI and BSA".

3. Participation in the "Bulgarian Cardiovascular Association in Support of Science" 17.10.2020, National Palace of Culture, Bulgaria Sofia - "Evaluation of changes in cardiac structure and function in pregnant women with gestational hypertension and preeclampsia".

4. Accepted abstract for oral presentation "Cardiovascular Biomarkers and their changes in hypertensive disorders of pregnancy" at the international congress "XXVIII. BALKAN CLINICAL LABORATORY FEDERATION MEETING and XIII. NATIONAL CONFERENCE OF CLINICAL LABORATORY", which will be held 08-11.09.2021 in Sofia, Bulgaria.

Participations in scientific projects

Project of Medical University - Plovdiv DPDP 19/2019 " Assessment of changes in cardiac structure and function in pregnant women with preeclampsia and gestational hypertension" with chief investigator assoc. prof. Dr. Dolina Gencheva, under the supervision of Prof. Dr. Fedya Nikolov and Prof. Dr. Ekaterina Uchikova